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(54) 【発明の名称】 PD-L2分子：新規PD-1リガンドおよびその使用

(57) 【要約】

本発明は、PD-L2核酸分子と命名された単離核酸分子を提供し、該分子は、PD-1リガンドである新規B7関連分子をコードする。また本発明は、アンチセンス核酸分子、PD-L2核酸分子を含有する組換え発現ベクター、該発現ベクターが導入されている宿主細胞、ならびにPD-L2遺伝子が導入または破壊されている非ヒトトランスジェニック動物も提供する。さらに本発明は、単離PD-L2ポリペプチド、融合蛋白、抗原性ペプチドならびに抗PD-L2抗体を提供する。さらに本発明は、PD-L2とPD-1との間の相互作用を促進または阻害する方法を提供する。本発明の組成物を用いる診断および治療方法も提供される。

【特許請求の範囲】

【請求項 1】

- a) 第 1 の抗原濃度において、PD-L2 標的分子を発現する T 細胞を試験化合物と接触させ；
- b) 第 1 の抗原濃度において、T 細胞増殖またはサイトカイン産生をモジュレーションする試験化合物の能力を決定し；
- c) 第 2 の抗原濃度において、PD-L2 標的分子を発現する T 細胞を試験化合物と接触させ；ついで
- d) 第 1 の抗原濃度において、T 細胞増殖またはサイトカイン産生をモジュレーションする試験化合物の能力を決定し、
- そのことにより、第 1 および第 2 の抗原濃度において T 細胞活性化をモジュレーションする化合物を同定する
- ことを特徴とする、第 1 および第 2 の抗原濃度において T 細胞活性化をモジュレーションする化合物を同定する方法。

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【請求項 2】

PD-L2 標的分子が PD-1 である請求項 1 のアッセイ。

【発明の詳細な説明】

【0001】

関連出願

本願は、2000年6月28日出願の米国仮出願第60/214563号、2001年2月23日出願の米国仮出願第60/270822号、2001年2月23日出願の米国仮出願第60/271114号に対して優先権を主張するものである。これらの各出願の全内容をこの参照により本明細書に一体化させる。

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【0002】

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【0003】

発明の背景

T細胞が外来ポリペプチドに応答するためには、抗原提示細胞（APCs）により休止Tリンパ球に2つのシグナルが提供されなくてはならない（Jenkins, M. and Schwartz, R. (1987) J. Exp. Med. 165:302-319; Mueller, D. L. et al. (1990) J. Immunol. 144:3701-3709）。最初のシグナルは免疫応答に特異性を付与するものであり、主要組織適合抗原系において提示された外来抗原の認識後にT細胞受容体を介して伝達される。次のシグナルは同時刺激と呼ばれるものであり、T細胞が増殖し、機能的となることを誘導する（Lenschow et al. (1996) Annu. Rev. Immunol. 14:233）。同時刺激は抗原特異的でもなく、MHCにより制限されず、APCsにより発現される1またはそれ以上の異なる表面分子により提供され

と考えられている（Jenkins, M. K. et al. (1988) J. Immunol. 140:3324-3330; Linsley, P. S. et al. (1991) J. Exp. Med. 173:721-730; Gimmi, C. D. et al. (1991) Proc. Natl. Acad. Sci. USA 88:6575-6579; Young, J. W. et al. (1992) J. Clin. Invest. 90:229-237; Koulouva, L. et al. (1991) J. Exp. Med. 173:759-762; Reiser, H. et al. (1992) Proc. Natl. Acad. Sci. USA 89:271-275; van-Seventer, G. A. et al. (1990) J. Immunol. 144:4579-4586; LaSalle, J. M. e

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t al. (1991) J. Immunol. 147:774-80; Dustin, M. I. et al. (1989) J. Exp. Med. 169:503; Armitage, R. J. et al. (1992) Nature 357:80-82; Liu, Y. et al. (1992) J. Exp. Med. 175:437-445)。

【0004】

APCs上に発現されるCD80 (B7-1) およびCD86 (B7-2) 蛋白は重要な同時刺激分子である (Freeman et al. (1991) J. Exp. Med. 174:625; Freeman et al. (1989) J. Immunol. 143:2714; Azuma et al. (1993) Nature 366:76; Freeman et al. (1993) Science 262:909)。B7-2は一次免疫応答において主な役割を果たしていると思われ、B7-1は免疫応答の後期にアップレギュレーションされるものであり、一次T細胞応答の延長あるいは二次T細胞応答の同時刺激に重要である可能性がある (Bluestone (1995) Immunity 2:555)。

【0005】

B7-1およびB7-2が結合する1のリガンドであるCD28は休止T細胞上に構成的に発現され、活性化後の発現において増加する。T細胞受容体を介するシグナリング後、CD28と同時シグナルの伝達との連結により、T細胞の増殖およびIL-2分泌が誘導される (Linsley, P. S. et al. (1991) J. Exp. Med. 173:721-730; Gimmi, C. D. et al. (1991) Proc. Natl. Acad. Sci. USA 88:6575-6579; June, C. H. et al. (1990) Immunol. Today 11:211-6; Harding, F. A. et al. (1992) Nature 356:607-609)。CTLA4 (CD152) と呼ばれる第2のリガンドはCD28と相同的であるが、休止T細胞上には発現されず、T細胞活性化後に出現する (Brunet, J. F. et al. (1987) Nature 328:267-270)。CTLA4はT細胞応答の負の調節に重要であると思われる (Waterhouse et al. (1995) Science 270:985)。CTLA4のブロックは阻害シグナルを除去することがわかっているが、CTLA4の凝集は、T細胞応答をダウンレギュレーションする阻害シグナルを提供することがわかっている (Allison and Krummel (1995) Science 270:932)。B7分子はCD28よりもCTLA4に対して高いアフィニティーを有し (Linsley, P. S. et al. (1991) J. Exp. Med. 174:561-569)、B7-1およびB7-2はCTLA4分子の異なる領域に結合し、CTLA4への結合に関して異なるキネティクスを有することがわかっている (Linsley et al. (1994) Immunity 1:793)。CD28およびCTLA4に関連している新たな分子ICOSが、そのリガンドを有するものとして同定されており (Hutloff et al. (1999) Nature 397:263; WO 98/38216)、ICOSはB7ファミリーの新たなメンバーである (Aicher A. et al. (2000) J. Immunol. 164:4689-96; Mages H. W. et al. (2000) Eur. J. Immunol. 30:1040-7; Brodie D. et al. (2000) Curr. Biol. 10:333-6; Ling V. et al. (2000) J. Immunol. 164:1653-7; Yoshinaga S. K. et al. (1999) Nature 402:827-32)。T細胞が、さらなる同時刺激シグナルを受け取ることなくT細胞受容体を介して刺激されるだけならば、それらは無応答性で、アネルギー性となり、あるいは死滅して免疫応答のダウンレギュレーションを引き起こすようになる。

【0006】

免疫細胞は活性化シグナルを伝達する受容体を有している。例えば、T細胞はT細胞受容

体およびCD3複合体を有し、B細胞はB細胞受容体を有し、ミエロイド細胞はFc受容体を有している。さらに、免疫細胞は、同時刺激シグナルを提供するシグナルを伝達する受容体あるいは受容体により媒介されるシグナリングを阻害するシグナルを伝達する受容体を有している。例えば、CD28は同時刺激シグナルをT細胞に伝達する。T細胞受容体の連結後、CD28の連結は、例えば、IL-2 α 、IL-2 β およびIL-2 γ 受容体のアップレギュレーション、IL-2メッセンジャーRNA転写増加、ならびにサイトカイン（IL-2、IFN- γ 、GM-CSFおよびTNF- α を包含）遺伝子発現増加により特徴づけられる同時刺激シグナルを生じさせる。同時刺激シグナルの伝達により、細胞が細胞周期を経るようになり、かくして、T細胞増殖が増大する（Greenfield et al. (1998) Crit. Rev. Immunol. 18: 389）。同時刺激シグナルを細胞に伝達するT細胞上の受容体の同時刺激リガンドによる結合（例えば、サイトカイン分泌および／またはT細胞増殖を誘導する同時刺激受容体の連結）は同時刺激を引き起こす。かくして、同時刺激リガンドと同時刺激シグナルを免疫細胞に伝達する受容体との間の相互作用の阻害は、免疫応答のダウンレギュレーションおよび／または免疫細胞アネルギーと呼ばれる特異的無応答を引き起こす。例えば、抗-CD28 Fabフラグメント、B7ファミリー分子に対する抗体を用いて、あるいはB7ファミリーのメンバーの分子が競争的阻害剤として結合しうる可溶性形態の受容体（例えばCTLA4）を用いてこの相互作用の阻害が行なわれうる。

【0007】

同時刺激分子に結合する阻害性受容体も免疫細胞上において同定されている。例えば、CTLA4の活性化はT細胞に負のシグナルを伝達する。CTLA4を用いることはIL-2産生を阻害し、細胞周期停止を誘導しうる（Krummel and Allison (1996) J. Exp. Med. 183: 2533）。さらに、CTLA4欠損マウスはリンパ球増殖性の疾病を発症する（Tivol et al. (1995) Immunity 3: 541; Waterhouse et al. (1995) Science 270: 985）。抗体でのCTLA4のブロックは阻害性シグナルを除去する可能性があり、抗体とのCTLA4の凝集は阻害性シグナルを伝達する。それゆえ、同時刺激分子が結合する受容体に応じて（すなわち、CD28のごとき同時刺激受容体またはCTLA4のごとき阻害性受容体）、B7-4を包含するB7分子はT細胞同時刺激または阻害を促進しうる。

【0008】

PD-1は免疫グロブリン分子のファミリーのメンバーである（Ishida et al. (1992) EMBO J. 11: 3887; Shinohara et al. (1994) Genomics 23: 704）。PD-1は以前、プログラムされた細胞死のモジュレーターを同定するための設計されたアプローチに基づく引き算クローニングを用いて同定された（Ishida et al. (1992) EMBO J. 11: 3887-95; Woronicz et al. (1995) Curr. Top. Microbiol. Immunol. 200: 137）。PD-1は、リンパ球生存調節、例えば、クローン選択に間において役割を果たしている（Honjo (1992) Science 258: 591; Agata et al. (1996) Int. Immunology 8: 765; Nishimura et al. (1996) Int. Immunology 8: 773）。PD-1は免疫グロブリンスーパーファミリードメイン、膜貫通ドメインを含む細胞外領域、ならびに免疫受容体チロシンキナーゼに基づく阻害モチーフ（ITIM）を含む細胞内領域を有している（Ishida et al. (1992) supra; Shinohara et al. (1994) supra; US Patent 5,698,520）。この特徴は、免疫阻害性受容体と呼ばれる分子のより大きなファミリーも定義し、該ファミリーはgp49B、PIR-Bおよびキラー阻害性受容体（KIRs）をも包含する（Vivier and Daerou (1997) Immunology Today 18: 286）。これらの受容体のチロシルリン酸化ITIMモチーフが、阻害性シグナルを誘導するSH2ドメイン含有ホ

スファターゼと相互作用すると仮定されることが多い。これらの免疫阻害性受容体の一部のものはMHC分子、例えばKIRsに結合し、CTLA4はB7-1およびB7-2に結合する。MHCとB7遺伝子との間に系統発生的関連性があると提案されている(Henry et al. (1999) Immunology Today 20:285-288)。

【0009】

PD1はB細胞応答のレギュレーターとしても関与している(Nishimura (1998) Int. Immunology 10:1563)。T細胞上でのみ見出されるCTLA4とは異なり、PD-1はB細胞(抗-IgMに応答)上ならびに胸腺細胞およびミエロイド細胞の一部のものの上にも見出される(Agata et al. (1996) supra; Nishimura et al. (1996) Int. Immunology 8:773)。

【0010】

B7:CD28/CTLA4同時刺激経路の重要性がインビトロおよびいくつかのインビボモデル系において示されている。この同時刺激経路のブロックは、メズミおよびヒトの系において抗原特異的耐性を発生させる(Harding, F. A. et al. (1992) Nature 356:607-609; Lenschow, D. J. et al. (1992) Science 257:789-792; Turka, L. A. et al. (1992) Proc. Natl. Acad. Sci. USA 89:11102-11105; Gimmi, C. D. et al. (1993) Proc. Natl. Acad. Sci. USA 90:6586-6590; Bousiotis, V. et al. (1993) J. Exp. Med. 178:1753-1763)。逆に、B7陰性ネズミ腫瘍細胞によるB7の発現は、T細胞により媒介される特異的免疫を生じさせ、それは腫瘍拒絶および腫瘍攻撃に対する長期防御を伴う(Chen, L. et al. (1992) Cell 71:1093-1102; Townsend, S. E. and Allison, J. P. (1993) Science 259:368-370; Baskar, S. et al. (1993) Proc. Natl. Acad. Sci. 90:5687-5690)。それゆえ、同時刺激経路を操作することにより、ヒトの免疫応答を刺激または抑制する大きな可能性が得られる。

【0011】

発明の概要

本発明は、少なくとも一部には、本明細書においてPD-L2核酸およびポリヌクレオチド分子と称される新規核酸分子およびかかる核酸分子によりコードされるポリペプチドの発見に基づくものであり、それらはB7ファミリーのメンバーでありPD-1のリガンドである。PD-L2とPD-1との相互作用は負のシグナルを免疫細胞に伝達し、免疫応答をダウンレギュレーションする。好ましいPD-L2分子はプロフェッショナル抗原提示細胞(例えば、Bリンパ球、単球、樹状細胞およびランゲルハンス細胞)および他の抗原提示細胞(例えば、ケラチノサイト、内皮細胞、星状細胞、線維芽細胞およびオリゴデンドロサイト)表面上に発現され、リンパ球活性化をダウンレギュレーションし、そして/あるいはPD-L2分子を認識する抗体により結合されるものである。本発明のPD-L2核酸およびポリペプチド分子は、例えば免疫応答のモジュレーションにおいて有用である。したがって、1の態様において、本発明はPD-L2ポリペプチドをコードする単離核酸分子ならびにPD-L2をコードする核酸の検出するためのプライマーまたはハイブリダイゼーションプローブとして適当な核酸フラグメントを提供する。

【0012】

1の具体例において、本発明のPD-L2核酸分子は配列番号:1または3に示すヌクレオチド配列またはその相補配列に対して(例えば、ヌクレオチド配列全長に対して)少なくとも約70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%またはそれ以上同一である。

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【0013】

好ましい具体例において、単離核酸分子は配列番号：1または3に示すヌクレオチド配列、あるいはその相補配列を包含する。もう1つの具体例において、核酸分子は配列番号：3に示す核酸配列および配列番号：1のヌクレオチド1～273を包含する。さらなる具体例において、核酸分子は配列番号：3に示す核酸配列および配列番号：1のヌクレオチド1096～1223を包含する。もう1つの好ましい具体例において、核酸分子は配列番号：1または3に示すヌクレオチド配列からなる。

【0014】

もう1つの具体例において、PD-L2核酸分子は、配列番号：2のアミノ酸配列に対して十分に同一であるアミノ酸配列を有するポリペプチドをコードするヌクレオチド配列を包含する。好ましい具体例において、PD-L2核酸分子は、配列番号：2のアミノ酸配列の全長に対して少なくとも約71%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%またはそれ以上同一であるアミノ酸配列を有するポリペプチドをコードするヌクレオチド配列を包含する。

【0015】

もう1つの好ましい具体例において、単離核酸分子はヒトPD-L2のアミノ酸配列をコードする。さらにもう1つの好ましい具体例において、核酸分子は配列番号：2のアミノ酸配列を有するポリペプチドをコードするヌクレオチド配列を包含する。さらにもう1つの好ましい具体例において、核酸分子は少なくとも約50、100、150、200、250、300、350、400、450、500、550、600、650、700、750、800、850、900、950、1000、1050、1100、1150ヌクレオチドまたはそれ以上の長さである。さらなる好ましい具体例において、核酸分子は少なくとも約50、100、150、200、250、300、350、400、450、500、550、600、650、700、750、800、850、900、950、1000、1050、1100、1150ヌクレオチドまたはそれ以上の長さであり、PD-L2活性（本明細書に記載）を有するポリペプチドをコードする。

【0016】

本発明のもう1つの具体例は核酸分子、好ましくはPD-L2核酸分子に関するものであり、それらは非PD-L2ポリペプチドをコードする核酸分子と比較してPD-L2核酸分子を検出する。例えば、1の具体例において、かかる核酸分子は少なくとも880、900、950、1000、1050、1100、1150ヌクレオチドまたはそれ以上の長さであり、ストリンジェントな条件下で配列番号：1に示すヌクレオチド配列を含む核酸分子またはその相補配列とハイブリダイゼーションする。もう1つの具体例において、かかる核酸分子は少なくとも20、30、40、50、100、150、200、250、300ヌクレオチドまたはそれ以上の長さであり、ストリンジェントな条件下で配列番号：1のヌクレオチド1～358を含む核酸分子またはその相補配列とハイブリダイゼーションする。さらなる具体例において、かかる核酸分子は少なくとも20、30、40、50、100、150、200、250、300、350、400、450、500、550、600、650、700、750、800、850、900、950、1000、1050、1100、1150ヌクレオチドまたはそれ以上の長さであり、配列番号：1のヌクレオチド1～358を含む配列の少なくとも15個のヌクレオチドまたはその相補配列を含み、ストリンジェントな条件下で配列番号：1に示すヌクレオチド配列を含む核酸分子またはその相補配列とハイブリダイゼーションする。

【0017】

好ましい具体例において、核酸分子は少なくとも約880ヌクレオチドの長さであり、ストリンジェントな条件下で配列番号：1に示すヌクレオチド分子（すなわち、配列番号：1の少なくとも880個の連続したヌクレオチド）またはその相補配列とハイブリダイゼーションする。他の好ましい具体例において、核酸分子は少なくとも約15ヌクレオチドの長さであり、ストリンジェントな条件下で配列番号：1に示すヌクレオチド分子のヌクレオチド1～358（すなわち、配列番号：1のヌクレオチド1～358の少なくとも15

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個の連続したヌクレオチド) またはその相補配列とハイブリダイゼーションする。さらなる好ましい具体例において、核酸分子は少なくとも15ヌクレオチドの長さであり、配列番号: 1のヌクレオチド1~358またはその相補配列を含む配列の少なくとも15個(すなわち、連続した15個)のヌクレオチドを含み、ストリンジェントな条件下で配列番号: 1に示すヌクレオチド配列またはその相補配列を含む核酸分子とハイブリダイゼーションする。

【0018】

さらに他の好ましい具体例において、核酸分子は配列番号: 2のアミノ酸配列を含むポリペプチドの天然に存在する対立遺伝子変種をコードし、該核酸分子は配列番号: 1または3を含む核酸分子の相補配列あるいはその相補配列とストリンジェントな条件下でハイブリダイゼーションする。 10

【0019】

本発明のもう1つの具体例は、PD-L2核酸分子に対するアンチセンスである、例えば、配列番号: 1または3に示すPD-L2核酸分子のコーディング鎖に対してアンチセンスである単離核酸分子を提供する。

【0020】

本発明のもう1つの態様は、PD-L2核酸分子を含むベクターを提供する。ある具体例において、ベクターは組換え発現ベクターである。もう1つの具体例において、本発明は、本発明のベクターを含む宿主細胞を提供する。さらにもう1つの具体例において、本発明は、本発明の核酸分子を含む宿主細胞を提供する。また本発明は、適当な培地中で宿主細胞、例えば組換え発現ベクターを含む本発明の非ヒト哺乳動物細胞のごとき哺乳動物宿主細胞を培養して、ポリペプチドを得ることによる、ポリペプチド、好ましくはPD-L2ポリペプチドの製造方法を提供する。 20

【0021】

本発明のもう1つの態様は、単離または組換えPD-L2ポリペプチド(例えば、蛋白、ポリペプチド、ペプチドまたはそれらのフラグメントもしくは部分)に関する。1の具体例において、単離PD-L2ポリペプチドは少なくとも1またはそれ以上の下記ドメインを含む: シグナルペプチドドメイン、IgVドメイン、IgCドメイン、細胞外ドメイン、膜貫通ドメイン、および細胞質ドメイン。

【0022】

好ましい具体例において、PD-L2ポリペプチドは少なくとも1またはそれ以上の下記ドメインを含み、配列番号: 2のアミノ酸配列に対して少なくとも約71%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%またはそれ以上同一であるアミノ酸配列を有する: シグナルペプチドドメイン、IgVドメイン、IgCドメイン、細胞外ドメイン、膜貫通ドメイン、および細胞質ドメイン。もう1つの好ましい具体例において、PD-L2ポリペプチドは少なくとも1またはそれ以上の下記ドメインを含み、PD-L2活性(本明細書に記載)を有する: シグナルペプチドドメイン、IgVドメイン、IgCドメイン、細胞外ドメイン、膜貫通ドメイン、および細胞質ドメイン。 30

【0023】

さらにもう1つの好ましい具体例において、PD-L2ポリペプチドは少なくとも1またはそれ以上の下記ドメインを含み、配列番号: 1または3のヌクレオチド配列を含む核酸分子の相補配列とストリンジェントな条件下でハイブリダイゼーションするヌクレオチド配列を有する核酸分子によりコードされる: シグナルペプチドドメイン、IgVドメイン、IgCドメイン、細胞外ドメイン、膜貫通ドメイン、および細胞質ドメイン。 40

【0024】

もう1つの具体例において、本発明は、配列番号: 2のアミノ酸配列を有するポリペプチドのフラグメントまたは部分に関し、フラグメントは配列番号: 2のアミノ酸配列の少なくとも15個のアミノ酸(すなわち、連続したアミノ酸)を含む。もう1つの具体例において、PD-L2ポリペプチドは配列番号: 2のアミノ酸配列を含むか、あるいはそれよ 50

りなる。

【0025】

もう1つの具体例において、本発明は、配列番号：1または3のヌクレオチド配列に対して少なくとも約70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%またはそれ以上同一であるヌクレオチド配列、またはその相補配列からなる核酸分子によりコードされるPD-L2ポリペプチドに関する。さらに本発明は、配列番号：1または3のヌクレオチド配列を含む核酸分子の相補配列にストリンジェントな条件下でハイブリダイゼーションするヌクレオチド配列からなる核酸分子によりコードされるPD-L2ポリペプチドに関する。

【0026】

本発明のポリペプチドまたはその部分、例えば、その生物学的に活性のある分部を、非PD-L2ポリペプチド（例えば、異種アミノ酸配列）に作動可能に連結して、融合ポリペプチドを得ることができる。さらに本発明は、本発明のポリペプチド、好ましくはPD-L2ポリペプチドに特異的に結合するモノクローナルまたはポリクローナル抗体のごとき抗体に関する。さらに、PD-L2ポリペプチド（またはその生物学的に活性のあるフラグメント）またはPD-L2分子のモジュレーターを、医薬上許容される担体を含んでもよい医薬組成物中に含有させることができる。

【0027】

もう1つの態様において、本発明は、生物学的試料をPD-L2核酸分子を検出する作用剤と接触させて、生物学的試料中のPD-L2核酸分子、蛋白またはポリペプチドを検出することによる、生物学的試料中のPD-L2核酸分子、蛋白またはポリペプチドの存在を検出する方法を提供する。

【0028】

もう1つの態様において、本発明は、生物学的試料をPD-L2活性のインジケーターを検出する作用剤と接触させて、生物学的試料中のPD-L2活性の存在を検出することによる、生物学的試料中のPD-L2活性の存在を検出する方法を提供する。

【0029】

もう1つの態様において、本発明は、PD-L2を発現する細胞をPD-L2活性をモジュレーションする作用剤と接触させて、細胞中のPD-L2活性をモジュレーションすることを特徴とする、PD-L2活性をモジュレーションする方法を提供する。1の具体例において、作用剤はPD-L2活性を阻害する。もう1つの具体例において、作用剤はPD-L2活性を刺激する。さらなる具体例において、作用剤はPD-L2ポリペプチドとその本来の結合パートナー、例えばPD-1との間の相互作用を妨害または促進する。好ましい具体例において、結合パートナーはPD-1である。1の具体例において、作用剤はPD-L2ポリペプチドに特異的に結合する抗体である。さらなる具体例において、作用剤は、PD-L2に特異的に結合する抗体と、PD-L1ポリペプチドに特異的に結合する抗体との組み合わせである。もう1つの具体例において、作用剤はPD-L2ポリペプチドに結合するペプチド、ペプチド模倣物、または他の小型分子である。さらにもう1つの具体例において、作用剤は、PD-L2とPD-1との間の相互作用をモジュレーションするもう1つのPD-1リガンドである。さらにもう1つの具体例において、作用剤は、PD-L2遺伝子の転写、PD-L2 mRNAの翻訳、あるいはPD-L2ポリペプチドの翻訳後修飾をモジュレーションすることによりPD-L2の発現をモジュレーションする。もう1つの具体例において、作用剤はPD-L2 mRNAまたはPD-L2遺伝子のコーディング鎖に対するアンチセンスであるヌクレオチド配列を有する核酸分子である。

【0030】

1の具体例において、PD-L2モジュレーターである作用剤を対象に投与することにより、異常な、不十分な、あるいは望ましくないPD-L2ポリペプチドまたは核酸の発現または活性により特徴づけられる疾病または症状を有する対象を治療するために本発明が用いられる。1の具体例において、PD-L2モジュレーターはPD-L2ポリペプチド

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である。もう1つの具体例において、PD-L2モジュレーターはPD-L2核酸分子である。さらなる具体例において、PD-L2モジュレーターはPD-L2ポリペプチドに特異的に結合する抗体である。もう1つの具体例において、PD-L2モジュレーターはPD-L2ポリペプチドに特異的に結合する抗体と、PD-L1ポリペプチドに特異的に結合する抗体との組み合わせである。さらにもう1つの具体例において、PD-L2モジュレーターはペプチド、ペプチド模倣物、または他の小型分子である。好ましい具体例において、異常な、不十分な、あるいは望ましくないPD-L2ポリペプチドまたは核酸の発現または活性により特徴づけられる疾病または症状は、PD-L2活性のモジュレーションにより恩恵を受けるであろう免疫応答疾患または症状である。もう1つの具体例において、本発明は、免疫応答をモジュレーションするさらなる作用剤を用いて対象を治療することをさらに提供する。 10

【0031】

さらにもう1つの具体例において、本発明は、抗原と、PD-L2活性を低下あるいは阻害する作用剤とを含むワクチンを提供する。好ましい具体例において、ワクチンはPD-L2とその本来の結合パートナーとの間の相互作用を阻害する。より好ましい具体例において、結合パートナーはPD-1である。

【0032】

また本発明は、(i) PD-L2ポリペプチドをコードする遺伝子の異常な修飾または変異；(ii) 該遺伝子の誤調節；および(iii) PD-L2ポリペプチドの異常な翻訳後修飾により特徴づけられる遺伝学的変化の存在または不存在を同定するための診断アッセイを提供し、ここに該遺伝子の野生型形態はPD-L2活性を有するポリペプチドをコードするものである。 20

【0033】

本発明のもう1つの態様は、PD-L2活性を有するPD-L2ポリペプチドを含むインジケータ組成物を用意し、インジケータ組成物を試験化合物と接触させ、ついで、インジケータ組成物中のPD-L2活性に対する試験化合物の影響を調べて、PD-L2ポリペプチドの活性をモジュレーションする化合物を同定することによる、PD-L2ポリペプチドに結合し、あるいはその活性をモジュレーションする化合物を同定する方法を提供する。

【0034】

もう1つの態様において、本発明は、PD-1とPD-L2との間の相互作用をモジュレーションすることによる、免疫応答をモジュレーションする方法を提供する。 30

【0035】

1の態様において、本発明は、PD-L2を発現する抗原提示細胞を、PD-L2の1形態およびPD-1の1形態からなる群より群より選択される作用剤、あるいはPD-L2とその本来の結合パートナーとの相互作用をモジュレーションする作用剤と接触させて、PD-L2と免疫細胞上のその本来の結合パートナーとの相互作用をモジュレーションすることを特徴とする、PD-L2と免疫細胞上のその本来の結合パートナーとの相互作用をモジュレーションする方法に関する。好ましい具体例において、PD-L2と免疫細胞上のその本来の結合パートナー（例えばPD-1）との相互作用をモジュレーションする作用剤は、PD-L2に特異的に結合する抗体である。もう1つの好ましい具体例において、作用剤はPD-L2ポリペプチドに特異的に結合する抗体と、PD-L1ポリペプチドに特異的に結合する抗体との組み合わせである。 40

【0036】

1の具体例において、PD-L2と免疫細胞上のその本来の結合パートナーとの相互作用がアップレギュレーションされる。もう1つの具体例において、PD-L2と免疫細胞上のその本来の結合パートナーとの相互作用がダウンレギュレーションされる。

【0037】

1の具体例において、該方法は、免疫細胞または抗原提示細胞を、免疫応答をモジュレーションするさらなる作用剤と接触させることを特徴とする。 50

1 の具体例において、接触工程がインビトロで行なわれる。もう 1 つの具体例において、接触工程がインビボで行なわれる。

1 の具体例において、免疫細胞は T 細胞、B 細胞およびミエロイド細胞からなる群より選択される。

1 の具体例において、PD-L2 結合パートナーは PD-1 である。

【0038】

もう 1 つの態様において、本発明は、細胞における PD-L2 の活性または発現を増大させて免疫細胞活性化を阻害することの特徴とする、非アポトーシス機構による免疫細胞における活性化の阻害方法に関する。

【0039】

さらにもう 1 つの態様において、本発明は、抗原および PD-L2 とその本来の結合パートナーとの間の相互作用を阻害する作用剤を含むワクチンに関する。

さらにもう 1 つの態様において、本発明は、抗原および PD-L2 とその本来の結合パートナーとの間の相互作用を促進する作用剤を含むワクチンに関する。

1 の具体例において PD-L2 結合パートナーは PD-1 である。

【0040】

もう 1 つの態様において、本発明は、免疫応答のアップレギュレーションにより恩恵を受けるであろう症状を有する対象の治療方法に関し、該方法は、PD-L2 と対象細胞上のその本来の結合パートナーとの間の相互作用を阻害する作用剤を投与して、免疫応答のアップレギュレーションにより恩恵を受けるであろう症状を治療することの特徴とする。

【0041】

1 の具体例において、作用剤は、PD-L2 に結合し、PD-L2 とその本来の結合パートナーとの間の相互作用を阻害するブロッキング抗体または小型分子を含む。もう 1 つの具体例において、作用剤は、PD-L2 ポリペプチドに特異的に結合する抗体と、PD-L1 ポリペプチドに特異的に結合する抗体との組み合わせである。

【0042】

もう 1 つの具体例において、該方法は、免疫応答をアップレギュレーションする第 2 の作用剤を対象に投与することをさらに特徴とする。

1 の具体例において、症状は腫瘍、病原体感染または免疫抑制性疾患からなる群より選択される。

もう 1 つの具体例において、PD-L2 結合パートナーは PD-1 である。

【0043】

1 の態様において、本発明は、免疫応答のダウンレギュレーションにより恩恵を受けるであろう症状を有する対象の治療方法に関し、該方法は、PD-L2 と対象細胞上のその本来の結合パートナーとの間の相互作用を刺激する作用剤を投与して、免疫応答のダウンレギュレーションにより恩恵を受けるであろう症状を治療することの特徴とする。

【0044】

1 の具体例において、作用剤は、PD-L2 に結合し、PD-L2 とその本来の結合パートナーとの間の相互作用を刺激するブロッキング抗体または小型分子を含む。

【0045】

もう 1 つの具体例において、該方法は、免疫応答をダウンレギュレーションする第 2 の作用剤を対象に投与することをさらに特徴とする。

1 の具体例において、症状は移植、アレルギー、および自己免疫疾患からなる群より選択される。

もう 1 つの具体例において、PD-L2 結合パートナーは PD-1 である。

【0046】

もう 1 つの態様において、本発明は、PD-L2 標的分子を発現する細胞を試験化合物と接触させ、ついで、PD-L2 標的分子の活性をモジュレーションする試験化合物の能力を調べることを特徴とする、PD-L2 の活性をモジュレーションする化合物のスクリー

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ニングのための細胞に基づくアッセイに関する。

【0047】

さらにもう1つの態様において、本発明は、PD-L2ポリペプチドまたはその生物学的に活性のある部分を試験化合物と接触させ、ついで、PD-L2ポリペプチドまたはその生物学的に活性のある部分への試験化合物の結合能を調べることを特徴とする、PD-L2の標的分子への結合をモジュレーションする化合物のスクリーニングのための無細胞アッセイに関する。

【0048】

もう1つの具体例において、本発明は、PD-L2標的分子を発現するT細胞を第1の抗原濃度において試験化合物と接触させ、第1の濃度においてT細胞増殖またはサイトカイン産生をモジュレーションする試験化合物の能力を調べ、PD-L2標的分子を発現するT細胞を第2の抗原濃度において試験化合物と接触させ、ついで、第2の濃度においてT細胞増殖またはサイトカイン産生をモジュレーションする試験化合物の能力を調べ、そのことにより第1および第2の抗原濃度においてT細胞活性化をモジュレーションする化合物を同定することを特徴とする、第1および第2の抗原濃度においてT細胞活性化をモジュレーションする化合物を同定する方法に関する。

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1の具体例において、PD-L2標的分子はPD-1である。

【0049】

本発明の他の特徴および利点は、以下の詳細な説明および特許請求の範囲から明らかであろう。

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【0050】

発明の詳細な説明

すでに特徴付けられているBリンパ球活性化抗原、例えば、B7-1およびBN7-2のほかに、抗原提示細胞（例えば、B細胞、単球、樹状細胞、ランゲルハンス細胞、ケラチノサイト、内皮細胞、星状細胞、線維芽細胞、およびオリゴデンドロサイト）の表面上に、B細胞、T細胞、および他の免疫細胞の活性化をモジュレーションする他の抗原が存在する。本発明は、少なくとも一部には、本明細書でPD-L2ポリペプチドと称される新規分子の発見にも基づくものであり、該分子はPD-1受容体に結合し、これらの免疫細胞の活性化をダウンレギュレーションし、そして／あるいは免疫応答をダウンレギュレーションする。これらの新規分子は免疫応答のモジュレーションにおいて役割を果たす。

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【0051】

PD-L2がPD-1に結合するという今回の知見は、PD-L2を阻害性リガンドのファミリー中に置くものであり、配列の分析はPD-L2をB7ファミリー中に置くものである。同時刺激受容体の使用は免疫細胞中に同時刺激シグナルを生じさせるが、阻害性受容体、例えば、CTLA-4またはPD-1の使用（架橋による、あるいは凝集による）は、免疫細胞において阻害性シグナルの伝達を誘導し、免疫細胞応答のダウンレギュレーションおよび／または細胞アネルギーを生じさせる。免疫細胞における阻害性シグナルの伝達は免疫細胞応答のダウンレギュレーションを導く（そして免疫応答全体をダウンレギュレーションする）が、免疫細胞における阻害性シグナルの妨害（例えば、PD-1に対する非活性化抗体（non-activating antibody）を用いることによる）は、免疫細胞応答のアップモジュレーションを誘導する（そして免疫応答のアップモジュレーションを引き起こす）。

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【0052】

PD-L2は、すでに記載されているPD-1に対するリガンドであるPD-L1に対して相同性を有する（同時係属米国出願第09/644,934号；国際公開WO 01/14557；Dong, H. et al. (1999) Nat. Med. 5:1365-1369；and Freeman, G. J. et al. (2000) J. Exp. Med. 192:1027-1034参照、参照により各内容を本明細書に一体化させる）。

【0053】

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本発明は、PD-L2の活性および／または発現をモジュレーションするのに有用な作用剤；PD-L2とその本来の結合パートナー（例えば、PD-1）との間の相互作用をモジュレーションするための作用剤、ならびにPD-L2とその本来の結合パートナー（例えば、PD-1）との間の相互作用のモジュレーションにより免疫応答をモジュレーションするための作用剤を利用するものである。これらの方法に使用される典型的な調節作用剤を以下においてさらに説明する。

【0054】

定義

本明細書の用語「免疫細胞」は、造血系起源の細胞であって、免疫応答において役割を果たす細胞を包含する。免疫細胞はB細胞およびT細胞のごときリンパ球；ナチュラルキラー細胞；ならびに単球、マクロファージ、好酸球、肥満細胞、好塩基球、および顆粒球のごときミエロイド細胞を包含する。

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【0055】

本明細書の用語「T細胞」はCD4⁺T細胞およびCD8⁺T細胞を包含する。また用語T細胞はTヘルパー1タイプのT細胞およびTヘルパー2タイプのT細胞の両方を包含する。用語「抗原提示細胞」は、プロフェッショナル抗原提示細胞（例えば、Bリンパ球、単球、樹状細胞およびランゲルハンス細胞）ならびに他の抗原提示細胞（例えば、ケラチノサイト、内皮細胞、星状細胞、線維芽細胞およびオリゴデンドロサイト）を包含する。

【0056】

本明細書の用語「免疫応答」は、T細胞同時刺激のモジュレーションにより影響を受ける、T細胞により媒介される免疫応答および／またはB細胞により媒介される免疫応答を包含する。典型的な免疫応答はB細胞応答（例えば、抗体産生）、T細胞応答（例えば、サイトカイン産生、および細胞性の細胞毒性）およびサイトカイン応答細胞、例えばマクロファージの活性化を包含する。本明細書の免疫応答に関する用語「ダウンレギュレーション」は、1またはそれ以上の免疫応答の減少を包含し、免疫応答に関する用語「アップレギュレーション」は、1またはそれ以上の免疫応答の増加を包含する。1のタイプの免疫応答のアップモジュレーションは別のタイプの免疫応答における対応ダウンモジュレーションを誘導しうることが理解されよう。例えば、ある種のサイトカイン（例えば、IL-10）の産生のアップモジュレーションは細胞性免疫応答のダウンモジュレーションを誘導しうる。

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【0057】

本明細書の用語「同時刺激受容体」は、同時刺激シグナルを免疫細胞に伝達する受容体、例えば、CD28またはICOSを包含する。本明細書の用語「阻害性受容体」は、免疫細胞に負のシグナルを伝達する受容体を包含する（例えば、CTLA-4またはPD-1）。

【0058】

本明細書の用語「同時刺激」は、活性化された免疫細胞に関しては、増殖またはエフェクター機能を誘導する、第2の、活性化しない（non-activating）、受容体により媒介されるシグナル（「同時刺激シグナル」）を提供する同時刺激分子の能力を包含する。例えば、同時刺激シグナルはサイトカイン分泌を引き起こし、例えば、T細胞受容体により媒介されるシグナルを受け取ったT細胞におけるサイトカインにおけるサイトカイン分泌を引き起こす。細胞受容体により媒介、例えば、活性化受容体を介して伝達されるシグナルを受け取った免疫細胞を、本明細書において、「活性化された免疫細胞」という。

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【0059】

同時刺激受容体（CD28またはICOSのごとき）が免疫細胞上に存在しない場合でさえも、阻害性受容体により伝達される阻害性シグナルが生じることがあり、それは同時刺激分子の結合に関する阻害性受容体と同時刺激受容体との間の競争の単なる関数ではない（Fallarino et al. (1998) J. Exp. Med. 188: 205）。免疫細胞への阻害性シグナルの伝達は免疫細胞における無応答、アネルギーまた

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はプログラムされた細胞死を生じさせる。好ましくは、阻害性シグナルの伝達は、アポトーシスに関連しない機構を通して機能するものである。用語「アポトーシス」は、当該分野で知られた方法を用いて特徴づけされうるプログラムされた細胞死を包含する。例えば、細胞収縮、膜の水泡、および細胞断片化の際の顕著なクロマチン濃縮により、アポトーシスによる細胞死を特徴づけることができる。アポトーシスを受けている細胞は特徴的なヌクレオソーム間DNA開裂のパターンも示す。

【0060】

受容体に結合するPD-L2分子の形態に応じて、例えば、受容体に結合するためのPD-L2分子の活性化形態との競争により、シグナルが伝達（例えば、受容体の架橋を生じさせる多価形態のPD-L2分子により、あるいは抗原提示細胞上のFc受容体に結合する可溶性のPD-L2により）あるいは阻害（例えば、当該分野で知られた方法を用いて抗原提示細胞上のFc受容体に結合しないように変化させられた可溶性の1価形態のPD-L2分子または可溶性形態のPD-L2により）されうる。しかしながら、可溶性分子が刺激性でありうる例が存在する。本明細書に記載のように常套的なスクリーニングアッセイを用いて種々のモジュレーション作用剤の効果を容易に示すことができる。

【0061】

本明細書の用語「活性化受容体 (activating receptor)」は、抗原、複合体化抗原（例えば、MHC分子の意味において）、または抗体に結合する免疫細胞受容体を包含する。かかる活性化受容体はT細胞受容体(TCRs)、B細胞受容体(BCRs)、サイトカイン受容体、LPS受容体、補体受容体、ならびにFc受容体を包含する。

【0062】

例えば、T細胞受容体はT細胞上に存在し、CD3分子と結合する。T細胞受容体はMHC分子の意味において抗原により（ならびにポリクローナルT細胞活性化試薬により）刺激される。TCRを介するT細胞活性化は多くの変化を生じさせ、例えば、蛋白リン酸化、膜脂質変化、イオンフラックス、環状ヌクレオチド変化、RNA転写変化、蛋白合成変化、ならびに細胞体積変化を生じさせる。

【0063】

本明細書の用語「B細胞受容体(BCR)」は、B細胞上に見られる膜Ig(mIg)と他の膜貫通ポリペプチド（例えば、IgαおよびIgβ）との間の複合体を包含する。mIgのシグナル伝達機能は、オリゴマーまたはマルチマー抗原による受容体分子の架橋により引き金を引かれる。B細胞は抗-免疫グロブリン抗体によっても活性化される。BCR活性化により、チロシンリン酸化をはじめとする多くの変化がB細胞において生じる。

【0064】

用語「Fc受容体」(FcRs)は免疫グロブリン分子(Igs)のFc部分に対する細胞表面受容体を包含する。Fc受容体は免疫応答に関与する多くの細胞上に見られる。これまで同定されたヒトFcRsにはIgGを認識するもの(FcγRと命名された)、IgEを認識するもの(FcεR1)、IgAを認識するもの(FcαR)、およびポリマー化IgM/Aを認識するもの(FcμαR)がある。FcRsは下記の細胞タイプにおいて見られる：FcεRI(肥満細胞)、FcεRII(多くの白血球)、FcαR(好中球)、およびFcμαR(顆粒内皮、肝細胞)(Hogg, N. (1988) Immunol. Today 9:185-86)。広く研究されたFcγRsは細胞性免疫防御の中枢であり、炎症メディエータおよび自己免疫疾患の発症に関与している加水分解酵素の放出を刺激する(Unkeless, J. C. (1988) Annu. Rev. Immunol. 6:251-87)。FcγRsは、エフェクター細胞とIgを分泌するリンパ球との間の重要なリンクを提供する。なぜならマクロファージ/単球、多型核白血球、およびナチュラルキラー(NK)細胞のFcγRsはIgGにより媒介される特異的認識のエレメントを付与するからである。ヒト白血球はIgGに対する少なくとも3つの異なる受容体を有する：それらはhFcγRI(単球/マクロファージ上に見られる)、hFcγRII(単球、好中球、好酸球、血小板、おそらくB細胞、およびK56

2細胞系)、およびhFcγRIII(NK細胞、好中球、好酸球、およびマクロファージ)である。

【0065】

T細胞に関して、T細胞への同時刺激シグナルの伝達には、シクロスポリンAにより阻害されないシグナリング経路が用いられる。さらに、同時刺激シグナルはT細胞におけるサイトカイン分泌を誘導することができ、そして／あるいは抗原に対する無応答の誘導、アレルギーの誘導、あるいはT細胞における細胞死の誘導を防止することができる。

【0066】

本明細書の用語「阻害性シグナル」は、免疫細胞上の阻害性受容体(例えば、CTLA4またはPD-1)分子を介して伝達されるシグナルをいう。かかるシグナルは、活性化受容体を介する(例えば、TCR、CD3、BCR、またはFc分子)シグナルに拮抗し、例えば、下記のもの阻害を引き起こす:第2のメッセンジャー発生、増殖、または免疫細胞におけるエフェクター機能、例えば減少したファゴサイトーシス、抗体産生、あるいは細胞性の細胞毒性、あるいはサイトカイン(例えばIL-2)および／またはアレルギー性応答のメディエ이터のごときメディエータを免疫細胞が産生しないこと、あるいはアレルギーの発生。

【0067】

本明細書の用語「無応答」は、刺激、例えば、活性化受容体またはサイトカインを介する刺激に対する免疫細胞の無反応を包含する。無応答は、例えば、免疫抑制剤または高用量の抗原への曝露により生じうる。本明細書の用語「アレルギー」または「寛容」は、活性化受容体により媒介される刺激への無反応を包含する。一般的には、かかる無反応は抗原特異的であり、寛容化剤への曝露が終わってからでも持続する。例えば、T細胞におけるアレルギー(無応答に対抗するものとしての)はサイトカイン、例えばIL-2産生欠乏により特徴づけられる。T細胞アレルギーは、T細胞が抗原に曝露され、第2のシグナル(同時刺激シグナル)の不存在下で最初のシグナル(T細胞受容体またはCD3により媒介されるシグナル)を受け取った場合に生じる。これらの条件下において、同じ抗原への細胞の再曝露(同時刺激分子の存在下で再曝露が行なわれたとしても)は、サイトカイン無産生を引き起こし、かくして、増殖が起こらなくなる。しかしながら、アレルギー性T細胞は無関係な抗原に対して応答をマウントでき、サイトカイン(例えばIL-2)とともに培養された場合に増殖できる。例えば、ELISAにより測定する場合にはTリンパ球によるIL-2産生欠如により、あるいはインジケータ細胞系を用いる増殖アッセイにより、T細胞アレルギーを観察できる。別法として、リポーター遺伝子構築物を用いることもできる。例えば、アレルギー性T細胞は、5'IL-2遺伝子エンハンサーの制御下の異種プロモーターによりあるいはエンハンサー中に見出されうるAP1配列のマルチマーにより誘導されるIL-2遺伝子転写を開始させることができない(Kang et al. (1992) Science 257:1134)。

【0068】

同時刺激シグナルのモジュレーションは免疫細胞のエフェクター機能のモジュレーションを引き起こす。かくして、用語「PD-L2活性」は、PD-L2ポリペプチドがその本来の結合パートナー、例えばPD-1に結合する能力、免疫細胞同時刺激または阻害性シグナルをモジュレーションする能力、ならびに免疫応答をモジュレーションする能力を包含する。

【0069】

PD-1に関して、用語「活性」は、例えば抗原提示細胞上の本来のリガンドを用いることにより、活性化された免疫細胞における阻害性シグナルをモジュレーションするPD-1の能力を包含する。PD-1は、CTLA4と類似の様式で、阻害性シグナルを免疫細胞に伝達する。免疫細胞における阻害性シグナルのモジュレーションは、免疫細胞の増殖および／または免疫細胞によるサイトカイン分泌のモジュレーションを引き起こす。PD-1は、その本来のリガンドを求めて同時刺激受容体と競争することにより、同時刺激シグナルをモジュレーションすることもできる。かくして、用語「PD-1活性」は、PD

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ー 1 ポリペプチドがその本来のリガンドに結合する能力、免疫細胞同時刺激または阻害性シグナルをモジュレーションする能力、ならびに免疫応答をモジュレーションする能力を包含する。

【0070】

本明細書の用語「天然に存在する」核酸分子は、天然に存在するヌクレオチド配列を有するRNAまたはDNA分子（例えば、天然蛋白をコードするもの）をいう。

【0071】

本明細書の用語「アンチセンス」は、蛋白をコードする「センス」核酸に対して相補的なヌクレオチド配列、例えば、二本鎖cDNA分子のコーディング鎖に対して相補的な配列、mRNA配列に対して相補的な配列、あるいは遺伝子のコーディング鎖に対して相補的な配列を含む。したがって、アンチセンス核酸分子はセンス核酸分子に水素結合しうる。

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【0072】

本明細書の用語「コーディング領域」は、アミノ酸残基中に翻訳されるコドンを含むヌクレオチド配列の領域をいい、用語「非コーディング領域」はアミノ酸に翻訳されないヌクレオチド配列の領域（例えば、5'および3'非翻訳領域）をいう。

【0073】

本明細書の用語「ベクター」は、別の核酸分子が結合しており、それを輸送可能な核酸分子をいう。1のタイプのベクターは「プラスミド」であり、それは環状二本鎖DNAループをいい、その中にさらなるDNAセグメントが連結されうる。もう1つのタイプのベクターはウイルスベクターであり、その中のウイルスゲノム中にさらなるDNAセグメントが連結されうる。ある種のベクターは、それらが導入された宿主細胞中で自律的複製が可能である（例えば、細菌の複製開始点を有する細菌ベクターおよびエピソード性哺乳動物ベクター）。他のベクター（例えば、非エピソード性哺乳動物ベクター）は、宿主細胞中に導入されると、宿主細胞ゲノム中に組み込まれ、そのことにより、宿主ゲノムとともに複製される。そのうえ、ある種のベクターは、作動可能に連結された遺伝子の発現を指令しうる。かかるベクターを本明細書において「組換え発現ベクター」または単に「発現ベクター」という。一般的には、組換えDNA法において有用な発現ベクターはプラスミド形態であることが多い。プラスミドはベクターの形態として最も普通に用いられるので、本明細書において「プラスミド」および「ベクター」を混用する。しかしながら、本発明は、同等の機能を果たすウイルスベクター（例えば、複製欠陥レトロウイルス、アデノウイルスおよびアデノ随伴ウイルス）のような他の形態の発現ベクターも包含する。

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【0074】

本明細書の用語「宿主細胞」は、本発明の組換え発現ベクターのごとき本発明の核酸分子が導入された細胞をいう。用語「宿主細胞」および「組換え宿主細胞」を本明細書において混用する。かかる用語は特定の対象細胞のみならずかかる細胞の子孫または潜在的子孫をもいうことが理解されるべきである。突然変異または環境の影響によりある種の修飾が継代の間に生じうるので、実際には、かかる子孫は親細胞と同一でないかもしれないが、やはり本明細書に用いられる用語の範囲内である。

【0075】

本明細書の用語「トランスジェニック動物」は、非ヒト動物、好ましくは哺乳動物、より好ましくはマウスであり、その動物の1またはそれ以上の細胞が「導入遺伝子」を含んでいるものである。用語「導入遺伝子」は、トランスジェニック動物が発生する細胞のゲノム中に組み込まれており、成熟動物のゲノム中に残り、例えば、トランスジェニック動物の1またはそれ以上の細胞タイプまたは組織においてコードする遺伝子産物の発現を指令する外来DNAをいう。

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【0076】

本明細書の用語「相同組換え動物」は、トランスジェニック非ヒト動物、好ましくは哺乳動物、より好ましくはマウスをいい、動物中において、動物の発生に先立って動物の細胞、例えば動物の胚性細胞中に導入された外来DNA分子と内在遺伝子との間の相同組換えによって内在遺伝子に変化しているものをいう。

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【0077】

本明細書の用語「単離蛋白」は、細胞から単離された場合、あるいは組換えDNA法により製造された場合に他の蛋白、細胞性物質および培地を実質的に含まず、あるいは化学合成された場合に化学前駆体または他の化学物質を実質的に含まない蛋白をいう。

【0078】

「単離」または「精製」された蛋白またはその生物学的に活性のある部分は、PD-L2蛋白が得られた細胞または組織ソースに由来する細胞性物質または他の混入蛋白を実質的に含まず、あるいは化学合成された場合には化学前駆体または他の化学物質を実質的に含まない。用語「細胞性物質を実質的に含まない」は、PD-L2蛋白が単離または組換え法により得られた細胞の細胞性成分からPD-L2蛋白が分離されているPD-L2調合物を包含する。1の具体例において、用語「細胞性物質を実質的に含まない」は、約30%（乾燥重量）未満の非PD-L2蛋白（本明細書において「混入蛋白」ともいう）を有する、より好ましくは約20%未満の非PD-L2蛋白を有する、さらにより好ましくは約10%未満の非PD-L2蛋白を有する、最も好ましくは約5%未満の非PD-L2蛋白を有するPD-L2の調合物を包含する。PD-L2蛋白またはその生物学的に活性のある部分を組換え法により製造する場合、それが培地を実質的に含まないことが好ましく、すなわち、培地が蛋白調合物の体積の約20%未満、より好ましくは約10%未満、最も好ましくは約5%未満を占める。

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【0079】

用語「化学前駆体または他の化学物質を実質的に含まない」は、蛋白合成に使用される化学前駆体または他の化学物質から蛋白が分離されているPD-L2調合物を包含する。1の具体例において、用語「化学前駆体または他の化学物質を実質的に含まない」は、約30%（乾燥重量）未満の化学前駆体または非PD-L2化学物質を有し、より好ましくは約20%未満の化学前駆体または非PD-L2化学物質を有し、さらにより好ましくは約10%未満の化学前駆体または非PD-L2化学物質を有し、最も好ましくは約5%未満の化学前駆体または非PD-L2化学物質を有するPD-L2蛋白の調合物を包含する。

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【0080】

本明細書の用語「抗体」は、抗体の「抗原結合部分」（あるいは単に「抗体部分」）ならびに抗体分子全体を包含する。本明細書の用語「抗原結合部分」は、抗原（例えば、PD-L2）に特異的に結合する能力を保持している抗体の1またはそれ以上のフラグメントをいう。完全長抗体のフラグメントによって抗体の抗原結合機能が果たされることが示されている。抗体の「抗原結合部分」なる用語に包含される結合フラグメントの例は、（i）VL、VHCLおよびCH1ドメインからなる1価フラグメントであるFabフラグメント；（ii）ヒンジ領域においてジスルフィド結合により連結された2つのFabフラグメントを含む2価フラグメントであるF(ab')₂フラグメント；（iii）VHおよびCH1ドメインからなるFdフラグメント；（iv）抗体の単一アームのVLおよびVHドメインからなるFvフラグメント；（v）VHドメインからなるdAbフラグメント（Ward et al. (1989) Nature 341:544-546）；および（vi）単離された相補性決定領域（CDR）を包含する。さらにそのうえ、Fvフラグメントの2つのドメイン、VLおよびVHは別個の遺伝子によりコードされるが、組換え法を用いて、それらを単一蛋白鎖にすることのできる合成リンカーによりVLとVH領域のペアーを1価分子にすることにより、それらを結合することができる（1本鎖Fv（scFv）として知られる。例えば、Bird et al. (1988) Science 242:423-426；and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883；and Osbourn et al. 1998 Nat. Biotechnol. 16:778参照）。かかる1本鎖抗体も、抗体の「抗原結合部分」なる用語に包含される。特定のscFvのVHおよびVL配列をヒト免疫グロブリン不変領域cDNAまたはゲノム配列に連結して、完全なIgG分子または他のイソタイプをコードする発現ベクターを得ることができる。蛋白化学または組換えDNA法のいずれかを用いて、VHおよびVLをFa

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b、Fv、または免疫グロブリンの他のフラグメントの生成に使用することもできる。ダイアボディー (diabody) のとき他の形態の1本鎖抗体も包含される。ダイアボディーは2価の二特異性抗体であり、その中で、VHおよびVLドメインが1本のポリペプチド鎖上に発現されるが、同じ鎖上の2つのドメイン間の対合を可能にするには短かすぎるリンカーを用いて、それらのドメインを別の鎖の相補的ドメインと対合させて2つの抗原結合部位が創生されている (例えば、Holliger, P. et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J. et al. (1994) Structure 2:1121-1123 参照)。

【0081】

さらに、抗体またはその抗原結合部分は、1またはそれ以上の他の蛋白またはペプチドとの抗体または抗体部分の共有結合または非共有結合により形成されるより大きな免疫接着分子の一部であってもよい。かかる免疫接着分子の例は、テトラマー scFv を生成するためのストレプトアビジンコア領域の使用 (Kipriyanov, S. M. et al. (1995) Hum. Antibodies Hybridomas 6:93-101) ならびに2価のビオチン化 scFv 分子を生成するためのシステイン残基、マーカペプチドおよびC末端ポリヒスチジンタグの使用 (Kipriyanov, S. M. et al. (1994) Mol. Immunol. 31:1047-1058) を包含する。抗体全体のパインまたはペプシン消化のとき慣用的方法を用いて Fab および F(ab')₂ フラグメントのとき抗体部分を抗体全体から調製することができる。そのうえ、本明細書記載の標準的な組換えDNA法を用いて抗体、抗体部分および免疫接着分子を得ることができる。

【0082】

抗体はポリクローナルまたはモノクローナル; 異種、同種、または同系; あるいはそれらの修飾形態、例えば、ヒト化、キメラ等であってもよい。好ましくは、本発明の抗体はPD-L2分子に特異的あるいは実質的に特異的に結合する。本明細書の用語「モノクローナル抗体」および「モノクローナル抗体組成物」は、抗原の特定のエピトープと免疫反応しうるだた1つの種の抗原結合部位を含む抗体分子の集団をいい、用語「ポリクローナル抗体」または「ポリクローナル抗体組成物」は特定抗原と相互作用しうる複数種の抗原結合部位を含む抗体分子の集団をいう。モノクローナル抗体組成物は、典型的には、それが免疫反応する特異抗原に対する単一の結合アフィニティーを示す。

【0083】

本明細書の用語「ヒト化抗体」は、ヒト細胞により作られるであろう抗体に近似するように変化させられた可変領域および不変領域を有する非ヒト細胞により作られる抗体を包含する。例えば、非ヒト抗体アミノ酸配列を変化させてヒト生殖系免疫グロブリン配列に見られるアミノ酸を含むようにしてもよい。本発明のヒト化抗体は、ヒト生殖系免疫グロブリン配列によりコードされないアミノ酸残基 (例えば、インビトロでのランダムあるいは部位特異的突然変異による、あるいはインビボでの体細胞突然変異による) を、例えばCDRs中に含んでいてもよい。本明細書の用語「ヒト化抗体」は、マウスのごとき別の哺乳動物種の生殖系に由来するCDR配列がヒトフレームワーク配列上に移植された抗体も包含する。

【0084】

本明細書の用語「単離抗体」は、異なる抗原特異性を有する他の抗体を実質的に含まない抗体をいう (例えば、特異的にPD-L2に結合する単離抗体は、PD-L2以外の抗原に特異的に結合する抗体を実質的に含まない)。そのうえ、単離抗体は他の細胞性物質および/または化学物質を実質的に含まないものであってもよい。

【0085】

PD-L2 核酸およびポリペプチド分子

用語「ファミリー」は、本発明のポリペプチドおよび核酸分子についていう場合、共通の構造ドメインまたはモチーフを有し、本明細書で定義するように十分なアミノ酸またはヌ

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クレオチド配列相同性を有する2種またはそれ以上のポリペプチドまたは核酸分子を意味する。かかるファミリーのメンバーは天然または非天然のものであってよく、同じまたは異なる種に由来するものであってもよい。例えば、ファミリーはヒト起源の第1のポリペプチドならびにヒト起源の他の異なるポリペプチドを含んでいてもよく、あるいは非ヒト起源のホモログ、例えば、サルポリペプチドを含んでいてもよい。ファミリーのメンバーは共通の機能的特徴を有していてもよい。

【0086】

例えば、本発明のPD-L2ポリペプチドは、好ましくは、少なくとも1つの「シグナルペプチドドメイン」を含む。本明細書の用語「シグナル配列」または「シグナルペプチド」は、分泌および膜結合ポリペプチドのN末端に存在し、多くの疎水性アミノ酸残基を含んでいる約15個またはそれ以上のアミノ酸を含むペプチドを包含する。例えば、シグナル配列は少なくとも約10～30個のアミノ酸残基、好ましくは約15～25個のアミノ酸残基、より好ましくは約18～20個のアミノ酸残基、さらにより好ましくは約19個のアミノ酸残基を含み、少なくとも約35～65%、好ましくは約38～50%、より好ましくは約40～45%の疎水性アミノ酸残基（例えば、バリン、ロイシン、イソロイシンまたはフェニルアラニン）を有する。かかる「シグナル配列」は、当該分野において「シグナルペプチド」とも呼ばれ、かかる配列を含むポリペプチドを脂質二重層に指向させるのに役立ち、分泌および膜結合ポリペプチド中において開裂される。シグナル配列は配列番号：2（図3）のアミノ酸ほぼ1～19であり、ネイティブなヒトPD-L2のアミノ酸配列中に確認された。シグナル配列は配列番号：5（図3）のアミノ酸ほぼ1～19であり、ネイティブなマウスPD-L2のアミノ酸配列中にも確認された。

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【0087】

本発明のもう1つの具体例において、本発明のPD-L2ポリペプチドが「膜貫通ドメイン」の存在に基づいて同定される。本明細書の用語「膜貫通ドメイン」は、細胞質膜を貫通する長さ約15アミノ酸残基のアミノ酸配列を包含する。より詳細には、膜貫通ドメインは少なくとも約20、25、30、35、40または45個のアミノ酸を含み、細胞質膜を貫通する。膜貫通ドメインは疎水性残基に富み、典型的には、 α -ヘリックス構造を有する。好ましい具体例において、膜貫通ドメインの少なくとも50%、60%、70%、80%、90%、95%またはそれ以上のアミノ酸が疎水性、例えば、ロイシン、イソロイシン、チロシンまたはトリプトファンである。膜貫通ドメインは、例えば、Zagotta, W. N. et al. (1996) Annu. Rev. Neurosci. 19: 235-263に記載されており、参照によりその内容を本明細書に一体化させる。ネイティブなヒトPD-L2ポリペプチドのアミノ酸残基220～243、ならびに推定成熟ポリペプチドのアミノ酸残基201～243は膜貫通ドメインを構成すると推定される（図3参照）。ネイティブなマウスPD-L2ポリペプチドのアミノ酸残基220～242、ならびに推定成熟ポリペプチドのアミノ酸残基201～223は膜貫通ドメインを構成すると推定される（図3参照）。したがって、ヒトPD-L2の膜貫通ドメインに対して少なくとも71～80%、より好ましくは約80～90%の相同性を有するPD-L2ポリペプチドは本発明の範囲内である。

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【0088】

もう1つの具体例において、ポリペプチドまたは対応核酸分子中の「IgCドメイン」または「IgVドメイン」の存在に基づいて本発明のPD-L2分子が同定される。本明細書の用語、IgCドメインおよびIgVドメインはIgスーパーファミリーのメンバーのドメインとして当該分野において認識されている。これらのドメインは、Igフォールドと呼ばれる特徴的なフォールディングパターンを有する構造ユニットに対応する。Igフォールドは2つの β シートのサンドイッチから構成されており、各々は5～10個のアミノ酸の逆平行 β 鎖からなり、すべてではないが大部分のドメインにおいて2つの β シート間に保存されたジスルフィド結合がある。Ig、TCRおよびMHC分子のIgCドメインは同じタイプの配列パターンを共有し、Igスーパーファミリー中のC1セットと呼ばれている。他のIgCドメインは他のセットに属する。IgVドメインも配列パターンを

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共有しており、Vセットドメインと呼ばれている。ネイティブなヒトPD-L2ポリペプチドのアミノ酸残基20～120、ならびに推定成熟ポリペプチドのアミノ酸残基1～101はIgVドメインを含むと推定される（図3参照）。ネイティブなマウスPD-L2ポリペプチドのアミノ酸残基20～120、ならびに推定成熟ポリペプチドのアミノ酸残基1～101もIgVドメインを含むと推定される（図3参照）。ネイティブなヒトPD-L2ポリペプチドのアミノ酸残基121～219、ならびに推定成熟ポリペプチドのアミノ酸残基102～200はIgCドメインを含むと推定される（図3参照）。ネイティブなマウスPD-L2ポリペプチドのアミノ酸残基121～219、ならびに推定成熟ポリペプチドのアミノ酸残基102～200もIgCドメインを含むと推定される（図3参照）。好ましい具体例において、IgVドメインの存在はPD-L2のその本来の結合パートナー、例えばPD-1への結合に必要とされる。

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【0089】

もう1つの具体例において、ポリペプチドまたは対応核酸分子中の「細胞外ドメイン」の存在に基づいて本発明のPD-L2分子が同定される。本明細書の用語「細胞外ドメイン」は、細胞表面から尾部として伸長しているN末端アミノ酸を示す。本発明の細胞外ドメインはIgVドメインおよびIgCドメインを含み、シグナルペプチドドメインを含んでもよい。ネイティブなヒトPD-L2ポリペプチドのアミノ酸残基1～219、ならびに推定成熟ポリペプチドのアミノ酸残基1～200は細胞外ドメインを含むと推定される（図3参照）。ネイティブなマウスPD-L2ポリペプチドのアミノ酸残基1～219、ならびに推定成熟ポリペプチドのアミノ酸残基1～200も細胞外ドメインを含むと推定される（図3参照）。

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【0090】

さらにもう1つの具体例において、ポリペプチドまたは対応核酸分子中の「細胞質ドメイン」の存在に基づいて本発明のPD-L2分子が同定される。本明細書の用語「細胞質ドメイン」は、細胞の細胞質中の尾部として伸長しているC末端アミノ酸を示す。ネイティブなヒトPD-L2ポリペプチドのアミノ酸残基244～273、ならびに推定成熟ポリペプチドのアミノ酸残基225～273は細胞質ドメインを含むと推定される（図3参照）。ネイティブなマウスPD-L2ポリペプチドのアミノ酸残基243～247、ならびに推定成熟ポリペプチドのアミノ酸残基224～228も細胞質ドメインを含むと推定される（図3参照）。

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好ましい具体例において、本発明のPD-L2分子は少なくとも1個またはそれ以上の下記ドメインを含む：シグナルペプチドドメイン、IgVドメイン、IgCドメイン、細胞外ドメイン、膜貫通ドメイン、および細胞質ドメイン。

【0091】

本発明の単離ポリペプチド、好ましくはPD-L2ポリペプチドは配列番号：2のアミノ酸配列に対して十分に同一であるアミノ酸配列を有し、あるいは配列番号：1または3に対して十分に同一であるヌクレオチド配列によりコードされる。本明細書の用語「十分に同一」は、第2のアミノ酸またはヌクレオチド配列に対して十分または最小の数の同一または同等なアミノ酸残基（例えば、類似の側鎖を有するアミノ酸残基）またはヌクレオチドを含む第1のアミノ酸またはヌクレオチド配列をいい、第1および第2のアミノ酸またはヌクレオチド配列は共通の構造ドメインまたはモチーフおよび／または共通の機能活性を有する。例えば、ドメインのアミノ酸配列間において少なくとも30%、40%または50%の同一性、好ましくは60%の同一性、より好ましくは70～80%の同一性、さらに好ましくは90～95%の同一性を有し、少なくとも1つ、好ましくは少なくとも2つの構造ドメインまたはモチーフを含む共通の構造ドメインを共有するアミノ酸またはヌクレオチド配列は十分に同一であると定義される。さらにそのうえ、少なくとも30%、40%または50%の同一性、好ましくは60%の同一性、より好ましくは70～80%の同一性、さらに好ましくは90～95%の同一性を有し、共通の機能的活性を共有するアミノ酸またはヌクレオチド配列は十分に同一であると定義される。

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【0092】

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本明細書の用語「PD-L2 活性」、「PD-L2 の生物学的活性」または「PD-L2 の機能的活性」は、PD-L2 応答性細胞または組織に対し、あるいはPD-L2 ポリペプチド結合パートナーに対し、PD-L2 蛋白、ポリペプチドまたは核酸分子により発揮される活性であって、標準的方法によりインビボまたはインビトロにおいて決定される活性をいう。1 の具体例において、PD-L2 活性は、PD-L2 結合パートナーとの結合のごとき直接的活性である。本明細書の用語「標的分子」または「結合パートナー」は、本来的にPD-L2 ポリペプチドが結合または相互作用してPD-L2 により媒介される機能が発揮される分子である。典型的な具体例において、PD-L2 標的分子は受容体PD-1 である。あるいはまた、PD-L2 活性は、PD-L2 ポリペプチドとその本来の結合パートナー、例えばPD-1 との相互作用により媒介される細胞シグナリング活性のごとき間接的活性である。PD-L2 の生物学的活性は本明細書に記載されている。例えば、本発明のPD-L2 ポリペプチドは1 またはそれ以上の下記活性を有しうる：1) 受容体PD-1 または他のPD-L2 の本来の結合パートナーに結合し、そして／あるいはその活性をモジュレーションする、2) 細胞内または細胞間シグナリングをモジュレーションする、3) 免疫細胞、例えば、Tリンパ球の活性化をモジュレーションする、4) 生物、例えばマウスまたはヒト生物の免疫応答をモジュレーションする。

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【0093】

したがって、本発明のもう1つの具体例は、PD-L2 活性を有する単離PD-L2 蛋白およびポリペプチドに関する。他の好ましいポリペプチドは、1 またはそれ以上の下記ドメインを有するPD-L2 ポリペプチドであり、好ましくはPD-L2 活性を有するもの

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【0094】

さらなる好ましいPD-L2 ポリペプチドは少なくとも1つの細胞外ドメイン、および1つまたはそれ以上のシグナルペプチドドメイン、IgVドメイン、IgCドメイン、膜貫通ドメイン、および細胞質ドメインを有し、好ましくは、ストリンジェントな条件下において配列番号：1、3、4または6のヌクレオチド配列の相補配列を含む核酸分子とハイブリダイゼーションするヌクレオチド配列を有する核酸分子によりコードされる。

【0095】

単離ヒトPD-L2 cDNAのヌクレオチド配列およびヒトPD-L2 ポリペプチドの推定アミノ酸配列はそれぞれ図1ならびに配列番号：1および2に示されている。単離マウスPD-L2 cDNAのヌクレオチド配列およびマウスPD-L2 ポリペプチドの推定アミノ酸配列はそれぞれ図2ならびに配列番号：4および5に示されている。

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【0096】

ヒトPD-L2 遺伝子は約1223ヌクレオチドの長さであり、約30.0kDの分子量を有し、約273アミノ酸の長さのポリペプチドをコードする。マウスPD-L2 遺伝子は約1655ヌクレオチドの長さであり、約27.2kDの分子量を有し、約247アミノ酸の長さのポリペプチドをコードする。

【0097】

本発明の種々の態様を以下のサブセクションにおいてさらに詳細に説明する：

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【0098】

I. 単離核酸分子

本発明の1の態様は、PD-L2 ポリペプチドまたはその生物学的に活性のある部分をコードする単離核酸分子、ならびにPD-L2 をコードする核酸分子（例えば、PD-L2 mRNA）を同定するためのハイブリダイゼーションプローブとして使用するに十分な核酸フラグメントおよびPD-L2 核酸分子の増幅または突然変異のためのPCRプライマーとして使用されるフラグメントに関する。本明細書の用語「核酸分子」は、DNA分子（例えば、cDNAまたはゲノムDNA）およびRNA分子（例えば、mRNA）ならびにヌクレオチドアナログを用いて得られるDNAまたはRNAのアナログをいう。核酸分子は1本鎖または2本鎖でありうるが、好ましくは2本鎖DNAである。

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【0099】

用語「単離核酸分子」は、核酸の天然ソース中に存在する他の核酸から分離されている核酸分子を包含する。例えば、ゲノムDNAに関して、用語「単離」は、ゲノムDNAは本来的に結合している染色体から分離されている核酸分子を包含する。好ましくは、「単離」核酸分子は、当該核酸が由来した生物のゲノムDNA中の本来的に隣接している配列（すなわち、当該核酸分子の5'および3'末端に存在する配列）を含まない。例えば、種々の具体例において、単離PD-L2核酸分子は、その核酸分子が由来した細胞のゲノムDNA中のその核酸分子に隣接した約5kb、4kb、3kb、2kb、1kb、0.5kbまたは0.1kb未満のヌクレオチド配列を含むことができる。そのうえ、組換え法により製造される場合にはDNA分子のごとき「単離」核酸分子は細胞性物質または培地を実質的に含まないものであってもよく、化学合成される場合には化学前駆体または他の化学物質を実質的に含まないものであってもよい。

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【0100】

標準的な分子生物学の方法および本明細書に示される配列の情報を用いて、本発明の核酸分子、例えば、配列番号：1、3、4または6のヌクレオチド配列またはその一部を有する核酸分子を単離することができる。配列番号：1、3、4または6の核酸配列の全部または一部をハイブリダイゼーションプローブとして使用し、標準的なハイブリダイゼーションおよびクロニング方法を用いて、PD-L2核酸分子を単離することができる（例えば、Sambrook, J. et al. Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989に記載されたようにして）。

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そのうえ、配列番号：1、3、4または6の配列に基づいて設計された合成オリゴヌクレオチドプライマーを用いるポリメラーゼ連鎖反応（PCR）により、配列番号：1、3、4または6の全部または一部を含む核酸分子を単離することができる。

【0101】

cDNA、mRNAを鋳型として用いて、別法としてゲノムDNAを鋳型として用い、適当なオリゴヌクレオチドプライマーを用いて、標準的なPCR増幅法により本発明の核酸分子を増幅することができる。そのようにして増幅された核酸分子を適当なベクター中にクローン化して、DNA配列分析により特徴づけることができる。さらにそのうえ、標準的な合成方法により、例えば、自動DNA合成装置を用いてPD-L2ヌクレオチド配列に対応するオリゴヌクレオチドを調製することができる。

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【0102】

好ましい具体例において、本発明の単離核酸分子は配列番号：1、3、4または6に示すヌクレオチド配列を含む。このcDNAは配列番号：1のヒトPD-L2ポリペプチドをコードする配列（すなわち、ヌクレオチド274～1092のコーディング領域）ならびに5'非翻訳配列（ヌクレオチド1～273）および3'非翻訳配列（ヌクレオチド1093～1223）を含んでいてもよい。あるいは、核酸分子は、配列番号：1のコーディング領域のみ（すなわち、ヌクレオチド274～1092、配列番号：3に対応）を含んでいてもよい。したがって、もう1つの具体例において、本発明の単離核酸分子は配列番号：3および配列番号：1のヌクレオチド1～274を含む。さらにもう1つの具体例において、単離核酸分子は配列番号：3および配列番号：1のヌクレオチド1093～1223を含む。さらにもう1つの具体例において、核酸分子は配列番号：1または配列番号：3に示すヌクレオチド配列からなる。さらにもう1つの具体例において、核酸分子は配列番号：1のコーディング領域（例えば、ヌクレオチド274～1092、配列番号：3に対応）ならびに停止コドン（例えば、配列番号：1のヌクレオチド1093～1095）を含んでいてもよい。

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【0103】

このcDNAは、配列番号：4のマウスPD-L2をコードする配列（すなわち、ヌクレ

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オチド 2 1 0 ~ 9 5 0 のコーディング領域) ならびに 5' 非翻訳配列 (ヌクレオチド) および 3' 非翻訳配列 (ヌクレオチド 1 ~ 2 0 9) を含んでいてもよい。あるいは、核酸分子は、配列番号: 4 のコーディング領域のみ (すなわち、ヌクレオチド 2 1 0 ~ 9 5 0、配列番号: 6 に対応) を含んでいてもよい。したがって、もう 1 つの具体例において、本発明の単離核酸分子は配列番号: 6 および配列番号: 4 のヌクレオチド 1 ~ 2 1 0 を含む。さらにもう 1 つの具体例において、単離核酸分子は配列番号: 6 および配列番号: 4 のヌクレオチド 9 5 1 ~ 1 6 5 5 を含む。さらにもう 1 つの具体例において、核酸分子は配列番号: 4 または配列番号: 6 に示すヌクレオチド配列からなる。さらにもう 1 つの具体例において、核酸分子は配列番号: 4 のコーディング領域 (例えば、ヌクレオチド 2 1 0 ~ 9 5 0、配列番号: 4 に対応) ならびに停止コドン (例えば、配列番号: 4 のヌクレオチド 9 5 1 ~ 9 5 3) を含んでいてもよい。

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【0 1 0 4】

もう 1 つの好ましい具体例において、本発明の単離核酸分子は、配列番号: 1、3、4 または 6 に示すヌクレオチド配列またはそれらのヌクレオチド配列の一部の相補配列である核酸分子を含む。配列番号: 1、3、4 または 6 に示すヌクレオチド配列に対して相補的な核酸分子は、配列番号: 1、3、4 または 6 に示すヌクレオチド配列に対して十分に相補的である核酸分子であり、その結果、それはそれぞれ配列番号: 1、3、4 または 6 に示すヌクレオチド配列にハイブリダイゼーションでき、そのことにより安定な 2 本鎖を形成する。

【0 1 0 5】

さらにもう 1 つの具体例において、本発明の単離核酸分子は、配列番号: 1、3、4 または 6 に示すヌクレオチド配列の全長またはこれらのヌクレオチド配列の一部に対して少なくとも約 7 0 %、7 5 %、8 0 %、8 5 %、9 0 %、9 1 %、9 2 %、9 3 %、9 4 %、9 5 %、9 6 %、9 7 %、9 8 %、9 9 % またはそれ以上同一であるヌクレオチド配列を含む。

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そのうえ、本発明の核酸分子は配列番号: 1、3、4 または 6 の核酸配列の一部のみ、例えば、プローブまたはプライマーとして使用できるフラグメントあるいは P D - L 2 ポリペプチドの一部、例えば P D - L 2 ポリペプチドの生物学的に活性のある部分をコードするフラグメントのみを含んでいてもよい。ヒト P D - L 2 遺伝子のクローニングにより決定されたヌクレオチド配列は、他の P D - L 2 ファミリーのメンバーならびに他の種由来の P D - L 2 ホモログの同定および/またはクローニングに使用するように設計されたプローブおよびプライマーの取得を可能にする。典型的には、プローブ/プライマーは実質的に精製されたオリゴヌクレオチドを含む。典型的には、オリゴヌクレオチドは、配列番号: 1、3、4 または 6 のセンス配列の、あるいは配列番号: 1、3、4 または 6 のアンチセンス配列の、あるいは配列番号: 1、3、4 または 6 の天然に存在する対立遺伝子変種の少なくとも約 1 2 または 1 5 個の、好ましくは約 2 0 または 2 5 個の、より個の約 3 0、3 5、4 0、4 5、5 0、5 5、6 0、6 5 または 7 5 個の連続したヌクレオチドに、ストリンジェントな条件下でハイブリダイゼーションするヌクレオチド配列の領域を含む。

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【0 1 0 6】

1 の具体例において、本発明の核酸分子は、約 5 0 - 1 0 0、1 0 0 - 1 5 0、1 5 0 - 2 0 0、2 0 0 - 2 5 0、2 5 0 - 3 0 0、3 0 0 - 3 5 0、3 5 0 - 4 0 0、4 0 0 - 4 5 0、4 5 0 - 5 0 0、5 0 0 - 5 5 0、5 5 0 - 6 0 0、6 0 0 - 6 5 0、6 5 0 - 7 0 0、7 0 0 - 7 5 0、7 5 0 - 8 0 0、8 0 0 - 8 5 0、8 5 0 - 9 0 0、9 0 0 - 9 5 0、9 5 0 - 1 0 0 0、1 0 0 0 - 1 0 5 0、1 0 5 0 - 1 1 0 0、1 1 0 0 - 1 1 5 0 ヌクレオチドまたはそれ以上のものよりも大きく、ストリンジェントな条件下で配列番号: 1、3、4 または 6 の核酸分子にハイブリダイゼーションするヌクレオチド配列またはその相補配列を含む。さらなる具体例において、本発明の核酸分子は約 8 8 0 - 9 0 0、9 0 0 - 9 5 0、9 5 0 - 1 0 0 0、1 0 0 0 - 1 0 5 0、1 0 5 0 - 1 1 0 0、1 1 0 0 - 1 1 5 0 ヌクレオチドまたはそれ以上のものよりも大きく、ストリンジェントな

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条件下で配列番号：1または3の核酸分子にハイブリダイゼーションするヌクレオチド配列またはその相補配列を含む。さらにもう1つの具体例において、本発明の核酸分子は約50-100、100-150、150-200、200-250、250-300ヌクレオチドまたはそれ以上のものよりも大きく、ストリンジェントな条件下で配列番号：1のヌクレオチド1～358を含む核酸分子にハイブリダイゼーションするヌクレオチド配列またはその相補配列を含む。さらなる具体例において、本発明の核酸分子は約50-100、100-150、150-200、200-250、250-300、300-350、350-400、400-450、450-500、500-550、550-600、600-650、650-700、700-750、750-800、850-900、900-950、950-1000-1050-1100、1100-1150ヌクレオチドまたはそれ以上の長さであり、配列番号：1のヌクレオチド1～358を含む配列の少なくとも約15個の（すなわち、連続した15個の）ヌクレオチドまたはその相補配列を含み、ストリンジェントな条件下で配列番号：1に示すヌクレオチド配列を含む核酸分子またはその相補配列にハイブリダイゼーションするヌクレオチド配列を含む。

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【0107】

PD-L2ヌクレオチド配列に基づくプローブを用いて同じまたは相同的なポリペプチドをコードする転写物またはゲノム配列を検出することができる。好ましい具体例において、プローブは、それに結合した標識基をさらに含み、例えば、標識基は放射性同位元素、蛍光化合物、酵素、または酵素コファクターであってもよい。かかるプローブを診断試験キットの一部として用いて、例えば、対象からの細胞試料中のPD-L2をコードする核酸のレベル、例えば、PD-L2 mRNAレベルを測定し、あるいはゲノムPD-L2遺伝子に変異または欠失しているかどうかを調べることにより、PD-L2ポリペプチドを誤って発現する細胞または組織を同定することができる。

【0108】

PD-L2の生物学的活性（例えば、その本来の結合パートナー（例えばPD-1）に結合し、そして／あるいは免疫細胞活性をモジュレーションする能力）を有するポリペプチドをコードする配列番号：1、3、4または6のヌクレオチド配列の一部分を単離し、PD-L2ポリペプチドのコードされた部分を発現させ（例えば、インビトロでの組換え発現により）、次いで、PD-L2ポリペプチドのコードされた部分の活性を評価することにより、「PD-L2ポリペプチドの生物学的に活性のある部分」をコードする核酸フラグメントを調製することができる。

【0109】

さらに本発明は、遺伝暗号の縮重により配列番号：1、3、4または6に示すヌクレオチド配列とは異なっているが、配列番号：1、3、4または6に示すヌクレオチド配列によりコードされるのと同じPD-L2ポリペプチドをコードする核酸分子を包含する。もう1つの具体例において、本発明の単離核酸分子は配列番号：2または5に示すアミノ酸配列を有するポリペプチドをコードするヌクレオチド配列を有する。

【0110】

配列番号：1、3、4または6に示すPD-L2ヌクレオチド配列のほかに、PD-L2ポリペプチドのアミノ酸配列の変化を導くDNA配列多型が集団（例えば、ヒト集団）中に存在してもよいことが当業者に理解されよう。PD-L2遺伝子中のかかる遺伝学的多型が、天然の対立遺伝子変化により集団中の個体間に存在してもよい。本明細書の用語「遺伝子」および「組換え遺伝子」は、PD-L2ポリペプチド、好ましくは哺乳動物PD-L2ポリペプチドをコードする読み取り枠を含み、さらに非コーディング調節配列およびイントロンを含んでもよい核酸分子をいう。

【0111】

ヒトまたはマウスのPD-L2の対立遺伝子変種は機能的ならびに非機能的PD-L2ポリペプチドを包含する。機能的対立遺伝子変種は、本来のPD-L2結合パートナー、例えばPD-1に結合し、そして／あるいはリンパ球活性化をモジュレーションする能力を維持しているヒトまたはマウスのPD-L2ポリペプチドの天然に存在するアミノ酸配列

変種である。典型的には、機能的対立遺伝子変種は、配列番号：2または5の1個またはそれ以上のアミノ酸の保存的置換のみを含み、あるいはポリペプチドの重要でない領域の重要でない残基の置換、欠失または挿入を含む。

【0112】

非機能的対立遺伝子変種は、本来のPD-L2結合パートナー、例えばPD-1に結合せず、本明細書記載のPD-L2活性をモジュレーションしないヒトまたはマウスのPD-L2ポリペプチドの天然に存在するアミノ酸配列変種である。典型的には、非機能的対立遺伝子変種は、配列番号：2または5のアミノ酸配列の非保存的置換、欠失または挿入あるいは未成熟な末端切断、あるいはポリペプチドの重要な残基または重要な領域、例えばIgV領域における置換、挿入または欠失を含む。

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【0113】

さらに本発明は、ヒトまたはマウスPD-L2ポリペプチドの非ヒト、非マウスオースログを提供する。ヒトまたはマウスPD-L2ポリペプチドのオースログは、非ヒト、非マウス生物から単離され、PD-L2ポリペプチドと同じPS-1結合活性および／またはリンパ球活性化モジュレーション活性を有するポリペプチドである。ヒトまたはマウスPD-L2ポリペプチドのオースログは配列番号：2または5と実質的に同じアミノ酸配列を含むものと容易に同定される。

【0114】

そのうえ、他のPD-L2ファミリーのメンバーをコードする核酸分子、よって配列番号：1、3、4または6のPD-L2配列とは異なるヌクレオチド配列を有する核酸分子は本発明の範囲内にある。例えば、マウスまたはヒトのPD-L2のヌクレオチド配列に基づいて別のPD-L2 cDNAを同定することができる。そのうえ、異なる種由来のPD-L2ポリペプチドをコードする核酸分子、かくして配列番号：1、3、4または6のPD-L2配列とは異なるヌクレオチド配列を有する核酸分子は本発明の範囲内である。例えば、マウスまたはヒトPD-L2のヌクレオチド配列に基づいてサルPD-L2 cDNAを同定することができる。

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【0115】

ストリンジェントなハイブリダイゼーション条件下において標準的なハイブリダイゼーション法により、本明細書開示のcDNAあるいはその一部分をハイブリダイゼーションプローブとして用いて、本明細書開示のPD-L2核酸に対する相同性に基づいて、本発明のPD-L2 cDNAの天然の対立遺伝子変種およびホモログに対応する核酸分子を単離することができる。さらに、PD-L2遺伝子と同じ染色体または遺伝子座にマッピングすることにより、本発明のPD-L2 cDNAの天然の対立遺伝子変種およびホモログに対応する核酸分子を単離することができる。

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【0116】

したがって、もう1つの具体例において、本発明の単離核酸分子は少なくとも15、20、25、30ヌクレオチドまたはそれ以上の長さであり、ストリンジェントな条件下で配列番号：1のヌクレオチド配列のヌクレオチド1～358または配列番号：3のヌクレオチド1～85を含む核酸分子にハイブリダイゼーションする。他の具体例において、核酸は少なくとも880～900、900～950、950～1000、1000～1050、1050～1100、1100～1150ヌクレオチドまたはそれ以上の長さである。

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【0117】

本明細書の用語「ストリンジェントな条件下でハイブリダイゼーションする」は、有意に同一または相同的であるヌクレオチド配列が互いにハイブリダイゼーションしたままであるハイブリダイゼーションおよび洗浄の条件をいう。好ましくは、少なくとも約70%、好ましくは少なくとも約80%、さらに好ましくは少なくとも約85%または90%同一である配列が互いにハイブリダイゼーションしたままである条件である。かかるストリンジェントな上官は当業者に知られており、Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley & Sons, Inc. (1995), セクション 2, 4 および 6

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中に見られる。さらなるストリンジェントな条件はMolecular Cloning : A Laboratory Manual, Sambrook et al., Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), 第7, 9 および 11 章中に見られる。ストリンジェントなハイブリダイゼーション条件の好ましい非限定的な例は、4X または 6X 塩化ナトリウム／クエン酸ナトリウム (SSC) 中、約 65-70℃でのハイブリダイゼーション (あるいは 4X SSC プラス 50% ホルムアミド中、約 42-50℃でのハイブリダイゼーション)、次いで、1X SSC 中、約 65-70℃での1回またはそれ以上の回数の洗浄を包含する。ストリンジェントなハイブリダイゼーション条件のさらなる好ましい非限定的な例は、6X SSC、45℃でのハイブリダイゼーション、次いで、0.2X SSC, 0.1% SDS 中、65℃での1回またはそれ以上の回数の洗浄を包含する。非常にストリンジェントなハイブリダイゼーション条件の好ましい非限定的な例は、1X SSC 中、約 65-70℃でのハイブリダイゼーション (あるいは 1X SSC プラス 50% ホルムアミド中、約 42-50℃でのハイブリダイゼーション)、次いで、0.3X SSC 中、約 65-70℃での1回またはそれ以上の回数の洗浄を包含する。ストリンジェンシーを減じたハイブリダイゼーション条件の好ましい非限定的な例は、4X または 6X SSC 中、約 50-60℃でのハイブリダイゼーション (あるいは 6X SSC プラス 50% ホルムアミド中、約 40-45℃でのハイブリダイゼーション)、次いで、2X SSC 中、約 50-60℃での1回またはそれ以上の回数の洗浄を包含する。上記値の間の範囲、例えば、65-70℃または42-50℃もまた本発明の範囲内である。ハイブリダイゼーションおよび洗浄バッファー中のSSC (1x SSC は 0.15M NaCl および 15mM クエン酸ナトリウム) に代えてSSPE (1x SSPE は 0.15M NaCl, 10mM NaH₂PO₄, および 1.25mM EDTA, pH 7.4) を用いることができ、ハイブリダイゼーション完了後、各回15分の洗浄を行なう。長さ50塩基対未満と予想されるハイブリッドのハイブリダイゼーション温度はハイブリッドの融解温度 (T_m) よりも5-10℃低くすべきである。ここにT_mは以下の等式により決定される。長さ18塩基対未満のハイブリッドの場合、T_m (℃) = 2 (A + T 塩基数) + 4 (G + C 塩基数)。長さ18ないし49塩基対のハイブリッドの場合、T_m (℃) = 81.5 + 16.6 (log₁₀ [Na⁺]) + 0.41 (%G+C) - (600/N)、ここにNはハイブリッド中の塩基数、[Na⁺] はハイブリダイゼーションバッファー中のナトリウムイオン濃度 (1x SSC の [Na⁺] は0.165M) である。ブロッキング剤 (例えばBSAまたはサケもしくはニシンの精子キャリアDNA)、界面活性剤 (例えばSDS)、キレート剤 (例えばEDTA)、Ficoll、PVP等のさらなる試薬 (これらに限らない) をハイブリダイゼーションおよび/または洗浄バッファーに添加して核酸分子の膜、例えばニトロセルロースまたはナイロン膜への非特異的ハイブリダイゼーションを減少させてもよいことが当業者により理解されるであろう。ナイロン膜を用いる場合、詳細には、ストリンジェントなハイブリダイゼーション条件のさらなる好ましい非限定的な例は、0.25-0.5M NaH₂PO₄, 7% SDS 中、約65℃でのハイブリダイゼーション、次いで、0.02M NaH₂PO₄, 1% SDS 中、65℃での1回またはそれ以上の回数の洗浄である。例えば、Church and Gilbert (1984) Proc. Natl. Acad. Sci. USA 81:1991-1995 参照 (あるいは 0.2X SSC, 1% SDS)。

【0118】

好ましくは、ストリンジェントな条件下で配列番号：1、3、4または6の配列にハイブリダイゼーションする本発明の単離核酸分子は、天然に存在する核酸分子に対応する。本明細書の用語「天然に存在する」核酸分子は、天然に存在するヌクレオチド配列を有するRNAまたはDNA分子をいう。

【0119】

集団中に存在してもよいPD-L2配列の天然に存在する対立遺伝子変種のほかに、当業

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者は、配列番号：1、3、4または6のヌクレオチド配列中に突然変異により変化を導入し、そのことによりPD-L2ポリペプチドの機能能力を変化させることなく、コードされるPD-L2ポリペプチドのアミノ酸配列における変化を導くことが可能であることをさらに認識するであろう。例えば、「非必須」アミノ酸残基におけるアミノ酸置換を導くヌクレオチド置換を配列番号：1、3、4または6において作成することができる。「非必須」アミノ酸残基は、生物学的活性を変化させることなくPD-L2の野生型配列（例えば、配列番号：2または5の配列）から変化させることのできる残基であり、「必須」アミノ酸残基は生物学的活性に必要とされる。例えば、本発明のPD-L2ポリペプチド間で保存されているアミノ酸残基、例えば、細胞外ドメイン中に存在するものは特に変化に適さないと推定される。さらにそのうえ、本発明のPD-L2間およびPD-L2ファミリーの他のメンバー間で保存されているさらなるアミノ酸残基も変化に適する可能性が低い。

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【0120】

したがって、本発明のもう1つの態様は、活性に必須でないアミノ酸残基中に変化を含むPD-L2ポリペプチドをコードする核酸分子に関する。かかるPD-L2ポリペプチドは配列番号：2または5のアミノ酸配列において異なっているが、やはり生物学的活性を保持している。1の具体例において、単離核酸分子は、ポリペプチドをコードするヌクレオチド配列を含み、ポリペプチドは配列番号：2または5に対して少なくとも約71%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%またはそれ以上同一であるアミノ酸配列を含む。

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【0121】

配列番号：2または5のポリペプチドと同一のPD-L2ポリペプチドをコードする単離核酸を、配列番号：1、3、4または6のヌクレオチド配列中に1またはそれ以上のヌクレオチド置換、付加または欠失を導入することにより作成して、1またはそれ以上のアミノ酸置換、付加または欠失がコードされるポリペプチド中に導入されるようにすることができる。部位特異的突然変異方およびPCRによる突然変異法のごとき標準的方法により配列番号：1、3、4または6中に変異を導入することができる。好ましくは、保存的アミノ酸置換は、1つまたはそれ以上の非必須アミノ酸残基で起こる。「保存的アミノ酸置換」は、アミノ酸残基を同様の側鎖を有するアミノ酸残基で置換する方法の1つである。同様の側鎖を有するアミノ酸残基のファミリーは、当該分野で定義され、塩基性側鎖（例えば、リシン、アルギニン、ヒスチジン）、酸性側鎖（例えば、アスパラギン酸、グルタミン酸）、非電荷の極性側鎖（例えば、グリシン、アスパラギン、グルタミン、セリン、トレオニン、チロシン、システイン）、非極性側鎖（例えば、アラニン、バリン、ロイシン、イソロイシン、プロリン、フェニルアラニン、メチオニン、トリプトファン）、ベータ分岐側鎖（例えば、トレオニン、バリン、イソロイシン）、および芳香族側鎖（例えば、チロシン、フェニルアラニン、トリプトファン、ヒスチジン）を含む。したがって、好ましくは、PD-L2ポリペプチド中の非必須なアミノ酸残基は、同様の側鎖ファミリーの他のアミノ酸残基に置換される。あるいは、もう1つの具体例において、例えば飽和突然変異法によりPD-L2コーディング配列の全体または一部にランダムに変異を導入することができ、得られた変異体をPD-L2生物学的活性に関してスクリーニングして、活性を保持している変異体を同定することができる。配列番号：1、3、4または6の変異後、コードされるポリペプチドを組換え方により発現させ、ポリペプチドの活性を調べることができる。

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【0122】

好ましい具体例において、本来のPD-L2結合パートナー、例えばPD-1への結合および／またはその活性をモジュレーションする能力、細胞内または細胞間シグナリングをモジュレーションする能力、Tリンパ球の活性化をモジュレーションする能力、ならびに／あるいは生物の免疫応答をモジュレーションする能力に関して変異PD-L2ポリペプチドをアッセイすることができる。

【0123】

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さらにもう1つの本発明の態様は、PD-L2融合蛋白をコードする単離核酸分子に関する。非PD-L2ポリペプチド、ポリペプチドまたはペプチドをコードする第2のヌクレオチド配列に作動可能に結合される、少なくともPD-L2ポリペプチド、ポリペプチドまたはペプチドをコードする第1のヌクレオチド配列を含むこのような核酸分子は、標準的な組み換えDNA法により調製できる。

【0124】

上記PD-L2ポリペプチドをコードする核酸分子のほかに、本発明のもう1つの態様は、それらに対してアンチセンスである単離核酸分子に関する。「アンチセンス」核酸は蛋白をコードする「センス」核酸に相補的なヌクレオチド配列、例えば、二本鎖cDNA分子のコーディング鎖に相補的なヌクレオチド配列、またはmRNA配列に相補的なヌクレオチド配列を含む。したがって、アンチセンス核酸は、センス核酸と水素結合しうる。アンチセンス核酸は、PD-L2コーディング鎖全体、あるいはその蛋白にのみ相補的であり得る。1の具体例において、アンチセンス核酸分子は、PD-L2をコードするヌクレオチド配列のコーディング鎖の「コーディング領域」に対してアンチセンスである。用語「コーディング領域」は、アミノ酸残基に翻訳されるコドンを含むヌクレオチド配列の領域をいう。他の具体例において、アンチセンス核酸分子は、PD-L2をコードするヌクレオチド配列のコーディング鎖の「非コーディング領域」にアンチセンスである。用語「非コーディング領域」は、アミノ酸に翻訳されないコーディング領域に隣接する5'および3'配列をいう（5'および3'非翻訳領域ともいう）。

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【0125】

本明細書で開示されたヒトまたはマウスのPD-L2をコードするコーディング配列（例えば、それぞれ配列番号：3または配列番号：6）が得られたならば、本発明のアンチセンス核酸を、WatsonおよびCrickの塩基対合の法則により設計できる。アンチセンス核酸分子は、PD-L2 mRNAの全コーディング領域に相補的であってよい、より好ましくは、PD-L2 mRNAのコーディング領域または非コーディング領域だけにアンチセンスなオリゴヌクレオチドである。例えば、アンチセンスオリゴヌクレオチドは、PD-L2 mRNAの翻訳開始部位の周辺領域に相補的であってよい。アンチセンスオリゴヌクレオチドは、例えば、約5、10、15、20、25、30、35、40、45または50ヌクレオチドの長さであってよい。本発明のアンチセンス核酸は、化学合成および当該分野で公知の工程を使用する酵素により連結反応を使用して構築できる。例えば、天然に存するヌクレオチドまたは分子の生物学的安定性を増加させ、アンチセンスとセンス核酸の間に形成される二重鎖の物理的安定性を増加させるように設計された様々に修飾されたヌクレオチドを使用してアンチセンス核酸分子（例えば、アンチセンスヌクレオチド）を化学的に合成することができ、例えば、ホスホロチオエート誘導体およびアクリジン置換ヌクレオチドを使用することができる。アンチセンス核酸の生成に使用しうる修飾ヌクレオチドの例は、5-フルオロウラシル、5-ブロモウラシル、5-クロロウラシル、5-ヨウドウラシル、ヒポキサンチン、キサンチン、4-アセチルシトシン、5-（カルボキシヒドロキシメチル）ウラシル、5-カルボキシメチルアミノメチル-2-チオウリジン、5-カルボキシメチルアミノメチルウラシル、ジヒドロウラシル、ベータ-D-ガラクトシルクエオシン、イノシン、N6-イソペンテニルアデニン、1-メチルグアニン、1-メチルリノシン、2,2-ジメチルグアニン、2-メチルアデニン、2-メチルグアニン、3-メチルシトシン、5-メチルシトシン、N6-アデニン、7-メチルグアニン、5-メチルアミノメチルウラシル、5-メトキシアミノメチル-2-チオウラシル、ベータ-D-マンノシルクエオシン、5'-メトキシカルボキシメチルウラシル、5-メトキシウラシル、2-メチルチオ-N6-イソペンテニルアデニン、ウラシル-5-オキシ酢酸（v）、ワイブトキソシン、プソイドウラシル、クエオシン、2-チオシトシン、5-メチル-2-チオウラシル、2-チオウラシル、4-チオウラシル、5-メチルウラシル、ウラシル-5-オキシ酢酸メチルエステル、ウラシル-5-オキシ酢酸（v）、5-メチル-2-チオウラシル、3-（3-アミノ-3-N-2-カルボキシプロピル）ウラシル、（acp3）w、および2,6-ジアミノプリンを包含する。別法と

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して、核酸をアンチセンス方向（すなわち、挿入核酸から転写されたRNAは、対象とする標的核酸に対してアンチセンス方向であろう。これは以下のサブセクションにさらに記載する。）にサブクロニングする発現ベクターを使用してアンチセンス核酸を生物学的に生産できる。

【0126】

本発明のアンチセンス核酸分子は、典型的には、対象に投与され、または *in situ* において産生され、これらはハイブリッド形成し、またはPD-L2ポリペプチドをコードする細胞のmRNAおよび／またはゲノムDNAに結合し、そのことにより、例えば、転写および／または翻訳を阻害することにより、蛋白の発現を阻害する。ハイブリッド形成は、保存ヌクレオチド相補性により、あるいは例えば、DNA二本鎖に結合するアンチセンス核酸分子の場合、二重螺旋の主要な溝での特別な相互作用により、安定な二本鎖を形成することができる。本発明のアンチセンス核酸分子の投与経路の例は、組織部位における直接注入を含む。別法として、アンチセンス核酸分子を修飾して選択された細胞を標的化し、ついで、全身投与することができる。例えば、全身投与するために、例えば、アンチセンス核酸分子を、細胞表面の受容体または抗原に結合するペプチドまたは抗体に結合することにより、アンチセンス分子が選択された細胞表面で発現した受容体または抗原に結合するように特異的に修飾することができる。また、アンチセンス核酸分子を、本明細書記載のベクターを使用して細胞にデリバリーすることができる。アンチセンス分子の十分な細胞内濃度を実現させるため、アンチセンス核酸分子が強力なp0111またはp0111プロモーターの制御下に置かれるベクター構造が好ましい。

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【0127】

さらなる具体例において、本発明のアンチセンス核酸分子は、 α -アノマー核酸分子である。 α -アノマー核酸分子は、相補的なRNAと特異的な二本鎖ハイブリッドを形成し、そこでは通常の β -ユニットとは対照的に鎖は互いに平行に伸長する（Gaultier et al. (1987) Nucleic Acids. Res. 15:6625-6641）。また、アンチセンス核酸分子は、2'-O-メチルリボヌクレオチドを含んでいてもよく（Inoue et al. (1987) Nucleic Acids Res. 15:6431-6148）あるいはキメラRNA-DNAアナログを含んでいてもよい（Inoue et al. (1987) FEBS Lett. 215:327-330）。

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【0128】

さらなる他の具体例において、本発明のアンチセンス核酸分子は、リボザイムである。リボザイムは、それに相補的な領域を有するmRNAのごとき一本鎖核酸分子を切断することができるリボヌクレアーゼ活性を有する触媒RNA分子である。したがって、リボザイム（例えば、ハンマーヘッドリボザイム（hammerhead ribozymes）（Haseloff and Gerlach (1988) Nature 334:585-591に記載）を用いてPD-L2 mRNA転写物を触媒的に切断することができる。そのことによりPD-L2 mRNAの翻訳を阻害することができる。PD-L2をコードする核酸に対して特異性を有するリボザイムを、本明細書に記載のPD-L2のヌクレオチド配列（すなわち、配列番号：1、3、4または6）に基づいて設計することができる。例えば、Tetrahymena L-19 IVS RNAの誘導体を、活性部位のヌクレオチド配列がPD-L2をコードするmRNA中で切断されるヌクレオチド配列に相補的であるように構築できる。例えば、Cechらの米国特許第4,987,071号；およびCechらの米国特許第5,116,742号参照。別法として、PD-L2 mRNAを用いて、RNA分子のプールから特異的なリボヌクレアーゼ活性を有する触媒RNAを選択することができる。例えば、Bartel, D. and Szostak, J. W. (1993) Science 261:1411-1418参照。

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【0129】

別法として、PD-L2の調節領域（例えば、PD-L2プロモーターおよび／またはエンハンサー；例えば、配列番号：1のヌクレオチド1～273または配列番号：4のヌク

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レオチド1～209)に相補的なヌクレオチド配列を標的化して、標的細胞中のPD-L2遺伝子の転写を防ぐ三重螺旋構造を形成することにより、PD-L2遺伝子発現を阻害することができる。一般的には、Helene, C. (1991) *Anticancer Drug Des.* 6(6): 569-84; Helene, C. et al (1992) *Ann. N. Y. Acad. Sci.* 660: 27-36; and Maher, L. J. (1992) *Bioessays* 14(12): 807-15 参照。

【0130】

さらなる他の具体例において、本発明のPD-L2核酸分子を、塩基部分、糖部分、またはリン酸骨格において修飾して、例えば、安定性、ハイブリッド形成、または分子の溶解性を改善することができる。例えば、核酸分子のデオキシリボースリン酸骨格を修飾してペプチド核酸を得ることができる(Hyrup, B. and Nielsen, P. E. (1996) *Bioorg. Med. Chem.* 4(1): 5-23 参照)。本明細書の用語「ペプチド核酸」または「PNA」は、デオキシリボースリン酸骨格を、擬似ペプチド骨格により置換し、4つの天然ヌクレオベースだけが維持された核酸模倣物、例えば、DNA模倣物を意味する。PNAの天然の骨格は、低イオン強度の環境下でDNAおよびRNAに特異的なハイブリッド形成を可能にすることが示されている。PNAオリゴマーの合成は、標準的な、Hyrup and Nielsen (1996) *supra* and Perry-O'Keefe et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 14670-675に記載のように固相ペプチド法を使用して実行できる。

【0131】

PD-L2核酸分子のPNAを治療および診断用に使用できる。例えば、PNAは、例えば、転写または翻訳の停止を誘発し、または複製を阻害することにより、アンチセンスまたは抗原作用剤として、遺伝子発現の配列特異的なモジュレーションに使用可能である。また、PD-L2核酸分子のPNAは、遺伝子中の単塩基対突然変異体の分析に(例えば、PNA-指向性PCRクランピング)、他の酵素と組み合わせて使用される場合には「人工制限酵素」として(例えば、上記S1ヌクレアーゼ(上で引用したHyrup and Nielsen (1996)))、あるいはDNA配列またはハイブリッド形成用のプローブまたはプライマーとして(上で引用したHyrup and Nielsen (1996); Perry-O'Keefe et al. (1996) *supra*)使用可能である。

【0132】

PD-L2核酸分子のPNAを治療および診断用途に使用することができる。例えば、転写または翻訳停止を誘導することにより、あるいは複製を阻害することにより、遺伝子発現の配列特異的なモジュレーションのためのアンチセンスまたは抗原としてPNAを使用することができる。また、PD-L2核酸分子のPNAを、遺伝子中の単一塩基対変異の分析に(例えば、PNAに指向されたPCRクランピングにより);他の酵素(例えば、S1ヌクレアーゼ(上記Hyrup and Nielsen (1996)))と組み合わせた場合に人工制限酵素として;あるいはDNA配列決定またはハイブリダイゼーションのためのプローブまたはプライマーとして(上記Hyrup and Nielsen (1996); 上記Perry-O'Keefe (1996))使用することもできる。

【0133】

他の具体例において、親油性または他のヘルパー基をPNAに付着させることにより、PNA-DNAキメラの形成により、またはリポソームまたは当業者に公知の薬剤デリバリーの他の方法により、PD-L2のPNAを修飾することができる(例えば、安定性または細胞摂取を高めるために)。例えば、PNAおよびDNAの有利な特性を組み合わせることができるPD-L2核酸分子のPNA-DNAキメラを得ることができる。このようなキメラは、DNA認識酵素(例えば、RNAse HおよびDNAポリメラーゼ)に、DNA部分との相互作用を可能ならしめ、その一方で、PNA部分は、高い結合アフィニ

ティーおよび特異性を提供するであろう。塩基スタッキング、ヌクレオベース間の結合の数、および方向に関して選択される適当な長さのリンカーを使用してPNA-DNAキメラを連結することができる(Hyrup B. and Nielsen (1996) *supra*)。PNA-DNAキメラの合成を、Hyrup B. and Nielsen (1996) *supra* および Finn P. J. et al. (1996) *Nucleic Acids Res.* 24 (17) : 3357-63に記載のように行うことができる。例えば、標準的なホスホラミダイトカップリング化学物質を使用して、固体支持体上でDNA鎖を合成することができる。修飾ヌクレオシドアナログ(例えば、5'-((4-メトキシトリチル)アミノ-5'-デオキシチミジンホスホラミダイト)を、PNAとDNAの5'末端との間のリンカーとして使用できる(Mag, M. et al. (1989) *Nucleic acid Res.* 17:5973-88)。ついで、キメラ分子を生成する段階的な方法で、PNAモノマーを5' PNAセグメントおよび3' DNAセグメントとカップリングさせる(上記Finn P. J. et al. (1996))。別法として、5' DNAセグメントおよび3' PNAセグメントを用いてキメラ分子を合成することができる(Peterser, K. H. et al. (1975) *Bioorganic Med. Chem. Lett.* 5:1119-11124)。

【0134】

他の具体例において、オリゴヌクレオチドは、ペプチドのごとき他の付加基(例えば、インビボでの宿主細胞受容体の標的化のために)、あるいは細胞膜(例えば、Letting et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaître et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT出願WO88/09810参照)または血液脳関門(例えば、PCT出願WO89/10134)を越えた輸送を容易ならしめる作用剤を包含する。さらに、オリゴヌクレオチドを、ハイブリッド形成により誘発される開裂剤(例えば、Krol et al. (1988) *Bio techniques* 6:958-976参照)または挿入剤(例えば、Zon (1988) *Pharm. Res.* 5:539-549参照)を用いて修飾することができる。この目的のために、オリゴヌクレオチドを、他の分子(例えば、ペプチド、ハイブリッド形成により誘発される架橋剤、輸送剤またはハイブリッド形成により誘発される切断剤)に結合させることができる。

【0135】

別法として、安定な細胞系またはクローン化された微生物のゲノム中に異種DNA調節エレメントを挿入して、挿入された調節エレメントが内在性PD-L2遺伝子に作動可能に連結されるようにすることにより、細胞系または微生物中の内在性PD-L2遺伝子の発現特性を修飾してもよい。例えば、通常には「転写的にはサイレント」な内在性PD-L2遺伝子、すなわち、通常には細胞系または微生物において発現されないかあるいは非常に低レベルでしか発現されないPD-L2遺伝子を、当該細胞系または微生物において通常に発現される遺伝子産物の発現を促進しうる調節エレメントを挿入することにより活性化させてもよい。別法として、転写的にサイレントな内在性PD-L2遺伝子を、細胞タイプを超えて作用する汎用調節エレメントを挿入することにより活性化させてもよい。

【0136】

当業者によく知られ、例えば、Chappel, 米国特許第5,272,071号; PCT公開第WO 91/06667号(1991年5月16日公開)に記載されている標的化相同組換え法のごとき方法を用いて、異種調節エレメントを安定な細胞系またはクローン化された微生物中に挿入して、それが内在性PD-L2遺伝子に連結されるようにしてもよい。

【0137】

II. 単離PD-L2ポリペプチドおよび抗-PD-L2抗体

本発明の1の態様は、単離されたPD-L2ポリペプチド、およびその生物学的に活性の

ある部分、ならびに抗PD-L2抗体を生じさせる免疫原としての使用に適したポリペプチドフラグメントに関する。1の具体例において、ネイティブなPD-L2ポリペプチドは、標準的な蛋白精製技術を用いた適当な精製計画により細胞または組織供給源から単離され得る。別の具体例において、PD-L2ポリペプチドは、組換えDNA技術により製造される。組換え発現に代わるものとして、PD-L2ポリペプチドまたはポリペプチドは、標準ペプチド合成技術を用いて化学合成され得る。

【0138】

「単離」または「精製」された蛋白またはその生物学的に活性のある蛋白は、PD-L2ポリペプチドが由来した細胞または組織源由来の細胞性物質または他の混入蛋白を実質的に含まず、あるいは化学合成の場合には化学前駆体または他の化学試薬を含まない。用語「細胞性物質を実質的に含まない」は、PD-L2ポリペプチドが単離されあるいは組み換え法により製造される細胞の細胞性成分から分離されているPD-L2ポリペプチドの調合物を意味する。1の具体例において、用語「細胞性物質を実質的に含まない」は、約30%（乾燥重量で）未満の非PD-L2ポリペプチド（本明細書において「混入蛋白」ともいう）、より好ましくは約20%の非PD-L2ポリペプチド、さらに好ましくは約10%未満の非PD-L2ポリペプチド、最も好ましくは約5%未満の非PD-L2ポリペプチドを有するPD-L2ポリペプチドの調合物を包含する。また、PD-L2ポリペプチドまたはその生物学的に活性のある部分が組み換え法により製造される場合、好ましくは、それは培地を含まないものであり、すなわち、培地が蛋白調合物の体積の約20%未満、より好ましくは約10%未満、最も好ましくは約5%未満である。

【0139】

用語「化学前駆体または他の化学物質を実質的に含まない」は、蛋白合成に使用される化学前駆体または化学物質から蛋白が分離されているPD-L2ポリペプチドの調合物を包含する。1の具体例において、用語「化学前駆体または他の化学物質を実質的に含まない」は約30%（乾燥重量で）未満の化学前駆体または非PD-L2化学物質、より好ましくは約20%未満の化学前駆体または非PD-L2化学物質、さらに好ましくは約10%未満の化学前駆体または非PD-L2化学物質、最も好ましくは約5%未満の化学前駆体または非PD-L2化学物質を有するPD-L2蛋白調合物を包含する。

【0140】

本明細書の用語、PD-L2ポリペプチドの「生物学的に活性のある部分」は、PD-L2分子と非PD-L2分子、例えば、PD-L2の本来のリガンド、例えばPD-1との間の相互作用に参与するPD-L2ポリペプチドのフラグメントを包含する。PD-L2ポリペプチドの生物学的に活性のある部分は、PD-L2ポリペプチドのアミノ酸配列、例えば配列番号：2または5に示すアミノ酸配列に対して十分に同一である、あるいはそれに由来するアミノ酸配列を含むペプチドを包含し、それは完全長のPD-L2ポリペプチドよりも少ないアミノ酸を含み、少なくとも1つのPD-L2ポリペプチドの活性を示す。典型的には、生物学的に活性のある部分は、少なくとも1つのPD-L2の活性、例えば、PD-1活性のモジュレーション活性を有するドメインまたはモチーフを含む。PD-L2ポリペプチドの生物学的に活性のある部分は、例えば、25、50、75、100、125、150、175、200、225個またはそれ以上のアミノ酸の長さである。PD-L2ポリペプチドの生物学的に活性のある部分を、PD-L2により媒介される活性、例えば、免疫細胞活性化をモジュレーションする作用剤の開発のための標的として使用することができる。

【0141】

1の具体例において、PD-L2ポリペプチドの生物学的に活性のある部分は少なくとも細胞外ドメインの一部を含む。本発明のPD-L2ポリペプチドの好ましい生物学的に活性のある部分が、少なくとも、1の細胞外ドメイン（例えば、IgVおよび／またはIgCドメインを含む）、および1またはそれ以上の下記ドメイン：シグナルペプチドドメイン、膜貫通ドメイン、および細胞質ドメインを含んでいてもよいことが理解されるはずである。そのうえ、ポリペプチドの他の領域が欠失されている他の生物学的に活性のある部

分を、組換え法により調製し、ネイティブなPD-L2ポリペプチドの1またはそれ以上の機能的活性について評価することができる。

【0142】

好ましい具体例において、PD-L2ポリペプチドは配列番号：2または5に示すアミノ酸配列を有し、配列番号：2または5のポリペプチドの機能的活性を保持しており、上のサブセクションIで詳述したように、自然発生的な対立遺伝子変化または突然変異によりアミノ酸配列が異なるものである。したがって、もう1つの具体例において、PD-L2ポリペプチドは、配列番号：2または5に対して少なくとも約71%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%またはそれ以上同一であるアミノ酸配列を含むポリペプチドである。

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【0143】

2つのアミノ酸配列または2つの核酸配列の同一性パーセントを測定するため、最適な比較を目的として配列を並置する（例、ギャップは、最適なアラインメント（並置）のために第1および第2アミノ酸または核酸配列の一方または両方に導入され得、非相同性配列は比較目的に関しては無視され得る）。好ましい態様において、比較目的用に並置させたレファレンス配列の長さは、レファレンス配列の長さの少なくとも30%、好ましくは少なくとも40%、さらに好ましくは少なくとも50%、さらに好ましくは少なくとも60%、さらに好ましくは少なくとも70%、80%または90%である（例えば、第2の配列を、273個のアミノ酸残基、少なくとも82個、好ましくは少なくとも109個、より好ましくは少なくとも137個、さらにより好ましくは少なくとも164個、ずっと好ましくは少なくとも191個、218個、246個またはそれ以上のアミノ酸残基を有する配列番号：2のヒトPD-L2アミノ酸配列と並置比較する場合；第2のアミノ酸配列を、247個のアミノ酸残基、少なくとも74個、好ましくは少なくとも99個、より好ましくは少なくとも124個、さらにより好ましくは少なくとも148個、ずっと好ましくは少なくとも173個、198個、222個またはそれ以上のアミノ酸残基を有する配列番号：2のヒトPD-L2アミノ酸配列と並置比較する場合）。次いで、対応する位置にある残基を比較し、一配列における位置を他の配列で対応する位置と同じ残基が占めるとき、分子はその位置において同一である。2つの配列間の同一性%は、2つの配列の最適並置比較のために導入される必要があるギャップの数および各ギャップの長さを考慮した上での、配列が共有する同一位置の数の関数である（本明細書で使用されているアミノ酸または核酸の「同一性」は、アミノ酸または核酸「相同性」と均等内容である）。2つの配列の最適並置比較を行なうために導入する必要のあるギャップ数および各ギャップの長さを考慮すると、2つの配列間の同一性パーセントは、2つの配列が共有する同一位置の数の関数である。

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【0144】

数学的アルゴリズムを用いて2つの配列間の配列の比較および同一性%の決定を行なうことができる。好ましい具体例において、GCGソフトウェアパッケージ（Genetics Computer Groupからオンラインで利用可能）中のGAPプログラム中に含まれるNeedleman and Wunschアルゴリズム（J. Mol. Biol. 48:444-453（1970））を用い、Blossum 62マトリックスまたはPAM250マトリックスのいずれかを用いて、ギャップ重量16、14、12、10、8、6、または4および長さ重量1、2、3、4、5、または6として、2つのアミノ酸配列間の同一性%が決定される。さらにもう1つの好ましい具体例において、GCGソフトウェアパッケージ（Genetics Computer Groupからオンラインで利用可能）中のGAPプログラムを用い、NWSgapdna.CMPマトリックスを用いて、ギャップ重量40、50、60、70、または80および長さ重量1、2、3、4、5、または6として、2つのヌクレオチド配列間の同一性%が決定される。もう1つの具体例において、ALIGNプログラム（バージョン2.0または2.0U）中に含まれるMeyers, E. and Moller, W.のアルゴリズム（Comput. Appl. Biosci. 4:11-17（1988））を用い、PAM120重

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量残基表、ギャップ長さペナルティー 12 およびギャップペナルティー 4 を用いて、2 つのアミノ酸またはヌクレオチド配列間の同一性 % が決定される。

【0145】

さらに本発明の核酸および蛋白配列を「クエリー (query) 配列」として使用することにより、パブリックデータベースに対する検索が遂行され、例えば他のファミリー構成員または関連配列が同定され得る。かかる検索は、Altschul ら (1990) J. Mol. Biol. 215:403-10 の NBLAST および XBLAST プログラム (バージョン 2.0) を用いて遂行され得る。BLAST ヌクレオチド検索を、NBLAST プログラム、スコア = 100、ワード長 = 12 で遂行することにより、本発明の PD-L2 核酸分子と相同的なヌクレオチド配列が得られる。BLAST 蛋白検索を、XBLAST プログラム、スコア = 100、ワード長 = 3 で遂行することにより、本発明の PD-L2 ポリペプチド分子と相同的なアミノ酸配列が得られる。比較目的用のギャップトアラインメントを得るためには、Altschul ら (1997) Nucleic Acids Res. 25(17):3389-3402 記載の要領でギャップト BLAST が利用され得る。BLAST およびギャップト BLAST プログラムを用いるとき、それぞれのプログラム (例えば、XBLAST および NBLAST) のデフォルトパラメーターが使用され得る。National Center for Biotechnology Information のインターネットウェブサイト参照。

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【0146】

本発明はまた、PD-L2 キメラまたは融合蛋白を提供する。ここで使用されている PD-L2 「キメラ蛋白」または「融合蛋白」は、非 PD-L2 ポリペプチドに作動可能に結合された PD-L2 ポリペプチドを含む。「PD-L2 ポリペプチド」は、PD-L2 ポリペプチドに対応するアミノ酸配列を有するポリペプチドを包含し、「非 PD-L2 ポリペプチド」は、PD-L2 ポリペプチドと実質的に相同的ではない蛋白、例えば PD-L2 ポリペプチドとは異なり、同一または異なる生物に由来する蛋白に対応するアミノ酸配列を有するポリペプチドを包含する。PD-L2 融合蛋白内では、PD-L2 ポリペプチドは、PD-L2 ポリペプチドの全部または一部に対応し得る。好ましい態様において、PD-L2 融合蛋白は、PD-L2 ポリペプチドの少なくとも 1 つの生物学的活性を有する部分を含む。もう 1 つの好ましい具体例において、PD-L2 融合蛋白は、PD-L2 ポリペプチドの少なくとも 2 つのドメインを含む。融合蛋白内で、「作動可能に結合された」の語は、PD-L2 ポリペプチドおよび非 PD-L2 ポリペプチドが互いにインフレーム (in-frame) で融合されていることを示すものとする。非 PD-L2 ポリペプチドを PD-L2 ポリペプチドの N-末端または C-末端に融合させることができ、非 PD-L2 ポリペプチドは PD-L2 ポリペプチドの溶解度、結合アフィニティー、安定性またはバレンシー (valency) を変化させる部分に対応する。

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例えば、1 の具体例において、融合蛋白は、PD-L2 配列が GST 配列の C 末端に融合した GST-PD-L2 融合蛋白である。かかる融合蛋白は、組換え PD-L2 の精製を容易ならしめることができる。

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【0147】

もう 1 つの具体例において、融合蛋白は、異種シグナル配列を N 末端に含む PD-L2 ポリペプチドである。ある種の宿主細胞 (例えば、哺乳動物宿主細胞) において、異種シグナル配列を使用することにより PD-L2 の発現および／または分泌を増加させることができる。

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【0148】

好ましい具体例において、融合蛋白は、PD-L2 配列が Ig 分子の一部分に融合した Ig-PD-L2 融合蛋白である。融合蛋白の Ig 部分は免疫グロブリン不変領域、例えば、ヒト C γ 1 ドメインまたは C γ 4 ドメイン (例えば、ヒト IgC γ 1 またはヒト IgC γ 4 ドメインのヒンジ、CH2 および CH3 領域) を含むことができる (例えば、Caponet al., U. S. Patent No. 5,116,964; 5,580,756; 5,844,095 等を参照、参照により本明細書に一体化させる)。得ら

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れる融合蛋白は変化したPD-L2溶解度、結合アフィニティー、安定性および／またはバレンシー（すなわち、1分子あたりの結合部位数）を有していてもよく、蛋白精製効率が高まっていてもよい。

【0149】

特に好ましいPD-L2 Ig融合蛋白は、免疫グロブリン不変領域（例えば、Fc領域）とカップリングしたPD-L2の細胞外部分を含む。免疫グロブリン不変領域は、免疫グロブリン構造における固有のエフェクター活性を低下または除去する遺伝学的修飾を含んでいてもよい。例えば、PD-L2ポリペプチドの細胞外部分をコードするDNAを、例えば、WO97/28267中に教示されたように部位特異的突然変異法により修飾されたヒトIgGγ1および／またはIgGγ4のヒンジ、CH2、およびCH3領域をコードするDNAに連結することができる。

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【0150】

本発明のPD-L2融合蛋白を医薬組成物中に含有させ、インビボにて対象に投与することができる。PD-L2融合蛋白を用いてPD-L2結合パートナー、例えば、PD-1の生体利用性に影響を及ぼしてもよい。PD-L2融合蛋白の使用は、免疫応答のモジュレーションにより利益を受けるであろう症状または疾病の治療に治療的に有用でありうる。

そのうえ、本発明のPD-L2融合蛋白を免疫原として用いて対象中に抗PD-L2抗体を産生させ、PD-L2結合蛋白を精製し、スクリーニングアッセイにおいて本来の結合パートナー、例えば、PD-1との相互作用を阻害する分子を同定することができる。

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【0151】

好ましくは、本発明のPD-L2キメラまたは融合蛋白は標準的な組換えDNA技術により製造される。例えば、異なるポリペプチド配列をコードするDNAフラグメントが、慣用的技術によりインフレームにて一緒に連結され、例えば平滑末端またはスタガー末端を用いて連結され、制限酵素消化により適当な末端が提供され、適当な場合には付着末端がフィルイン（fill-in）され、アルカリ性ホスファターゼ処理により望ましくない連結が回避され、酵素により連結される。別の態様において、融合遺伝子を自動DNA合成装置を含む慣用的技術により合成することができる。別法として、遺伝子フラグメントのPCR増幅を、アンカープライマーを用いて実施してもよく、これにより2つの連続遺伝子フラグメント間に相補的オーバーハングを生じさせ、次いで、アニーリング、再増幅を行なってキメラ遺伝子配列を得ることができる（例えば、Current Protocols in Molecular Biology、Ausubelら編、ジョン・ウィリー・アンド・サンズ：1992参照）。さらに、既に融合部分（例、GSTポリペプチドまたはHAエピトープ標識）をコードする多くの発現ベクターが市販されている。PD-L2をコードする核酸を、融合部分がPD-L2ポリペプチドにインフレームで融合されるように、かかる発現ベクター中へクローン化することができる。

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【0152】

また本発明は、PD-L2アゴニスト（模倣物）またはPD-L2アンタゴニストのいずれかとして機能するPD-L2ポリペプチドの変種に関する。突然変異法、例えば、PD-L2ポリペプチドの別個の点突然変異または末端切断によりPD-L2ポリペプチドの変種を得ることができる。PD-L2ポリペプチドのアゴニストは、天然に存在する形態のPD-L2ポリペプチドと実質的に同じ生物学的活性またはその一部の活性を保持しうる。PD-L2ポリペプチドのアンタゴニストは、例えば、PD-L2ポリペプチドのPD-L2により媒介される活性を競争的にモジュレーションすることにより、天然に存在する形態のPD-L2ポリペプチドの1またはそれ以上の活性を阻害しうる。かくして、機能が限定された変種で処理することにより、特定の生物学的効果が誘発されうる。1の具体例において、天然に存在する形態のポリペプチドの生物学的活性の一部を有する変種での対象の治療は、天然に存在する形態のPD-L2ポリペプチドでの治療に比べて対象における副作用が少ない。

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【0153】

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1 の具体例において、PD-L2 アゴニスト（模倣物）としてまたは PD-L2 アンタゴニストとして機能する PD-L2 ポリペプチドの変異体を、PD-L2 ポリペプチドアゴニストまたはアンタゴニスト活性について PD-L2 ポリペプチドの突然変異体、例えば末端切断突然変異体のコンビナトリアルライブラリーをスクリーニングすることにより同定することができる。1 の具体例において、PD-L2 変異体の雑多なライブラリーは、核酸レベルでの組み合わせ突然変異導入により生成され、雑多な (variegated) 遺伝子ライブラリーによりコードされる。例えば、合成オリゴヌクレオチドの混合物を遺伝子配列中に酵素的に連結させることにより、PD-L2 変異体の雑多なライブラリーを製造することができ、その場合、潜在的な PD-L2 配列の縮重セットは、個々のポリペプチドとして発現可能であり、あるいは別法として、PD-L2 配列のセットを含むより大きな融合蛋白のセットとして（例えば、ファージディスプレイ用に）発現可能である。縮重オリゴヌクレオチド配列から潜在的な PD-L2 変異体のライブラリーを製造するのに使用できる様々な方法がある。縮重遺伝子配列の化学合成を自動 DNA 合成装置で行なうことができ、次いで、合成遺伝子を適当な発現ベクターに結合することができる。遺伝子の縮重セットの使用により、1 の混合物中に、潜在的な PD-L2 配列の目的とするセットをコードする配列がすべて提供され得る。縮重オリゴヌクレオチドの合成方法は当該分野で公知である（例えば、Narang, S. A. (1983) Tetrahedron 39:3、Itakura ら (1984) Annu. Rev. Biochem. 53:323、Itakura ら (1984) Science 198:1056、Ike ら (1983) Nucleic Acid Res. 11:477 参照）。

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【0154】

さらに、PD-L2 ポリペプチドコーディング配列のフラグメントのライブラリーを用いて、PD-L2 フラグメントの雑多な集団を得ることにより、PD-L2 ポリペプチドの変異体をスクリーニングし、次いで、選択することができる。1 の具体例において、ニックングが 1 分子につき約 1 回だけ起こる条件下において PD-L2 をコードする配列の 2 本鎖 PCR フラグメントを処理し、2 本鎖 DNA を変性させ、DNA を再生させて、異なるニック産物由来のセンス／アンチセンス対を含みうる 2 本鎖 DNA を得て、S1 ヌクレアーゼ処理により再形成された 2 本鎖から 1 本鎖部分を除去し、次いで、得られたフラグメントライブラリーを発現ベクター中に連結することにより、コーディング配列フラグメントのライブラリーを得ることができる。この方法により、様々なサイズの PD-L2 ポリペプチドの N-末端、C-末端および内部フラグメントをコードする発現ライブラリーを誘導することができる。

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【0155】

点突然変異または末端切断により製造された組み合わせライブラリーの遺伝子産物をスクリーニングするための、ならびに選択された特性を有する遺伝子産物に関して cDNA ライブラリーをスクリーニングするためのいくつかの技術が当該分野において知られている。上記技術は、PD-L2 ポリペプチドのコンビナトリアル突然変異法により生成された遺伝子ライブラリーの迅速なスクリーニングに適合し得る。高処理量分析が可能な、大きな遺伝子ライブラリーのスクリーニングに最も広範に使用されている技術は、典型的には、遺伝子ライブラリーを複製可能な発現ベクターへクローニングし、生成したベクターのライブラリーで適当な細胞を形質転換し、そして目的とする活性の検出により、産物が検出された遺伝子をコードするベクターの単離が容易になる条件下でコンビナトリアル遺伝子を発現させることを含む。再帰アンサンブル突然変異導入法 (Recursive ensemble mutagenesis, REM) という、ライブラリーにおける機能性突然変異体の頻度を高める新規技術を、スクリーニングアッセイと組み合わせて使用することにより、PD-L2 変異体を同定することができる (Arkinn および Youvan (1992) Proc. Natl. Acad. Sci. USA 89:7811-7815、Delagrave ら (1993) Protein Eng. 6 (3):327-331)。

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【0156】

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1 の具体例において、細胞に基づくアッセイを利用することにより、雑多な PD-L2 ライブラリーを分析することができる。例えば、発現ベクターのライブラリーを細胞系中にトランスフェクションすることができる。次いで、トランスフェクションされた細胞を PD-1 発現細胞と接触させ、野生型 PD-L2 と本来のリガンド、例えば、PD-1 との相互作用による変異体の発現効果を検出することができる。次いで、PD-1 によるシグナリングの阻害（T 細胞アップレギュレーションを導く）あるいは PD-1 によるシグナリングの強化（T 細胞ダウンレギュレーションを導く）に関して評点をつけた細胞からプラスミド DNA を回収し、個々のクローンをさらに特徴づけることができる。

【0157】

天然に存するアミノ酸のみで構成される PD-L2 ポリペプチドに加えて、PD-L2 ペプチド模倣物も提供される。ペプチドアナログは、鑄型ペプチドの場合と類似した特性をもつ非ペプチド薬剤として製薬業界では常用されている。これらのタイプの非ペプチド化合物は、「ペプチド・ミメティクス」または「ペプチド模倣物」と呼ばれ (Fauchere, J. (1986) *Adv. Drug Res.* 15:29、Veber および Freidinger (1985) *TINS* 392 頁、および Evans ら、(1987) *J. Med. Chem.* 30:1229、出典明示により本明細書の一部とする)、通常には、コンピューター化分子モデリングの助けにより開発される。治療上有用なペプチドと構造的に類似したペプチド模倣物を用いることにより、同等内容の治療または予防効果が生じうる。一般的に、ペプチド模倣物は、パラダイムポリペプチド（すなわち、生物または薬理活性を有するポリペプチド）、例えばヒト PD-L2 と構造的に類似しているが、当業界では公知であり、さらに次の参考文献：Spatola, A. F.、*“Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins”* 中、Weinstein, B. 編、Marcel Dekker、ニューヨーク、267 (1983)、Spatola, A. F.、Vega Data (1983 年 3 月)、第 1 巻、3 刷、*“Peptide Backbone Modifications”* (レビュー)、Morley, J. S. (1980) *Trends Pharm. Sci.* 463-468 頁 (レビュー)、Hudson, D. ら (1979) *Int. J. Pept. Prot. Res.* 14:177-185 (-CH₂NH、CH₂CH₂-)、Spatola, A. F. ら (1986) *Life Sci.* 38:1243-1249 (-CH₂-S)、Hann, M. M. (1982) *J. Chem. Soc. Perkin Trans. I.* 307-314 (-CH-CH-、シスおよびトランス)、Almquist, R. G. ら (1990) *J. Med. Chem.* 23:1392-1398 (-COCH₂-)、Jennings-White, C. ら (1982) *Tetrahedron Lett.* 23:2533 (-COCH₂-)、Szelke, M. ら、ヨーロッパ出願 EP 45665 (1982) CA:97:39405 (1982) (-CH(OH)CH₂-)、Holladay, M. W. ら (1983) *Tetrahedron Lett.* (1983) 24:4401-4404 (-C(OH)CH₂-)、および Hruby, V. J. (1982) *Life Sci.* (1982) 31:189-199 (-CH₂-S-) に記載されている方法により (各々、出典明示により本明細書の一部とする)、-CH₂NH-、CH₂S-、-CH₂-CH₂-、-CH=CH- (シスおよびトランス)、-COCH₂-、-CH(OH)CH₂- および -CH₂SO- から成る群から選択された結合によって所望により置換されていてもよい 1 個またはそれ以上のペプチド結合を有する。特に好ましい非ペプチド結合は -CH₂NH- である。かかるペプチド模倣体は、例えば、生産性がより経済的である、化学的安定性が大きい、薬理学的特性（半減期、吸収、有効性、効力等）が高い、特異性が改変されている（例、広域スペクトルの生物活性）、抗原性が低いことなどを含め、ポリペプチド態様を凌ぐ顕著な利点を有し得る。ペプチド模倣体の標識は、直接的またはスペーサー（例、アミド基）を介して、定量的構造活性データおよび／または分子モデリングにより予測されるペプチド模倣物上の非干渉性位置 (non-interfering position) への 1 個またはそれ以上の標識の共有結合を通常伴う。上記非干渉性

位置は、一般的にペプチド模倣物が結合することにより治療効果を生じる高分子との直接接触を形成しない位置である。ペプチド模倣物の誘導体化（例えば、標識化）は、ペプチド模倣物の目的とする生物学的または薬理学的活性に実質的には干渉するものであってはならない。

【0158】

PD-L2 アミノ酸配列の1個またはそれ以上のアミノ酸を同じタイプのD-アミノ酸（例、L-リジンの代わりにD-リジン）と系統的に置換する方法を用いることにより、さらに安定したペプチドが生成され得る。さらに、PD-L2 アミノ酸配列または実質的に同一となる配列変化を含む束縛（constrained）ペプチドを、例えば、ペプチドを閉環する分子内ジスルフィド架橋を形成し得る内部システイン残基を付加することによる、当該分野で公知の方法により、得ることができる（RizooおよびGierasch（1992）Annu. Rev. Biochem. 61:387、出典明示により本明細書の一部とする）。

【0159】

本明細書において同定されたPD-L2 ポリペプチドのアミノ酸配列によると、当業者であれば、PD-L2 ペプチド配列およびその配列変異型に対応するポリペプチドを製造することが可能なはずである。多くの場合、PD-L2 ペプチド配列をコードするポリヌクレオチドの発現により、原核生物または真核生物宿主細胞中で上記ポリペプチドが大型ポリペプチドの一部として製造され得る。別法として、上記ペプチドを化学的方法により合成することもできる。組換え宿主における異種蛋白の発現、ポリペプチドの化学合成およびインビトロ翻訳に関する方法は、当該分野でよく知られており、Maniatisら、Molecular Cloning: A Laboratory Manual（1989）、第2版、コールドスプリングハーバー、ニューヨーク、BergerおよびKimmell、Methods in Enzymology、152巻、Guide to Molecular Cloning Techniques（1987）、アカデミック・プレス、インコーポレイテッド、サンディエゴ、カリフォルニア、Merrifield, J.（1969）J. Am. Chem. Soc. 91:501、Chaiken I. M.（1981）CRC Crit. Rev. Biochem. 11:255、Kaiserら（1989）Science 243:187、Merrifield, B.（1986）Science 232:342、Kent, S. B. H.（1988）Annu. Rev. Biochem. 57:957、およびOfford, R. E.（1980）Semisynthetic Proteins、ウィリー・パブリッシング（これらは出典明示により本明細書の一部とする）にさらに詳述されている。

【0160】

典型的には、ペプチドを直接化学合成により製造することができ、例えば、PD-L2 / PD-1 相互作用のアゴニストまたはアンタゴニストとして使用することができる。非ペプチド部分がN-末端および／またはC-末端への共有結合により結合している修飾ペプチドとして、ペプチドを製造することができる。ある好ましい具体例において、カルボキシ末端またはアミノ末端、またはその両方が化学的に修飾されている。末端アミノおよびカルボキシル基の最も一般的な修飾は、それぞれアセチル化およびアミド化である。アミノ末端修飾、例えばアシル化（例、アセチル化）またはアルキル化（例、メチル化）およびカルボキシ末端修飾、例えばアミド化、ならびに他の末端修飾、例えば閉環は、本発明の様々な態様に組込まれ得る。ある種のアミノ末端および／またはカルボキシ末端修飾および／またはコア配列へのペプチド伸長により、有利な物理的、化学的、生化学的および薬理学的特性、例えば高い安定性、強い効力および／または有効性、血清プロテアーゼに対する耐性、望ましい薬物動態学的特性などが提供され得る。ペプチドを治療的に使用して、例えば患者における共刺激を改変することによって病気を治療することができる。

【0161】

単離されたPD-L2 ポリペプチド、またはその一部もしくはフラグメント（またはかかるポリペプチドをコードする核酸分子）を免疫原として使用して、標準的なポリクローナ

ルおよびモノクローナル抗体製造技術を用いることによりPD-L2に結合する抗体を得ることができる。完全長PD-L2ポリペプチドを使用することもでき、あるいはまた、本発明は、免疫原として使用されるPD-L2の抗原性ペプチドフラグメントを提供する。1の具体例において、PD-L2の抗原性ペプチドは、配列番号：2または5に示すアミノ酸配列の少なくとも8個のアミノ酸残基を含み、PD-L2のエピトープを含むことから、ペプチドに対して産生した抗体はPD-L2との特異的免疫複合体を形成する。好ましくは、抗原性ペプチドは、少なくとも10個のアミノ酸残基、さらに好ましくは少なくとも15個のアミノ酸残基、さらに好ましくは少なくとも20個のアミノ酸残基、および最も好ましくは少なくとも30個のアミノ酸残基を含む。

抗原性ペプチドに含まれる好ましいエピトープは、蛋白表面に位置するPD-L2の領域、例えば親水性領域、ならびに高抗原性を有する領域である。 10

【0162】

典型的には、PD-L2免疫原を用いて、適当な対象（例、ウサギ、ヤギ、マウスまたは他の哺乳類）を免疫原で免疫することにより抗体が産生される。適当な免疫原性調合物は、例えば、組換え発現PD-L2ポリペプチドまたは化学合成PD-L2ペプチドを含み得る。さらにこの調合物は、アジュバント、例えばフロイント完全または不完全アジュバント、または同様の免疫刺激剤を含み得る。免疫原性PD-L2調合物で適当な対象を免疫化すると、ポリクローナル抗PD-L2抗体応答が誘導される。

【0163】

したがって、本発明のもう1つの態様は抗PD-L2抗体に関する。本明細書の用語「抗体」は、免疫グロブリン分子および免疫グロブリン分子の免疫学的に活性のある部分、すなわち、PD-L2のごとき抗原と特異的に結合（免疫反応）する抗原結合部を含む分子をいう。免疫グロブリン分子の免疫学的に活性のある部分の例は、F(ab)およびF(ab')₂フラグメントであり、それらはペプシンのごとき酵素で抗体を処理することにより得ることができる。本発明は、PD-L2分子に結合するポリクローナルおよびモノクローナル抗体を提供する。本明細書の用語「モノクローナル抗体」または「モノクローナル抗体組成物」は、PD-L2の特定のエピトープと免疫反応しうるたった1種の抗原結合部位を含む抗体分子の集団をいう。よって、典型的には、モノクローナル抗体組成物は、それが免疫反応する特定のPD-L2ポリペプチドに対する単一の結合アフィニティを示す。 20

【0164】

ポリクローナル抗PD-L2抗体を、適当な対象をPD-L2免疫原で免疫化することにより上記要領で製造することができる。免疫化対象における抗PD-L2抗体力価を、標準的技術、例えば固定化PD-L2ポリペプチドを用いた酵素結合免疫吸着アッセイ（ELISA）により時間経過とともにモニターすることができる。所望ならば、PD-L2ポリペプチドに対して指向した抗体分子を、哺乳類から（例えば、血液から）単離し、さらに公知技術、例えばプロテインAクロマトグラフィーにより精製すると、IgGフラクションが得られる。免疫後適当な時点で、例えば抗PD-L2抗体力価が最高であるときに、抗体産生細胞を対象から得ることができ、標準的技術、例えばKohlerおよびMilstein（1975）Nature 256：495-497により最初に報告されたハイブリドーマ技術（また、Brownら（1981）J. Immunol. 127：539-46、Brownら（1980）J. Biol. Chem. 255：4980-83、Yehら（1976）Proc. Natl. Acad. Sci. 76：2927-31、およびYehら（1982）Int. J. Cancer 29：269-75も参照）、さらに最近のヒトB細胞ハイブリドーマ技術（Kozborら（1983）Immunol. Today 4：72）、EBV-ハイブリドーマ技術（Coleら（1985）Monoclonal Antibodies and Cancer Therapy、アラン R. リス、インコーポレイテッド、77-96頁）またはトリオーマ技術によるモノクローナル抗体の製造に使用することができる。モノクローナル抗体ハイブリドーマ製造技術は公知である（一般的には、Kenneth, R. H.、Monocl 40

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onal Antibodies: A New Dimension In Biological Analyses、プレナム・パブリッシング・コーポレーション、ニューヨーク、ニューヨーク(1980)、Lerner, E. A. (1981) Yale J. Biol. Med. 54:387-402、Gefter, M. L. ら(1977) Somatic Cell Genet. 3:231-36 参照)。簡単に述べると、不死細胞系(典型的にはミエローマ)を、上記要領でPD-L2免疫原で免疫した哺乳類由来のリンパ球(典型的には脾臓細胞)に融合させ、生成したハイブリドーマ細胞の培養上清をスクリーニングして、PD-L2に結合するモノクローナル抗体を産生するハイブリドーマを同定する。

【0165】

リンパ球および不死化細胞系の融合に用いられる多くの公知プロトコルのいずれかを、抗PD-L2モノクローナル抗体産生目的に適用することができる(例えば、Galfré, G. ら(1977) Nature 266:55052、Gefter ら(1977) 前出、Lerner (1981) 前出、Kenneth (1980) 前出参照)。さらに、当業者にとって、同様に有用である上記方法の多くの変法があることは明らかである。典型的には、不死細胞系(例、骨髄種細胞系)は、リンパ球と同じ種の哺乳類に由来する。例えば、ネズミハイブリドーマは、本発明の免疫原性調合物により免疫化したマウスからのリンパ球を不死化マウス細胞系と融合させることにより製造され得る。好ましい不死細胞系は、ヒポキサンチン、アミノプテリンおよびチミジンを含む培養培地(「HAT培地」)に感受性を示すマウスミエローマ細胞系である。若干の骨髄種細胞系を標準的技術による融合相手として使用することができ、例えばP3-NS1/1-Ag4-1、P3-x63-Ag8.653またはSp2/O-Ag14骨髄種系がある。これらの骨髄種系は、メリーランド、ロックビルのアメリカン・タイプ・カルチャー・コレクション(ATCC)から入手可能である。典型的には、HAT感受性マウス骨髄種細胞を、ポリエチレングリコール(「PEG」)を用いてマウス脾臓細胞に融合させる。次いで、融合から生成したハイブリドーマ細胞はHAT培地を用いて選択し、この場合、非融合および非生産的融合骨髄種細胞は死滅する(非融合脾臓細胞は、形質転換されていないため数日後に死滅する)。本発明のモノクローナル抗体を産生するハイブリドーマ細胞は、例えば標準的なELISAアッセイを用いて、PD-L2分子と結合する抗体についてハイブリドーマ培養上清をスクリーニングすることにより検出される。

PD-L2に結合する抗体のさらなる製造方法を実施例4および5において説明する。

【0166】

モノクローナル抗体分泌性ハイブリドーマ製造のための別法として、PD-L2を伴う組換えコンビナトリアル免疫グロブリンライブラリー(例、抗体ファージディスプレイライブラリー)のスクリーニングによって、PD-L2ポリペプチドと結合する免疫グロブリンライブラリーのメンバーを単離することにより、モノクローナル抗PD-L2抗体を同定および単離することができる。ファージディスプレイライブラリーを作成し、スクリーニングするキットは市販されている(例えば、ファルマシア・レコンビナント・ファージ・アンチボディー・システム、カタログ番号27-9400-01、およびストラタジーンSurfZAP(商標)ファージ・ディスプレイ・キット、カタログ番号240612)。さらに、抗体ディスプレイライブラリーの作成およびスクリーニングにおいて特に使用しやすい方法および試薬の例は、例えばLadner ら、米国特許第5223409号、Kang ら、国際公開番号WO92/18619、Dower ら、国際公開番号WO91/17271、Winter ら、国際公開WO92/20791、Markland ら、国際公開番号WO92/15679、Breitling ら、国際公開WO93/01288、McCafferty ら、国際公開番号WO92/01047、Garrard ら、国際公開番号WO92/09690、Ladner ら、国際公開番号WO90/02809、Fuchs ら(1991) Biotechnology (NY) 9:1369-1372、Hay ら(1992) Hum. Antibod. Hybridomas 3:81-85、Huse ら(1989) Science 246:1275-1281、G

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r i f f i t h s ら (1993) E M B O J. 12:725-734、Hawkins ら (1992) J. Mol. Biol. 226:889-896、Clarkson ら (1990) Nature 352:624-628、Gram ら (1992) Proc. Natl. Acad. Sci. USA 89:3576-3580、Garrard ら (1991) Biotechnology (NY) 9:1373-1377、Hoogenboom ら (1991) Nucleic Acids Res. 19:4133-4137、Barbas ら (1991) Proc. Natl. Acad. Sci. USA 88:7978-7982、および McCafferty ら (1990) Nature 348:552-554において見出され得る。

【0167】

さらに、組換え抗PD-L2抗体、例えばキメラおよびヒト化モノクローナル抗体は、ヒトおよび非ヒト部分の両方を含み、標準的組換えDNA技術を用いて製造され得るものであり、本発明の範囲内に包含される。上記キメラおよびヒト化モノクローナル抗体は、例えばRobinson ら、国際特許公開PCT/US86/02269、Akira ら、欧州特許出願第184187号、Taniguchi, M.、欧州特許出願第171496号、Morrison ら、欧州特許出願第173494号、Neuberger ら、PCT出願WO86/01533、Cabilly ら、米国特許第4816567号、Cabilly ら、欧州特許出願第125023号、Better ら (1988) Science 240:1041-1043、Liu ら (1987) Proc. Natl. Acad. Sci. USA 84:3439-3443、Liu ら (1987) J. Immunol. 139:3521-3526、Sun ら (1987) Proc. Natl. Acad. Sci. 84:214-218、Nishimura ら (1987) Cancer Res. 47:999-1005、Wood ら (1985) Nature 314:446-449、およびShaw ら (1988) J. Natl. Cancer Inst. 80:1553-1559、Morrison, S. L. (1985) Science 229:1202-1207、Oi ら (1986) Biotechniques 4:214、Winter 米国特許5225539、Jones ら (1986) Nature 321:552-525、Verhoeyan ら (1988) Science 239:1534、およびBeidler ら (1988) J. Immunol. 141:4053-4060に記載された方法を用いることにより、当該分野で公知の組換えDNA技術により製造され得る。

【0168】

抗PD-L2抗体(例、モノクローナル抗体)を用いて、標準的技術、例えばアフィニティークロマトグラフィーまたは免疫沈降によりPD-L2ポリペプチドを単離することができる。抗PD-L2抗体により、細胞からの天然PD-L2ポリペプチド、および宿主細胞において発現される遺伝的組換えにより製造されたPD-L2ポリペプチドの精製は容易になり得る。そのうえ、抗PD-L2抗体を用いて、PD-L2ポリペプチドを検出(例えば、細胞溶解物または細胞上清において)することができる。抗PD-L2抗体を臨床試験手順の一部として診断的に用いて、組織中のポリペプチドレベルをモニターすることができ、例えば、特定の治療の有効性を調べることができる。検出可能な物質に抗体をカップリング(すなわち、物理的結合)させることにより検出は容易になり得る。従って、1の具体例において、本発明の抗PD-L2抗体は、検出可能な物質により標識される。検出可能な物質の例には、様々な酵素、補欠分子族、蛍光物質、ルミネセンス(発光)物質および放射性物質がある。適当な酵素の例には、セイヨウワサビペルオキシダーゼ、アルカリ性ホスファターゼ、 β -ガラクトシダーゼまたはアセチルコリンエステラーゼがあり、適当な補欠分子族複合体の例にはストレプトアビジン/ビオチンおよびアビジン/ビオチンがあり、適当な蛍光性物質の例には、ウンベリフェロン、フルオレセイン、フルオレセインイソチオシアネート、ローダミン、ジクロロトリアジニルアミンフルオレセイン、ダンシルクロリドまたはフィコエリスリンがあり、ルミネセンス物質の例にはルミノールがあり、そして適当な放射性物質の例には ^{125}I 、 ^{131}I 、 ^{35}S お

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よび³ Hがある。

【0169】

I I I. 組換え発現ベクターおよび宿主細胞

本発明のもう1つの態様は、PD-L2ファミリーの蛋白（またはその一部分）をコードする核酸分子を含むベクター、好ましくは発現ベクターに関する。ここで使用されている「ベクター」の語は、それに結合されている別の核酸を輸送し得る核酸分子を包含する。ベクターの1のタイプは「プラスミド」であり、付加的DNAセグメントが連結され得る環状二本鎖DNAループを包含する。別のタイプのベクターはウイルスベクターであり、その場合、付加的DNAセグメントはウイルスゲノムに連結され得る。ある種のベクターは、それらが導入される宿主細胞において自己複製し得る（例えば、細菌性複製起点を有する細菌性ベクターおよびエピソード性哺乳類ベクター）。他のベクター（例えば、非エピソード性哺乳類ベクター）は、宿主細胞への導入時に宿主細胞のゲノムへ組込まれ、それによって宿主ゲノムと一緒に複製される。さらに、ある種のベクターは、それらが作動可能に結合されている遺伝子の発現を指令し得る。上記ベクターは、ここでは「発現ベクター」と称される。一般に、組換えDNA技術において有用な発現ベクターはプラスミド形態であることが多い。本明細書の場合、プラスミドがベクターの最も一般的に使用される形態であることから、「プラスミド」および「ベクター」は混用され得る。しかしながら、本発明は、かかる他の形態の発現ベクターであって同様の機能を果たすもの、例えばウイルスベクター（例えば、複製能欠損レトロウイルス、アデノウイルスおよびアデノ随伴ウイルス）を包含する。

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【0170】

本発明の組換え発現ベクターは、宿主細胞における核酸の発現に適した形態の核酸を含み、このことは、組換え発現ベクターが、発現に使用される宿主細胞に基いて選択された1つまたはそれ以上の調節配列を含むことを意味するもので、調節配列は、発現されるべき核酸配列に作動可能に連結されている。組換え発現ベクター内で、「作動可能に連結されている」は、興味の対象であるヌクレオチド配列が、ヌクレオチド配列の（例えば、インビトロ転写／翻訳系における、またはベクターが宿主細胞へ導入されたとき宿主細胞における）発現を可能にする状態で調節配列（複数も可）に連結されていることを意味する。「調節配列」の語は、プロモーター、エンハンサーおよび他の発現制御エレメント（例えば、ポリアデニル化シグナル）を包含する。上記調節配列は、例えば Go edd el（1990）Methods Enzymol. 185: 3-7に記載されている。調節配列には、多くのタイプの宿主細胞におけるヌクレオチド配列の構成的発現を指令するものおよびある種の宿主細胞においてのみヌクレオチド配列の発現を指令するもの（例、組織特異的調節配列）がある。当業者であれば、発現ベクターの設計は、形質転換される宿主細胞の選択、目的蛋白の発現レベルなどの因子により異なり得ることを認識するはずである。本発明の発現ベクターを宿主細胞に導入することにより、本明細書に記載されたように（例えば、PD-L2ファミリーの蛋白、変異形態のPD-L2蛋白、融合蛋白など）、核酸によりコードされる融合蛋白またはペプチドを包含する蛋白またはペプチドを得ることができる。

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【0171】

本発明の組換え発現ベクターは、原核生物または真核生物細胞におけるPD-L2ポリペプチドの発現を目的として設計され得る。例えば、PD-L2ポリペプチドは、細菌細胞、例えばエシェリシア・コリ（E. coli）、昆虫細胞（バキュロウイルス発現ベクターを用いる）、酵母細胞または哺乳類細胞において発現され得る。適当な宿主細胞については、前出のGo edd el（1990）でさらに検討されている。別法として、組換え発現ベクターは、例えばT7プロモーター調節配列およびT7ポリメラーゼを用いてインビトロで転写および翻訳され得る。

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【0172】

原核生物における蛋白の発現を、融合または非融合蛋白の発現を指令する構成的または誘導性プロモーターを含むベクターによりエシェリシア・コリ（E. coli）において行

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なうことが最も多い。融合ベクターは、その中にコードされた蛋白に若干のアミノ酸を、通常には組換え蛋白のアミノ末端に付加する。上記融合ベクターは、典型的には、1) 組換え蛋白の発現を高める、2) 組換え蛋白の溶解性を高める、および3) アフィニティー精製におけるリガンドとして作用することにより組換え蛋白の精製で有用である、といった3つの目的に適うものである。多くの場合、融合発現ベクターにおいて、蛋白分解性開裂部位が融合部分および組換え蛋白の接合点に導入されることにより、融合蛋白の精製後に組換え蛋白を融合部分から分離することが可能となる。上記酵素およびそれらの同族認識配列には、因子Xa、トロンピンおよびエンテロキナーゼがある。典型的融合発現ベクターには、pGEX (ファルマシア・バイオテク・インコーポレイテッド、Smith, D. B. および Johnson, K. S. (1988) Gene 67: 31-40)、

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pMAL (ニューイングランド・バイオラブズ、ベヴァリー、マサチューセッツ) および pRIT5 (ファルマシア、ピスカタウェイ、ニュージャージー) があり、それぞれグルタチオンS-トランスフェラーゼ (GST)、マルトース結合蛋白または蛋白Aを標的組換え蛋白に融合させる。精製融合蛋白は、PD-L2 活性アッセイ (例、下記で詳述されている直接アッセイまたは競争的アッセイ) に、あるいは例えばPD-L2 ポリペプチドに特異的な抗体の生成に使用され得る。好ましい具体例において、本発明のレトロウイルス発現ベクター中で発現されたPD-L2 融合蛋白を用いて骨髓細胞に感染させ、次いで、それを放射線照射されたレシピエント中に移植する。次いで、十分時間 (例えば、6ヶ月) 経過後、対象レシピエントの病状を調べる。

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【0173】

適当な誘導性非融合エシェリシア・コリ (E. coli) 発現ベクターの例には、pTrc (Amannら (1988) Gene 69: 301-315) および pET11d (Studierら (1990) Methods Enzymol. 185: 60-89) がある。pTrcベクターからの標的遺伝子発現は、ハイブリッドtrp-lac融合プロモーターからの宿主RNAポリメラーゼ転写に依存する。pET11dベクターからの標的遺伝子発現は、共発現されたウイルス性RNAポリメラーゼ (T7gn1) が介在するT7gn10-lac融合プロモーターからの転写に依存する。このウイルス性ポリメラーゼは、lacUV5プロモーターの転写制御下T7gn1遺伝子をもつ内在プロファージから宿主株BL21 (DE3) またはHMS174 (DE3) により供給される。

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【0174】

エシェリシア・コリ (E. coli) における組換え蛋白発現を最大化させる1の戦略は、蛋白分解的に組換え蛋白を開裂する能力が損なわれた宿主細菌において蛋白を発現させることである (Gottesman, S. (1990) Methods Enzymol. 185: 119-128)。別の戦略は、発現ベクターへ挿入される核酸の核酸配列を改変することにより、各アミノ酸に関する個々のコドンが優先的にエシェリシア・コリ (E. coli) で利用されるものとなるようにする方法である (Wadaら (1992) Nucleic Acids Res. 20: 2111-2118)。本発明の核酸配列の上記改変は、標準的なDNA合成技術により実施され得る。

【0175】

もう1つの具体例において、PD-L2 発現ベクターは、酵母発現ベクターである。酵母S. cerevisiaeにおいて発現するベクターの例には、pYepSec1 (Baldariら (1987) EMBO J. 6: 229-234)、pMFa (KurjanおよびHerskowitz (1982) Cell 30: 933-943)、pJRY88 (Schultzら (1987) Gene 54: 113-123)、pYES2 (インビトロゲン・コーポレーション、サンディエゴ、カリフォルニア) およびpicZ (インビトロゲン・コーポレーション、サンディエゴ、カリフォルニア) がある。

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【0176】

別法として、バキュロウイルス発現ベクターを用いて昆虫細胞においてPD-L2 ポリペプチドを発現させることができる。培養昆虫細胞 (例えば、Sf9細胞) における蛋白発

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現に利用可能なバキュロウイルスベクターには、pAcシリーズ (Smithら (1983) Mol. Cell Biol. 3: 2156-2165) および pVLシリーズ (Lucklow, V. A. および Summers, M. D. (1989) Virology 170: 31-39) がある。

【0177】

さらに別の具体例において、本発明の核酸は、哺乳類発現ベクターを用いて哺乳類細胞で発現される。哺乳類発現ベクターの例には、pMex-NeoI、pCDM8 (Seed, B. (1987) Nature 329: 840) および pMT2PC (Kaufmanら (1987) EMBO J. 6: 187-195) がある。哺乳類細胞で使用する場合には、発現ベクターの制御機能がウイルス調節エレメントにより提供されることが多い。10
例えば、一般的に使用されるプロモーターは、ポリオーマ、アデノウイルス2、サイトメガロウイルスおよびシミアンウイルス40から誘導される。原核生物および真核生物の両細胞に関する他の適当な発現系については、Sambrook, J. ら、Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory、コールドスプリングハーバー・ラボラトリー・プレス、コールドスプリングハーバー、ニューヨーク (1989) の16および17章参照。

【0178】

もう1つの具体例において、組換え哺乳類発現ベクターは、特定の細胞タイプにおいて優先的に核酸の発現を指令し得る (例、組織特異的調節エレメントは核酸の発現に使用される)。組織特異的調節エレメントは当業界では公知である。適当な組織特異的プロモーターの非限定的な例には、アルブミンプロモーター (肝臓特異的、Pinkertら (1987) Genes Dev. 1: 268-277)、リンパ特異的プロモーター (Calameおよび Eaton (1988) Adv. Immunol. 43: 235-275)、特にT細胞受容体プロモーター (Winotoおよび Baltimore (1989) EMBO J. 8: 729-733) および免疫グロブリン (Banerjiら (1983) Cell 33: 729-740、Queenおよび Baltimore (1983) Cell 33: 741-748)、ニューロン特異的プロモーター (例、ニューロフィラメントプロモーター、Byrneおよび Ruddie (1989) Proc. Natl. Acad. Sci. USA 86: 5473-5477)、膵臓特異的プロモーター (Edlundら (1985) Science 230: 912-916)、および乳腺特異的プロモーター (例、乳漿プロモーター、米国特許第4873316号およびヨーロッパ出願公開第264166号) がある。また、発達の調節 (developmentally-regulated) プロモーター、例えばネズミホックス (hox) プロモーター (Kesselおよび Gruss (1990) Science 249: 374-379) およびα-フェトプロテインプロモーター (Campesおよび Tilghman (1989) Genes Dev. 3: 537-546) も包含される。20

【0179】

さらに本発明は、アンチセンス方向で発現ベクター中にクローン化された本発明のDNA分子を含む組換え発現ベクターを提供する。すなわち、DNA分子は、PD-L2 mRNA 40
NAに対してアンチセンスであるRNA分子の発現 (DNA分子の転写による) を可能にする形で調節配列に作動可能に結合されている。アンチセンス方向でクローン化された核酸に作動可能に結合された調節配列であって、様々な細胞タイプにおけるアンチセンスRNA分子の連続発現を指令するもの、例えばウイルスプロモーターおよび/またはエンハンサー、あるいはアンチセンスRNAの構成的、組織特異的または細胞型特異的発現を指令する調節配列が選択され得る。アンチセンス発現ベクターは、アンチセンス核酸が高効率調節領域の制御下で製造される組換えプラスミド、ファージミドまたは弱毒化ウイルス形態であってもよく、その活性はベクターが導入されている細胞タイプにより決定され得る。アンチセンス遺伝子を用いた遺伝子発現の調節を検討したものについては、Weintraub, H. ら (1986) "Antisense RNA as a molecu 50

ar tool for genetic analysis” Reviews-Trends in Genetics、第1巻(1)を参照。

【0180】

本発明のもう1つの態様は、本発明のPD-L2核酸分子、例えば、組換え発現ベクター中のPD-L2核酸分子、あるいは宿主細胞のゲノムの特異部位中に相同組換えが起こるようにする配列を含むPD-L2核酸分子が導入されている宿主細胞に関するものである。「宿主細胞」および「組換え宿主細胞」の語は、本明細書で混用される。上記の語は特定対象細胞だけでなくかかる細胞の子孫または潜在的子孫も包含するものと理解すべきである。突然変異または環境的影響故にある種の修飾が後続の世代で行われ得るため、上記子孫は事実上親細胞と同一ではないかもしれないが、依然としてここで使用されている語の範囲内に包含される。

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【0181】

宿主細胞は原核生物または真核生物であり得る。例えば、PD-L2ポリペプチドは、細菌細胞、例えばエシェリシア・コリ(E. coli)、昆虫細胞、酵母または哺乳類細胞(例えばチャイニーズハムスター卵巣細胞(CHO)またはCOS細胞)で発現され得る。他の適当な宿主細胞も当業者に公知である。

【0182】

ベクターDNAは、慣用的形質転換またはトランスフェクション技術により原核生物または真核生物細胞中に導入され得る。ここで使用されている「形質転換」および「トランスフェクション」の語は、リン酸カルシウムまたは塩化カルシウム共沈澱、DEAE-デキストラン介在トランスフェクション、リポフェクション(脂質小胞を介する遺伝子導入)または電気穿孔を含め、外来核酸(例、DNA)を宿主細胞へ導入するための様々な当業界公認の技術を包含する。宿主細胞の適当な形質転換またはトランスフェクション方法は、Sambrookら(Molecular Cloning: A Laboratory Manual, 2nd, ed., Cold Spring Harbor Laboratory, コールドスプリングハーバー・ラボラトリー・プレス、コールドスプリングハーバー、ニューヨーク、1989)、および他の研究室マニュアルに見出され得る。

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【0183】

哺乳類細胞の安定したトランスフェクションを行うために、使用される発現ベクターおよびトランスフェクション技術に応じて、細胞の小フラクションのみが外来DNAをそれらのゲノム中に組み込まれうるということが知られている。これらの成分を同定および選択するために、選択マーカー(例、抗生物質に対する耐性)をコードする遺伝子を、一般的に興味の対象である遺伝子と一緒に宿主細胞へ導入する。好ましい選択マーカーには、薬剤、例えばG418、ヒグロマイシンおよびメトトレキサートに対する耐性を付与するものがある。選択マーカーをコードする核酸を、PD-L2ポリペプチドをコードするものと同じベクターで宿主細胞へ導入することができ、あるいは別々のベクターで導入することができる。導入された核酸により安定してトランスフェクションされた細胞を薬剤選別により同定することができる(例えば、選択マーカー遺伝子が組込まれた細胞は生残り、他の細胞は死滅する)。

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【0184】

本発明の宿主細胞、例えば培養された原核生物または真核生物宿主細胞を、PD-L2ポリペプチドの製造(すなわち発現)に使用することができる。従って、本発明は、さらに本発明の宿主細胞を用いたPD-L2ポリペプチドの製造方法を提供する。1の具体例において、PD-L2ポリペプチドが製造されるように、本発明の宿主細胞(PD-L2ポリペプチドをコードする組換え発現ベクターが導入された)を適当な培養培地で培養することを含む。別の具体例において、前記方法はさらに、培地または宿主細胞からのPD-L2ポリペプチドの単離を含む。

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【0185】

ある種の宿主細胞を用いて、ヒト以外のトランスジェニック動物を製造することもできる。例えば、1の具体例において、宿主細胞は、PD-L2コーディング配列が導入された

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受精卵母細胞または胚性幹細胞である。次いで、上記宿主細胞を用いることにより、外来性PD-L2配列がゲノムに導入された非ヒトトランスジェニック動物あるいは内在性PD-L2配列が改変された相同的組換え動物が製造され得る。上記動物は、PD-L2ポリペプチドの機能および／または活性を研究ならびにPD-L2活性のモジュレーターを同定および／または評価するのに有用である。ここで使用されている「トランスジェニック動物」は、ヒト以外の動物、好ましくは哺乳類、さらに好ましくはげっ歯動物、例えばラットまたはマウスであり、この場合動物の細胞の1個またはそれ以上が導入遺伝子（トランスジーン）を含んでいる。トランスジェニック動物の他の例には、ヒト以外の霊長類、ヒツジ、イヌ、雌牛、ヤギ、ニワトリ、両生類などがある。導入遺伝子は、トランスジェニック動物が発生する細胞のゲノムに組込まれており、成熟動物のゲノムに残存している外性DNAであり、それによってトランスジェニック動物の1種またはそれ以上の細胞タイプまたは組織におけるコードされた遺伝子産物の発現が指令される。ここで使用されている「相同的組換え動物」は、ヒト以外の動物、好ましくは哺乳類、さらに好ましくはマウスであり、この場合内在性PD-L2遺伝子は、動物の発生前に、動物の1の細胞、例えば動物の胚性細胞へ導入された内在性遺伝子および外来性DNA分子間の相同的組換えにより改変されている。

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【0186】

トランスジェニック動物は、例えば、マイクロインジェクション、レトロウイルス感染により、PD-L2をコードする核酸分子を受精卵母細胞の雄性前核へ導入し、偽妊娠した雌フォスター（養育）動物において卵母細胞を発達させることによりトランスジェニック動物を製造することができる。配列番号1または4のPD-L2 cDNA配列を、非ヒト動物のゲノムへ導入遺伝子として導入することができる。別法として、ヒトPD-L2遺伝子の非ヒトホモログ、例えばマウスまたはラットPD-L2遺伝子を導入遺伝子として使用することができる。別法として、PD-L2遺伝子ホモログ、例えば別のPD-L2ファミリーのメンバーを、配列番号1、3、4または6のPD-L2 cDNA配列とのハイブリダイゼーションに基いて単離し（さらに上記サブセクションIで詳記されている）、導入遺伝子として使用することができる。また、イントロン配列およびポリアダニル化シグナルを導入遺伝子に含ませることにより、導入遺伝子の発現効率を高めることができる。組織特異的調節配列（複数も可）は、PD-L2導入遺伝子に作動可能に連結されることにより、特定細胞に対してPD-L2ポリペプチドの発現を指令し得る。胚マニピュレーションおよびマイクロインジェクションによるトランスジェニック動物、特に例えばマウスといった動物の製造方法は、当業界では既に慣用的なものになっており、例えば、両方ともLederらによる米国特許第4736866号および同4870009号、Wagnerらによる米国特許第4873191号およびHogan, B. Manipulating the Mouse Embryo（コールドスプリングハーバー・ラボラトリー・プレス、コールドスプリングハーバー、ニューヨーク、1986）で報告されている。他のトランスジェニック動物の製造には類似方法も使用される。トランスジェニック創始動物は、そのゲノムにおけるPD-L2導入遺伝子の存在および／または動物の組織または細胞におけるPD-L2 mRNAの発現に基いて同定され得る。次いで、トランスジェニック創始動物を用いることにより、導入遺伝子を有する動物が追加的に産み出され得る。さらに、PD-L2ポリペプチドをコードする導入遺伝子をもつトランスジェニック動物は、他の導入遺伝子をもつ他のトランスジェニック動物へとさらに繁殖され得る。

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【0187】

相同組換え動物を作製するために、PD-L2遺伝子の少なくとも一部分を含むもので、そこに欠失、付加または置換が導入されていることによりPD-L2遺伝子が改変、例えば機能的に崩壊されているベクターを製造する。PD-L2遺伝子はヒト遺伝子（例えば、配列番号1または3）であり得るが、さらに好ましくはヒトPD-L2遺伝子の非ヒト相同体である（例えば、配列番号1、3、4または6のヌクレオチド配列とのストリンジェントなハイブリダイゼーションにより単離されたcDNA）。例えば、マウスPD-L

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2 遺伝子（例えば、配列番号：3 または配列番号：6 の cDNA）を用いることにより、マウスゲノムにおいて内在性 PD-L2 遺伝子を改変するのに適した相同組換えベクターを構築することができる。好ましい具体例において、相同組換えが起こると内在性 PD-L2 遺伝子が機能崩壊されるようにベクターが設計される（すなわち、もはや機能性蛋白をコードしない、「ノックアウト」ベクターとも称される）。別法として、相同組換えが起こると内在性 PD-L2 遺伝子は突然変異誘発または他の形で改変されるが、依然として機能性蛋白をコードするようにベクターが設計され得る（例えば、上流調節領域を改変することにより、内在性 PD-L2 ポリペプチドの発現が改変され得る）。相同的組換えベクターでは、PD-L2 遺伝子の改変部分の 5' および 3' 両末端に PD-L2 遺伝子の付加的核酸配列が隣接していることにより、ベクターが担持する内在性 PD-L2 遺伝子と胚性幹細胞中の内在性 PD-L2 遺伝子との間で相同組換えが行われ得る。付加的な隣接 PD-L2 核酸配列は、内在性遺伝子との有効な相同的組換えにとって十分な長さを有する。典型的には、数千ベースのフランキング DNA（5' および 3' の両末端）がベクターに含まれている（例えば、相同的組換えベクターの記載については、Thomas, K. R. および Capecechi, M. R. (1987) *Cell* 51: 503 参照）。ベクターは胚性幹細胞系中に（例えば、電気穿孔により）導入され、導入された PD-L2 遺伝子が内在性 PD-L2 遺伝子と相同的に組換えられている細胞が選択される（例えば、Li, E. ら (1982) *Cell* 69: 915 参照）。次いで、選択された細胞は動物（例、マウス）の未分化胚芽細胞中に注入され、凝集キメラが形成される（例えば、Bradley, A., *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, E. J. 編 (IRL, オクスフォード, 1987) 113-152 頁参照）。次いで、キメラ胚を適当な偽妊娠雌養育（フォスター）動物へ移植し、胚を分娩させ得る。生殖細胞中に相同的組換え DNA をもつ子孫を用いることにより、動物の全細胞が導入遺伝子の生殖系列伝達により相同的組換え DNA を含む動物が産み出され得る。相同的組換えベクターおよび相同的組換え動物の構築方法は、Bradley, A. (1991) *Curr. Opin. Biotechnol.* 2: 823-829 および Le Mouelllec らによる PCT 国際公開第 WO90/11354 号、Smithies らによる同第 WO91/01140 号、Zijlstra らによる同第 WO92/0968 号、および Berns らによる同第 WO93/04169 号に詳述されている。

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【0188】

もう 1 つの具体例において、導入遺伝子の発現調節を可能にすべく選択された系を含むトランスジェニック非ヒト動物が製造され得る。かかる系の一例は、バクテリオファージ P1 の cre/loxP リコンビナーゼ系である。cre/loxP リコンビナーゼ系の記述については、例えば Lakso ら (1992) *Proc. Natl. Acad. Sci. USA* 89: 6232-6236 参照。リコンビナーゼ系の別の例は、*Saccharomyces cerevisiae* の FLP リコンビナーゼ系である (O'Gorman ら (1991) *Science* 251: 1351-1355)。cre/loxP リコンビナーゼ系を導入遺伝子の発現調節に使用する場合、Cre リコンビナーゼおよび選択された蛋白の両方をコードする導入遺伝子を含む動物が要求される。上記動物は、例えば、一方は選択された蛋白をコードする導入遺伝子を含み、他方はリコンビナーゼをコードする導入遺伝子を含む 2 種のトランスジェニック動物を交配させることにより、「二重」トランスジェニック動物の構築により提供され得る。

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【0189】

また、ここに記載されているヒト以外のトランスジェニック動物のクローンを、Wilmut, I. ら (1997) *Nature* 385: 810-813 および PCT 国際公開第 WO97/07668 号および同第 WO97/07669 号記載の方法により製造することができる。簡単に述べると、トランスジェニック動物からの細胞、例えば体細胞を単離し、成長周期を脱して G₀ 期へ入るように誘導することができる。次いで、例えば、電気パルスの使用により、静止細胞を、静止細胞が単離されたのと同種の動物に由来する除核

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卵母細胞と融合させることができる。次いで、再構築された卵母細胞を培養して桑実胚または芽細胞にまで発達するようにし、次いで、偽妊娠した雌フォスター動物に移す。この雌フォスター動物から生まれた子孫は、細胞、例えば体細胞が単離された動物のクローンとなる。

【0190】

I V. 医薬組成物

本発明のPD-L2分子、例えば、PD-L2核酸分子、PD-L2ポリペプチドのフラグメントおよび抗PD-L2抗体（本明細書において「活性化合物」または「モジュレーション作用剤」ともいう）を、投与に適した医薬組成物に含有させることができる。上記組成物は、典型的には核酸分子、蛋白または抗体および医薬的に許容される担体を含む。ここで使用されている「医薬的に許容される担体」の語は、医薬投与に適合したあらゆる溶媒、分散媒、コーティング、抗細菌および抗真菌剤、等張および吸収遅延剤などをすべて包含するものとする。医薬活性物質に関する上記媒質および薬剤の使用については当該分野では公知である。慣用的媒質または薬剤が活性化合物と不適合性である場合を除いて、組成物中におけるその使用が考えられる。補足的な活性化合物を組成物中に含有させることもできる。

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【0191】

本発明医薬組成物は、意図されたその投与経路と適合するように製剤化される。投与経路の例には、非経口、例えば静脈内、皮内、皮下、経口（例、吸入）、経皮（局所）、経粘膜および直腸投与がある。非経口、皮内または皮下適用に使用される溶液または懸濁液は、次の成分：滅菌希釈剤、例えば注射用水、食塩水、不揮発性油、ポリエチレングリコール、グリセリン、プロピレングリコールまたは他の合成溶媒、抗菌剤、例えばベンジルアルコールまたはメチルパラベン、酸化防止剤、例えばアスコルビン酸または重亜硫酸ナトリウム、キレート剤、例えばエチレンジアミン四酢酸、緩衝液、例えばアセテート、シトレートまたはホスフェートおよび等張性調節剤、例えば塩化ナトリウムまたはデキストロースを含み得る。酸または塩基、例えば塩酸または水酸化ナトリウムによりpHを調節することができる。非経口製剤を、ガラスまたはプラスチック製のアンプル、使い捨て注射器またはマルチブロードーズバイアル（multiple dose vial）に封入することができる。

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【0192】

注射可能用途に適した医薬組成物には、滅菌水溶液（水溶性の場合）または分散液および滅菌注射可能溶液または分散液をその場で製造するための滅菌粉末が含まれ得る。静脈内投与の場合、適当な担体には、生理食塩水、静菌水、クレモフォーEL（商標）（BASF、パーシッパニー、ニュージャージー）またはリン酸緩衝食塩水（PBS）がある。どの場合も、組成物は無菌状態でなくてはならず、容易に注射できる程度には流動性であるべきである。組成物は、製造および貯蔵条件下で安定していなくてはならず、微生物、例えば細菌および真菌の汚染作用に対して保存されなければならない。担体は、例えば水、エタノール、ポリオール（例えば、グリセリン、プロピレングリコールおよび液体ポリエチレングリコールなど）を含む溶媒または分散液媒質およびその適当な混合物であり得る。例えば、レシチンのごときコーティングの使用により、分散液の場合に必要なとされる粒子サイズの維持により、そして界面活性剤の使用により、適切な流動性が維持され得る。微生物作用の阻止は、様々な抗菌および抗真菌剤、例えばパラベン、クロロブタノール、フェノール、アスコルビン酸、チメロサルなどにより達成され得る。多くの場合、組成物中に等張剤、例えば糖類、ポリアルコール類、例えばマンニトール、ソルビトール、塩化ナトリウムを含むのが好ましい。注射可能組成物の長期間吸収は、吸収を遅延させる薬剤、例えばモノステアリン酸アルミニウムおよびゼラチンを組成物に含ませることにより達成され得る。必要ならば上記成分の1つまたは組み合わせとともに所望量の適当な溶媒に活性化合物（例えば、PD-L2核酸分子、PD-L2ポリペプチドのフラグメント、抗PD-L2抗体、または抗PD-L2抗体と抗PD-1抗体との組み合わせ）を含ませて、次いで、濾過滅菌することにより、滅菌注射可能溶液を調製することができ

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る。一般的には、塩基性分散媒質および上記で列挙されたものから必要とされる他の成分を含む滅菌賦形剤中に活性化合物を含ませることにより分散液を製造する。滅菌注射可能溶液製造用滅菌粉末の場合、好ましい製造方法は真空乾燥および凍結乾燥であり、予め滅菌濾過しておいたその溶液から有効成分に加えて所望の追加的成分からなる粉末を得ることができる。

【0193】

一般的には、経口組成物は不活性希釈剤または可食性担体を含む。それらをゼラチンカプセル中に封入、あるいは錠剤に圧縮成型することができる。経口治療投与を目的とする場合、活性化合物は賦形剤と混合され、錠剤、トローチまたはカプセル形態で使用され得る。口内洗浄剤として使用される流動担体を用いて経口組成物を製造することもでき、流動担体中の化合物は経口適用され、スウィッシュ（s w i s h）され、吐き出されるかまたは嚥下される。医薬的に適合し得る結合剤、および／またはアジュバント物質は、組成物の一部として含まれ得る。錠剤、丸薬、カプセル、トローチなどは、下記成分のいずれか、または似た性質の化合物：結合剤、例えば微晶性セルロース、トラガカントゴムまたはゼラチン、賦形剤、例えば澱粉または乳糖、崩壊剤、例えばアルギン酸、プリモゲルまたはコーンスターチ、滑沢剤、例えばステアリン酸マグネシウムまたはステロテス（S t e r o t e s）、滑剤、例えばコロイド状二酸化珪素、甘味剤、例えばしょ糖またはサッカリン、または香味料、例えばペパーミント、サリチル酸メチルまたはオレンジ香味料を含み得る。

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【0194】

吸入投与の場合、化合物は、適当な推進剤、例えば気体、例えば二酸化炭素を含む加圧容器またはディスペンサーからのエアゾールスプレー、またはネブライザーの形態でデリバリーされる。

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【0195】

全身投与を経粘膜または経皮手段で行なってもよい。経粘膜または経皮投与の場合、浸透させるべきバリアーに適した浸透剤を製剤中に使用する。上記浸透剤は一般的に当業界では公知であり、例えば、経粘膜投与の場合、デタージェント、胆汁酸塩、およびフシジン酸誘導体を包含する。鼻用スプレーまたは坐薬の使用により経粘膜投与を行なうことができる。経皮投与の場合、当該分野で一般的に知られているように、活性化合物を軟膏、膏薬、ゲルまたはクリームとして製剤化する。

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化合物を直腸デリバリー用の坐薬（例、慣用的坐薬基剤、例えばカカオバターおよび他のグリセリド類による）または停留浣腸形態として調合することもできる。

【0196】

1の具体例において、活性化合物は、体内からの急速な排出から化合物を保護する担体とともに調合され、例えばインプラントおよびマイクロカプセル封入デリバリー系を包含する除放性製剤がある。生物分解性、生物適合性ポリマー、例えばエチレンビニルアセテート、ポリ無水物、ポリグリコール酸、コラーゲン、ポリオルトエステルおよびポリ乳酸を使用することができる。上記製剤の製造方法は、当業者に明らかであろう。これらの材料を、アルザ・コーポレーションおよびノヴァ・ファーマシューティカルズ、インコーポレイテッドから購入することもできる。リポソーム懸濁液（ウイルス抗原に対するモノクローナル抗体により感染した細胞に標的化されたリポソームを含む）を医薬的に許容され得る担体として使用することもできる。例えば米国特許第4522811号に記載されたような当業者に公知の方法に従ってこれらを調合することができる。

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投与し易く用量を均一にするために単位用量形態で経口または非経口組成物を製剤するのは特に有利である。本明細書で使用されている単位用量形態とは、処置される対象にとって単位用量として適する物理的に独立した単位を包含しており、各単位は必要とされる医薬用担体と共に所望の治療効果を生じるように計算された予め定められた量の活性化合物を含むものをいう。本発明の単位用量形態に関する詳細は、活性化合物特有の特徴および達成すべき特定治療効果、および個体の処置を目的としたかかる活性化合物調合の当該分野における制限に直接的に左右される。

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【0197】

上記化合物の毒性および治療効率を細胞培養物または実験動物における標準製薬方法により測定して、例えば、LD50（集団の50％に対する致死用量）およびED50（集団の50％における治療有効量）を決定することができる。毒性効果と治療効果との間の用量比が治療指数であり、LD50／ED50比として表され得る。大きな治療指数を呈する化合物が好ましい。毒性副作用を呈する化合物を使用してもよい場合、かかる化合物を罹病組織の部位に標的化させるデリバリーシステムを設計して、未感染細胞に対する潜在的ダメージを最小化し、そのことにより副作用を減らすように注意を払うべきである。細胞培養アッセイおよび動物試験から得られたデータを、ヒトで使用するための範囲の用量を製剤化するために用いることができる。好ましくは、かかる化合物の用量は、毒性を全くまたはほとんど伴わないED50値を包含する循環濃度の範囲内である。使用される用量形態および使用される投与経路に応じて用量をこの範囲内で変化させてもよい。本発明方法で使用する化合物の場合、先ず、細胞培養アッセイから治療有効量を評価することができる。用量を動物モデルで製剤化することにより、細胞培養で測定されたIC50（すなわち、徴候の半最大阻害を達成する試験化合物の濃度）を包含する循環血漿濃度範囲が得られる。上記情報を使用することにより、ヒトにおける有用な用量をより正確に測定することができる。血漿レベルを、例えば、高速液体クロマトグラフィーにより測定することができる。

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【0198】

本明細書で定義するように、蛋白またはポリペプチドの治療上有効量（すなわち、有効用量）は、体重1kgあたり約0.001ないし30mg、好ましくは体重1kgあたり約0.01ないし25mg、より好ましくは体重1kgあたり約0.1ないし20mg、さらにより好ましくは体重1kgあたり約1ないし10mg、2ないし9mg、3ないし8mg、4ないし7mg、5ないし6mgの範囲である。疾病または疾患の重さ、以前受けていた治療、対象の一般的健康状態および／または年齢、ならびに存在する他の疾病（これらに限らない）を包含する特定の因子が、対象を有効に治療するために必要な容量に影響する可能性があることを、当業者は理解するであろう。そのうえ、治療上有効量の蛋白、ポリペプチド、あるいは抗体での対象の治療は、単一回の治療を包含しうるが、好ましくは一連の治療を包含しうる。

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【0199】

好ましい例において、体重1kgあたり約0.1ないし20mgの範囲の抗体、蛋白またはポリペプチドで、1週間に1回、約1ないし10週間にわたり、好ましくは2ないし8週間にわたり、より好ましくは約3ないし7週間、さらにより好ましくは約4、5または6週間にわたり対象を治療する。治療に使用される抗体、蛋白またはポリペプチドの有効量を個々の治療コースに応じて増加または減少させてもよいことも理解されよう。用量の変更は、本明細書記載の診断アッセイの結果によるものであり、該アッセイから明白なものとなる。

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【0200】

本発明は、PD-L2の発現または活性をモジュレーションする作用剤を包含する。例えば、作用剤は小型分子であってもよい。例えば、かかる小型分子は、ペプチド、ペプチド模倣物、アミノ酸、アミノ酸アナログ、ポリヌクレオチド、ポリヌクレオチドアナログ、ヌクレオチド、ヌクレオチドアナログ、1モルあたり約10000グラム未満の分子量を有する有機または無機化合物（すなわち、ヘテロ有機化合物および有機金属化合物を包含）、1モルあたり約5000グラム未満の分子量を有する有機または無機化合物、1モルあたり約1000グラム未満の分子量を有する有機または無機化合物、1モルあたり約500グラム未満の分子量を有する有機または無機化合物、ならびに塩、エステル、ならびに医薬上許容される形態のかかる化合物等であるが、これらに限らない。小型分子の適当な用量は当業者、獣医、または研究者の知識の範囲内の多くの因子に依存することが理解される。小型分子の用量は、例えば、治療すべき対象またはサンプルの同一性、サイズ、および状態に依存するであろうし、さらに、適用可能な場合には組成物が投与される経路

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、本発明の核酸またはポリペプチドにより得られる医師が望む小型分子の効果にも依存するであろう。

【0201】

典型的な用量は、対象またはサンプルの体重1 kgあたりミリグラムまたはマイクログラム量の小型分子（例えば、1 kgあたり約1マイクログラムないし約500ミリグラム、1 kgあたり約100マイクログラムないし約5ミリグラム、1 kgあたり約1マイクログラムないし約50マイクログラム）である。小型分子の用量は、モジュレーションすべき発現または活性に関する小型分子の有効性に依存することがさらに理解される。本明細書記載のアッセイを用いてかかる適当な用量を決定してもよい。1またはそれ以上のこれらの小型分子を動物（例えば、ヒト）に投与して本発明のポリペプチドまたは核酸の発現または活性をモジュレーションしようとする場合、医師、獣医、または研究者は、先ず比較的低用量を処方し、次いで、適当な応答が得られるまで用量を増加させてもよい。さらに、個々の動物対象に関する個々のレベルは、使用される個々の化合物の活性、対象の年齢、体重、一般的健康状態、性別および食事療法、投与期間、投与経路、排泄速度、併用薬剤、ならびにモジュレーションすべき発現または活性の程度を包含する種々の因子に依存するであろうことが理解される。

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【0202】

さらに、抗体（またはそのフラグメント）を、サイトトキシン、治療剤または放射性金属イオンのごとき治療部分に抱合させてもよい。サイトトキシンまたは細胞毒性作用剤は細胞に損傷を与える薬剤を包含する。例は、トクソール、サイトカラシンB、グラミシジンD、臭化エチジウム、エメチン、マイトマイシン、エトポサイド、テノポサイド、ビンクリスチン、ビンブラスチン、コルヒチン、ドキシソルビシン、ダウノルビシン、ジヒドロキシアントラシンジオン、ミトキサントロン、ミスラマイシン、アクチノマイシンD、1-デヒドロテストステロン、グルココルチコイド、ピロカイン、テトラカイン、リドカイン、プロパノロールおよびプロマイシン、ならびにそれらのアナログまたはホモログを包含する。治療剤は、アンチメタボライト（例えば、メトトレキサート、6-メルカプトプリン、6-チオグアニン、シタラビン、5-フルオロウラシルデカルバジン）、アルキル化剤（例えば、メクロレタミン、チオペアクロラムブシル、メルファラン、カルムスチン（BSNU）およびロムスチン（CCNU）、シクロソスファミド、ブスルファン、ジプロモマンニトール、ストレプトゾトシン、マイトマイシンC、および白金（II）cis-ジクロロジアミン（DDP）シスプラチン）、アンスラサイクリン（例えば、ダウノルビシン（以前はダウノマイシンと呼ばれた）およびドキシソルビシン）、抗生物質（例えば、ダクチノマイシン（以前はアクチノマイシンと呼ばれた）、ブレオマイシン、ミスラマイシン、およびアンスラマイシン（AMC））、ならびに抗分裂剤（例えば、ビンクリスチンおよびビンブラスチン）を包含するが、これらに限らない。

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【0203】

本発明の抱合体を用いてある種の生物学的応答を修飾することができ、その薬剤部分は古典的な化学療法剤に限らない。例えば、薬剤部分は消耗生物学的活性を有する蛋白またはポリペプチドであってもよい。かかるポリペプチドは、例えば、アブリン、リシンA、シュードモナス外毒素またはジフテリアトキシンのごときトキシン；腫瘍壊死因子、アルファインターフェロン、ベータインターフェロン、神経成長因子、血小板由来成長因子、組織プラスミノゲンアクチベーターのごとき蛋白；あるいは例えばリンホカイン、インターロイキン-1（IL-1）、インターロイキン-2（IL-2）、インターロイキン-6（IL-6）、顆粒球マクロファージコロニー刺激因子（GM-CSF）、顆粒球コロニー刺激因子（G-CSF）または他の成長因子のごとき生物学的応答修飾剤を包含する。

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【0204】

かかる治療部分を抗体に抱合させる方法はよく知られており、例えば、Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoc

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lonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies 84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985); および Thorpe et al. "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982) 参照。別法として、米国特許第4676980号において Segal により記載されたように、抗体を第2の抗体に抱合させて抗体ヘテロ抱合体を得てもよい。 10 20

【0205】

本発明核酸分子をベクター中に挿入し、遺伝子治療ベクターとして使用することができる。例えば静脈内注射、局所投与（米国特許5328470参照）または定位注射（例えば Chenら（1994）Proc. Natl. Acad. Sci. USA 91:3054-3057参照）により遺伝子治療ベクターを対象にデリバリーすることができる。遺伝子治療ベクターの医薬製剤は、許容される希釈液中に遺伝子治療ベクターを含んでもよく、あるいは遺伝子デリバリーベークルが埋封された遅速放出マトリックスを含んでもよい。別法として、完全な遺伝子デリバリーベクターを組換え細胞から無傷で製造できる場合（例えば、レトロウイルスベクター）、医薬製剤は遺伝子デリバリー系を生産する1個またはそれ以上の細胞を含んでもよい。 30 40

医薬組成物を投与に関する使用説明書と一緒に容器、パックまたはディスペンサー中に入れることができる。

【0206】

V. 本発明の用途および方法

PD-L2分子、例えば、本明細書記載のPD-L2核酸分子、ポリペプチド、ポリペプチドホモログ、および抗体を下記方法の1つまたはそれ以上に使用することができる：a) スクリーニングアッセイ；b) 予測医学（例えば、診断アッセイ、予後アッセイ、および臨床試験のモニタリング）；ならびにc) 治療方法（例えば、免疫応答をアップまたはダウンレギュレーションすることによる治療方法および予防方法）。本明細書に記載のように、本発明のPD-L2ポリペプチドは下記活性の1つまたはそれ以上を有する：1) 本来の結合パートナー、例えばPD-1に結合し、そして／あるいはその活性をモジュレーションする、2) 細胞内または細胞間シグナリングをモジュレーションする、3) Tリンパ球の活性化をモジュレーションする、4) 生物、例えば、マウスまたはヒトのごとき哺乳動物の免疫応答をモジュレーションする。 40 50

【0207】

本発明の単離核酸分子を用いて、例えば、本明細書においてさらに後で説明するように、PD-L2ポリペプチドを発現させ（例えば、遺伝子治療用途において宿主細胞中の組換え発現ベクターにより）、PD-L2 mRNA（例えば、生物学的試料中の）またはPD-L2遺伝子における遺伝学的変化を検出し、PD-L2活性をモジュレーションする 50

ことができる。PD-L2ポリペプチドを用いて、PD-L2ポリペプチドの不十分または過剰な発現あるいはPD-L2阻害剤の産生により特徴付けられる症状または疾病を治療することができる。さらに、PD-L2ポリペプチドを用いて、天然に存在するPD-L2結合パートナー（PD-1のほかに）をスクリーニングし、PD-L2活性をモジュレーションする薬剤または化合物をスクリーニングし、PD-L2ポリペプチドの不十分または過剰な産生あるいはPD-L2野生型ポリペプチドと比較して低下した、異常なまたは望ましくない活性を有するPD-L2ポリペプチド形態の産生により特徴付けられる症状または疾病（例えば、重篤な合併免疫不全、多発性硬化症、全身性狼瘡、I型糖尿病、リンパ球増殖症候群、炎症性腸疾患、アレルギー、喘息、移植片対宿主疾患および移植拒絶反応のごとき免疫系疾患；細菌およびウイルスのごとき感染性病原体に対する免疫応答；ならびにリンパ腫および白血病のごとき免疫系の癌）を治療することができる。そのうえ、本発明の抗PD-L2抗体を用いてPD-L2ポリペプチドを検出および単離し、PD-L2ポリペプチドの生体利用性を調節し、例えばPD-L2とその本来の結合パートナー（例えば、PD-1）との間の相互作用をモジュレーションすることによりPD-L2活性をモジュレーションすることができる。

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【0208】

A. スクリーニングアッセイ

本発明は、PD-L2ポリペプチドに結合し、例えば、PD-L2発現またはPD-L2活性に刺激または阻害効果を有し、あるいはPD-L2とその本来の結合パートナー、例えばPD-1との間の相互作用に刺激または阻害効果を有するモジュレーター、すなわち候補化合物または試験化合物または作用剤（例えば、ペプチド、ペプチド模倣物、小型分子または他の薬剤）を同定するための方法（本明細書で「スクリーニングアッセイ」という）を提供する。

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1の具体例において、本発明により、PD-L2ポリペプチドもしくはポリペプチドまたはその生物学的に活性のある部分に結合する、例えば、その本来の結合パートナーと相互作用するPD-L2ポリペプチドの能力をモジュレーションする候補もしくは試験化合物をスクリーニングするためのアッセイが提供される。好ましい具体例において、結合パートナーはPD-1である。もう1つの具体例において、本発明は、PD-L2蛋白またはポリペプチドまたは生物学的に活性のある部分（例えば、コファクターまたはコレンザイムアナログ、あるいは阻害性分子）に結合し、あるいはその活性をモジュレーションする候補化合物または試験化合物をスクリーニングするためのアッセイを提供する。

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【0209】

好ましい具体例において、本発明は、PD-L2とその本来の結合パートナーとの間の相互作用に対して刺激または阻害効果を有する候補化合物または試験化合物のアッセイ方法を提供する。典型的な具体例において、結合パートナーはPD-1である。本発明の試験化合物は、生物学的ライブラリー；空間的にアドレス可能な平行（spatially addressable parallel）固相または液相ライブラリー；デコンボリューションを要する合成ライブラリー法；「1-ビーズ1-化合物」ライブラリー法；およびアフィニティークロマトグラフィー選抜を用いる合成ライブラリー法を含む、当該分野で公知のコンビナトリアルライブラリー法における多くの方法のいずれかを用いて得ることができる。生物学的ライブラリー法はペプチドのライブラリーに限定されるが、他の4つの方法は、ペプチド、非ペプチドオリゴマーまたは化合物の小型分子ライブラリーに適用することができる（Lam, K. S. (1997) Anticancer Drug Des. 12:145）。

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【0210】

分子ライブラリーの合成方法の例は、当該分野において、例えばDeWitt et al. (1993) Proc. Natl. Acad. Sci. USA 90:6909; Erb et al. (1994) Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann et al. (1994) J. Med. Chem. 37:2678; Cho et al. (1993) Science

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261:1303; Carrell et al. (1994) Angew. Chem. Int. Ed. Engl. 33:2059; Carrell et al. (1994) Angew. Chem. Int. Ed. Engl. 33:2061; and in Gallop et al. (1994) J. Med. Chem. 37:1233において見出すことができる。

【0211】

化合物のライブラリーは、溶液中（例えば、Houghten (1992) Biotechniques 13:412-421）またはビーズ（Lam (1991) Nature 354:82-84）、チップ（Fodor (1993) Nature 364:555-556）、細菌（Ladner USP 5, 223, 409）、孢子（Ladner USP '409）、プラスミド（Cull et al. (1992) Proc. Natl. Acad. Sci. USA 89:1865-1869）またはファージ上に（Scott and Smith (1990) Science 249:386-390）提供され得る（Cwirlla et al. (1990) Proc. Natl. Acad. Sci. 87:6378-6382）；（Felici (1991) J. Mol. Biol. 222:301-310）；（Ladner 既出）。 10

【0212】

1の具体例において、アッセイは、PD-L2ポリペプチドまたはその生物学的に活性のある部分を発現している細胞を試験化合物と接触させること、および試験化合物がPD-L2活性をモジュレーションする能力を測定する、細胞に基づくアッセイである。例えば、試験化合物がPD-L2活性をモジュレーションする能力の決定は、例えば、PD-L2がその本来の結合パートナー、例えばPD-1に結合し、免疫細胞活性をモジュレーションする能力をモニターすることにより行うことができる。免疫細胞は、例えば、T細胞、B細胞またはミエロイド細胞であってよい。PD-L2のPD-1への結合をモジュレーションする試験化合物の能力の決定は、例えば、PD-1をラジオアイソトープまたは酵素標識とカップリングさせることにより行なうことができ、複合体中に標識PD-1を検出することによりPD-1のPD-L2への結合を検出することができる。別法として、PD-L2をラジオアイソトープまたは酵素標識とカップリングさせて、複合体中のPD-1へのPD-L2結合をモジュレーションする試験化合物の能力をモニターすることもできる。PD-L2に結合する試験化合物の能力の決定は、例えば、化合物をラジオアイソトープまたは酵素標識とカップリングさせることにより行なうことができ、複合体中の標識PD-L2化合物を検出することにより化合物のPD-L2への結合を検出することができる。例えば、化合物（例えば、PD-1）を¹²⁵I、³⁵S、¹⁴Cまたは³Hで、直接的または間接的に標識することができ、放射能を直接カウントすることにより、あるいはシンチレーション計測により検出することができる。別法として、化合物を、例えばセイヨウワサビペルキシダーゼ、アルカリホスファターゼまたはルシフェラーゼで標識し、適当な基質の生成物への変換を調べることにより酵素標識を検出することができる。 20 30

【0213】

相互作用物質を標識せずに化合物（例えば、PD-1）がPD-L2と相互作用する能力を調べることも本発明の範囲内である。例えば、マイクロフィジオメーターを用いて、PD-L2も標的分子も標識せずに、化合物とPD-L2との相互作用を検出することができる。McConnell, H. M. et al. (1992) Science 257:1906-1912。本明細書中に用いる「マイクロフィジオメーター」（例えば、サイトセンサー）は、細胞がその周囲を酸性化する速度を、光アドレス可能電位差センサー（light-addressable potentiometric sensor）（LAPS）を用いて測定する分析装置である。この酸性化速度の変化を、化合物とPD-L2との間の相互作用の指標として用いることができる。 40

【0214】

もう1つの具体例において、アッセイは、PD-L2結合パートナーを発現している細胞 50

を試験化合物と接触させ、次いで、試験化合物がPD-L2結合パートナーの活性をモジュレーションする（例えば、刺激または阻害する）能力を調べることを特徴とする細胞に基づくアッセイである。好ましい具体例において、結合パートナーはPD-1である。試験化合物がPD-L2結合パートナーの活性をモジュレーションする能力の決定は、例えば、PD-L2ポリペプチドがPD-L2結合パートナーに結合し、あるいは相互作用する能力を決定することにより行なうことができる。

【0215】

PD-L2ポリペプチドまたはその生物学的に活性のあるフラグメントがPD-L2結合パートナー、例えばPD-1に結合し、あるいは相互作用する能力の決定は、直接結合を調べるための上記方法の1つにより行なうことができる。好ましい具体例において、PD-L2ポリペプチドがPD-L2結合パートナーに結合あるいは相互作用する能力の決定は、結合パートナーの活性を調べることにより行なうことができる。例えば、細胞の第2のメッセンジャー（例えば、チロシンキナーゼまたはホスファターゼ活性）の誘導を検出することにより、適当な基質の触媒／酵素活性を検出することにより、リポーター遺伝子（検出可能マーカー、例えばルシフェラーゼをコードする核酸に作動可能に連結された標的応答性調節エレメントを含む）の誘導を検出することにより、あるいは標的により調節される細胞の応答を検出することにより、結合パートナーの活性を調べることができる。例えば、PD-L2ポリペプチドが本来のPD-L2結合パートナー、例えばPD-1に結合あるいは相互作用する能力の決定を、増殖アッセイにおいて免疫細胞同時刺激または阻害をモジュレーションする化合物の能力を測定することにより、あるいはPD-L2ポリペプチドの一部分を認識する抗体へのPD-L2ポリペプチドの結合能を妨害することにより、行なうことができる。1の具体例において、T細胞活性化をモジュレーションする化合物を、T細胞増殖またはサイトカイン産生をモジュレーションする化合物の能力を調べることにより同定することができる。好ましい具体例において、T細胞活性化をモジュレーションする化合物を、1またはそれ以上の抗原濃度においてT細胞増殖またはサイトカイン産生をモジュレーションする化合物の能力を調べることにより同定することができる。

【0216】

さらにもう1つの具体例において、本発明のアッセイは、PD-L2ポリペプチドまたはその生物学的に活性のある部分を試験化合物と接触させ、次いで、試験化合物がPD-L2ポリペプチドまたはその生物学的に活性な部分に結合する能力を決定する無細胞アッセイである。本発明のアッセイに用られるPD-L2ポリペプチドの好ましい生物学的に活性のある部分は、非PD-L2分子との相互作用に参与するフラグメント、例えば、PD-L2結合パートナーに結合する細胞外ドメインの少なくとも一部分を包含する。試験化合物のPD-L2ポリペプチドへの結合は、前記のように直接または間接的に決定することができる。好ましい具体例では、アッセイは、PD-L2ポリペプチドまたはその生物学的に活性な部分を、PD-L2ポリペプチドと結合してアッセイ混合物を形成する既知化合物と接触させること、アッセイ混合物を試験化合物と接触させること、次いで、試験化合物がPD-L2ポリペプチドと相互作用する能力を決定することを特徴とするが、該アッセイにおいては、試験化合物がPD-L2ポリペプチドと相互作用する能力を決定することは、既知化合物と比較して試験化合物がPD-L2ポリペプチドまたはその生物学的に活性な部分に選択的に結合する能力を決定することを特徴とする。

【0217】

もう1つの具体例では、アッセイは、PD-L2ポリペプチドまたはその生物学的に活性な部分を試験化合物と接触させ、次いで、試験化合物がPD-L2ポリペプチドまたはその生物学的に活性な部分の活性をモジュレーション（例えば、刺激または阻害）する能力を決定する無細胞アッセイである。試験化合物がPD-L2ポリペプチドの活性をモジュレーションする能力の決定は、例えば、PD-L2ポリペプチドがPD-L2結合パートナーに結合する能力を、前記の直接的に結合を決定するための方法の一つにより測定することにより行なうことができる。好ましい具体例において、結合パートナーはPD-1で

ある。PD-L2ポリペプチドがPD-L2結合パートナーに結合する能力の決定は、リアルタイム・バイオモレキュラー・インターアクション分析(BIA)などの方法を用いて行なうこともできる。Anal. Chem. 63:2338-2345 and Szabo et al. (1995) Curr. Opin. Struct. Biol. 5:699-705。本明細書中に用いる「BIA」は、生物特異的な相互作用をリアルタイムで、相互作用物質(例えばBIAコア)を標識することなく研究するための方法である。表面プラズモン共鳴(SPR)の視覚現象を、生物分子間のリアルタイムの反応の指標として用いることができる。

【0218】

別の具体例では、試験化合物がPD-L2ポリペプチドの活性を調節する能力の測定は、PD-L2ポリペプチドがPD-L2結合パートナーの下流エフェクター(例えば、PD-1の下流エフェクター)の活性をさらに調節する能力を測定することにより達成することができる。例えば、適当な標的に対するエフェクター分子の活性、または適当な標的へのエフェクターの結合を、前記のように決定することができる。

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【0219】

本発明の無細胞アッセイには、可溶性形態および／または膜結合形態の両方の蛋白(例えば、PD-L2ポリペプチドまたはその生物学的に活性のある部分、またはPD-L2が結合する結合パートナー、例えばPD-1)を用いることもできる。膜結合形態のポリペプチド(例えば、細胞表面PD-L2)を用いる無細胞アッセイの場合、膜結合形態の蛋白が溶液中で維持されるように可溶化剤を用いることが望ましい。そのような可溶化剤の例には、*n*-オクチルグルコシド、*n*-ドデシルマルトシド、オクタノイル-N-メチルグルカミド、デカノイル-N-メチルグルカミド、トライトン(登録商標)X-100、トライトン(登録商標)X-114、テシト(登録商標)、イソトリデシポリ(エチレングリコールエーテル)*n*、3-[(3-コラミドプロピル)ジメチルアミノオ]-1-プロパンスルホネート(CHAPS)、3-[(3-コラミドプロピル)ジメチルアミノオ]-2-ヒドロキシ-1-プロパンスルホネート(DHAPSO)または、N-ドデシル=N, N-ジメチル-3-アンモニオ-1-プロパンスルホネートなどの非イオン性界面活性剤が含まれる。

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【0220】

本発明の前記アッセイ法の1よりも多い具体例において、1または両方の蛋白の、複合体を形成していない形態からのその複合体形態の分離を促進するため、ならびにアッセイの自動化を図るために、PD-L2またはその結合パートナーいずれかを固定化することが望ましい。好ましい具体例において、結合パートナーはPD-1である。試験化合物のPD-L2ポリペプチドに対する結合、または候補化合物の存在下および不在下でのPD-L2ポリペプチドと標的分子との相互作用は、反応物を入れるのに適したいずれかの容器中で達成することができる。そのような容器の例には、マイクロタイタープレート、試験管、およびマイクロ遠心管が含まれる。1の具体例において、1または両方の蛋白がマトリックスに結合するのを可能とするドメインを付加する融合蛋白を提供することができる。例えば、グルタチオン-S-トランスフェラーゼ/PD-L2融合蛋白またはグルタチオン-S-トランスフェラーゼ/標的融合蛋白をグルタチオンセファローゼビーズ(Sigma Chemical, St. Louis, MO)またはグルタチオン誘導体化マイクロタイタープレートに吸着させ、次いでこれを試験化合物、または試験化合物および非吸着標的蛋白もしくはPD-L2ポリペプチドのいずれかと結合させ、次いで、混合物を複合体形成に資する条件下(例えば、塩およびpHの生理的条件)でインキュベーションする。インキュベーション後、ビーズまたはマイクロタイタープレートウェルを洗浄して、全ての結合していない成分を除き、ビーズの場合、マトリックスを固定化し、複合体形成を、例えば前記のように直接的または間接的に調べる。別法として、複合体をマトリックスから分離し、PD-L2結合または活性のレベルを標準的な方法を用いて決定することができる。

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【0221】

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蛋白をマトリックス上に固定するための他の方法を、本発明のスクリーニングアッセイに用いることもできる。例えば、PD-L2ポリペプチドまたはPD-L2結合パートナーのいずれかを、ビオチンおよびストレプトアビジンの結合を利用して固定化することができる。ビオチン化PD-L2ポリペプチドまたは標的分子は、当該分野で周知の方法（例えば、ビオチン化キット、Pierce Chemicals, Rockford, IL）を用いてビオチン-NHS（N-ヒドロキシスクシンイミド）から調製することができ、ストレプトアビジンでコートされた96ウェルのプレート（Pierce Chemical）に固定することができる。別法として、PD-L2ポリペプチドまたは結合パートナーと反応するが、PD-L2ポリペプチドのその標的分子への結合を妨害しない抗体をプレートのウェルに誘導体化して、未結合結合パートナーまたはPD-L2ポリペプチドをウェル中で抗体結合によりトラップすることができる。GST-固定化複合体に関する前記方法に加え、そのような複合体を検出するための方法には、PD-L2ポリペプチドまたは結合パートナーと反応する抗体を用いる複合体の免疫検出、ならびにPD-L2ポリペプチドまたは結合パートナーに関連する酵素活性を検出することに依存する酵素-結合アッセイが含まれる。

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【0222】

別の具体例において、試験化合物がPD-L2ポリペプチドの活性をモジュレーションする能力の決定は、PD-L2の下流で機能する分子の活性をモジュレーションする試験化合物の能力を調べることにより、例えば、PD-L2結合パートナー、例えばPD-1の細胞質ドメインと相互作用させることにより、行なうことができる。例えば、第2のメッ

【0223】

他の具体例では、細胞を候補化合物と接触させ、そしてPD-L2 mRNAまたは蛋白の細胞中での発現を決定する方法にて、PD-L2の発現のモジュレーターを同定する。候補化合物の存在下でのPD-L2 mRNAまたはポリペプチドの発現のレベルを、候補化合物の不在下でのPD-L2 mRNAまたはポリペプチドの発現のレベルと比較する。次いで、この比較に基づき、候補化合物をPD-L2の発現のモジュレーターとして同定することができる。例えば、PD-L2 mRNAまたはポリペプチドの発現が、候補化合物の存在下において、その不在下におけるよりも大きい（例えば、統計学的に有意に大きい）場合、候補化合物は、PD-L2 mRNAまたはポリペプチドの発現の刺激物質と同定される。あるいは、PD-L2 mRNAまたはポリペプチドの発現が、候補化合物の存在下において、その不在下におけるよりも少ない（例えば、統計学的に有意に少ない）場合、候補化合物はPD-L2 mRNAまたはポリペプチドの発現の阻害物質と同定される。細胞中でのPD-L2 mRNAまたはポリペプチドの発現のレベルは、本明細書中に開示するPD-L2 mRNAまたはポリペプチドを検出するための方法により決定することができる。

【0224】

本発明のさらに別の態様において、PD-L2ポリペプチドを、2-ハイブリッドアッセイまたは3-ハイブリッドアッセイにおいて「エサ（bait）蛋白」として用いて（U.S. 特許No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Biotechniques 14:920-924; Iwabuchi et al. (1993) Oncogene 8:1693-1696; and Brent WO 94/10300を参照されたい）、PD-L2と結合あるいは相互作用する他のポリペプチド（「PD-L2結合蛋白」、「PD-L2結合パートナー」または「PD-L2bp」）、ならびにPD-L2活性に関与する他のポリペプチドを同定することができる。そのような結合蛋白の例はPD-1である。かかるPD-L2結合蛋白は、例えば、PD-L2により媒介される情報伝達経路のダウンストリームエレメントとして、PD-

L2ポリペプチドまたはPD-L2標的によるシグナルの伝達に関与している可能性もある。あるいは、かかるPD-L2結合ポリペプチドはPD-L2阻害物質である可能性がある。

【0225】

2-ハイブリッドシステムは、分離可能なDNA結合および活性化ドメインからなる、大部分の転写因子の基本単位性に基づく。簡単には、アッセイは2つの異なるDNA構築物を利用する。一構築物において、PD-L2ポリペプチドをコードする遺伝子を公知の転写因子（例えば、GAL-4）のDNA結合ドメインをコードしている遺伝子に結合させる。他の構築物において、DNA配列のライブラリー由来の、同定されていないポリペプチド（「プレイ（prey）」または「サンプル」）をコードするDNA配列を、公知の転写因子の活性化ドメインをコードする遺伝子に結合させる。「バイト（bait）」と「プレイ」ポリペプチドがインビボで相互作用してPD-L2-依存性複合体を形成することができる場合、転写因子のDNA結合ドメインと活性化ドメインが接近する。この接近により、転写因子に反応する転写調節部位に作動可能な状態で連結されているレポーター遺伝子（例えば、lacZ）の転写が可能となる。レポーター遺伝子の発現は検出することができ、機能的転写因子を含んでいる細胞コロニーを単離し、そしてそれを用いてPD-L2ポリペプチドと相互作用するポリペプチドをコードするクローン化遺伝子を得ることができる。

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【0226】

もう1つの態様において、本発明は、本明細書記載の2またはそれ以上のアッセイの組み合わせに関する。例えば、細胞に基づくアッセイまたは無細胞アッセイを用いてモジュレーション物質を同定することができ、PD-L2ポリペプチドの活性をモジュレーションするその物質の能力をインビボにおいて、例えば、細胞変形または腫瘍発生に関する動物モデルのごとき動物において確認することができる。

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【0227】

本発明は、前記スクリーニングアッセイにより同定される新規な作用剤に関する。従って、本明細書中に開示するように同定された薬剤を適当な動物モデルでさらに用いることは、本発明の範囲内のことである。例えば、本明細書中に開示するように同定される作用剤（例えば、PD-L2モジュレーション剤、アンチセンスPD-L2核酸分子、PD-L2特異的抗体、またはPD-L2結合パートナー）を動物モデルに用いて、そのような作用剤を用いた治療の効力、毒性または副作用を決定することができる。別に、本明細書中に記載するように、同定された作用剤を動物モデルに用いて、そのような作用剤の活性のメカニズムを決定することができる。さらに、本発明は、前記スクリーニングアッセイにより同定される新規作用剤の、本明細書に記載する治療のための使用に関する。

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【0228】

B. 検出アッセイ

本明細書中で同定されるcDNA配列（および対応完全遺伝子配列）の部分またはフラグメントを、多くの方法でポリヌクレオチド試薬として用いることができる。例えば、これらの配列を用いて、（i）染色体上にそのそれぞれの遺伝子をマッピングし、かくして遺伝的疾患に関連する遺伝子領域の位置を確認し；（ii）微量な生物試料から個体を同定する（組織タイピング）；ならびに（iii）生物試料の法医学的同定に役立てることができる。これらの適用を、以下のサブセクションに記載する。

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【0229】

1. 染色体マッピング

遺伝子の配列（または配列の一部）が単離されたならば、この配列を用いて染色体上の遺伝子位置をマッピングすることができる。このプロセスは染色体マッピングと呼ばれる。したがって、本明細書記載のPD-L2ヌクレオチド配列の部分またはフラグメントを用いて染色体上のPD-L2遺伝子位置をマッピングすることができる。PD-L2配列の染色体へのマッピングは、これらの配列と疾病関連遺伝子とを関連づける重要な最初の工程である。

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【0230】

簡単に説明すると、PD-L2ヌクレオチド配列からPCRプライマー（好ましくは長さ15～25bp）を調製することによりPD-L2遺伝子を染色体にマッピングすることができる。PD-L2配列のコンピューター分析を用いて、ゲノムDNA中の1個よりも多いエキソンを含まないプライマーを予想することができ、かくして、増幅プロセスを複雑化することができる。次いで、これらのプライマーを、個々のヒト染色体を含む体細胞ハイブリッドのPCRスクリーニングに用いることができる。PD-L2配列に対応するヒト遺伝子を含むハイブリッドのみが増幅フラグメントを生じるであろう。

【0231】

異なる哺乳動物（ヒトおよびマウス細胞）由来の体細胞を融合させることにより体細胞ハイブリッドを調製する。ヒトおよびマウス細胞のハイブリッドが増殖し、分裂するにつれ、それらは徐々にランダムなオーダーでヒト染色体を失うが、マウス染色体を保持する。マウス細胞が増殖できない培地を用いることにより、所望酵素をコードする遺伝子を含む1のヒト染色体が保持されるであろう。なぜならマウス細胞は特定の酵素を欠いているが、ヒト細胞は有している可能性があるからである。種々の培地を用いることにより、ハイブリッド細胞系のパネルを確立することができる。パネル中の各細胞系は単一のヒト染色体または少数のヒト染色体のいずれか、ならびにマウス染色体の全セットを含み、個々の遺伝子を特定のヒト染色体に簡単にマッピングすることができる（D'Eustachio, P. et al. (1983) Science 220: 919-924）。トランスロケーションおよび欠失を有するヒト染色体を用いることにより、ヒト染色体のフラグメントのみを含む体細胞ハイブリッドを得ることもできる。

【0232】

体細胞ハイブリッドのPCRマッピングは、個々の染色体に個々の配列を割り当てるための迅速な方法である。単一のサーマルサイクルを用いて1日に3つまたはそれ以上の配列を割り当てることができる。オリゴヌクレオチドプライマーを設計するためにPD-L2ヌクレオチド配列を使用して、特定の染色体に由来するフラグメントのパネルを用いて、サブローカライゼーションを遂行することができる。PD-L2配列をその染色体にマッピングするために同様に使用される他のマッピング方法は、in situハイブリダイゼーション（Fan, Y. et al. (1990) Proc. Natl. Acad. Sci. USA 87: 6223-27）、標識フローソーテッド（flow-sorted）染色体を用いるスクリーニング、および染色体特異的cDNAライブラリーへのハイブリダイゼーションによるプレセクションを包含する。

【0233】

中期染色体スプレッドへのDNA配列の蛍光in situハイブリダイゼーション（FISH）をさらに用いて、1工程で正確な染色体位置を得ることができる。紡錘糸を破壊するコルセミドのごとき化学薬剤により中期で分裂を停止された細胞を用いることにより、染色体スプレッドを作成することができる。染色体を短時間トリプシン処理し、次いで、ギムザ染色することができる。明暗バンドのパターンが各染色体上に出現し、その結果、染色体を個々に同定することができる。500または600塩基程度の短いDNA配列にFISH法を使用することができ。しかしながら、1000塩基よりも大きいクローンは、十分なシグナル強度でもってユニークな染色体位置に結合する可能性が高く、検出が容易である。好ましくは、1000塩基以上、より好ましくは2000塩基以上であれば、合理的な時間内に良好な結果を得るに十分であろう。この方法のレビューには、Verma et al., Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988) 参照。

【0234】

染色体マッピング用試薬を個々に用いて、単一の染色体または染色体上の単一部位をマークすることができ、あるいは試薬のパネルを複数部位および／または複数染色体のマッピングに用いることができる。実際には、遺伝子の非コーディング領域に対応する試薬がマ

ッピング目的に好ましい。コーディング配列は遺伝子ファミリー内で保存されている可能性がさらに高く、よって、染色体マッピングの間にクロスハイブリダイゼーションのチャンスが高い。

【0235】

染色体上の配列の物理的な位置を、遺伝子マップデータと関連付けることができる。（そのようなデータは、例えば、Johns Hopkins University Welch Medical Libraryよりオンラインで入手できるV. McKusick, Mendelian Inheritance in Manに見出される。）同じ染色体領域にマップされた場合、遺伝子と疾患の間の関連性を、次いで、例えばEgeland, J. et al. (1987) Nature, 325: 783-787に開示されている連鎖分析（物理的に隣接した遺伝子の同時遺伝）により同定することができる。 10

【0236】

そのうえ、PD-L2遺伝子に関連する疾患に罹患している個体と罹患していない個体の間のDNA配列の相違を調べることができる。罹患している個体のいくつかまたはすべてに変異が認められるが、罹患していない個体には変異が全く認められない場合、変異が特定疾患の原因であることが考えられる。一般的には、罹患している個体と罹患していない個体の比較には、染色体スプレッドから見ることでできる、あるいはDNA配列に基づくPCRを用いて検出可能な欠失またはトランスロケーションのごとき染色体中の構造変化を先ず探すことを用いる。最終的には、いくつかの個体からの遺伝子の完全な配列決定を行なって、変異の存在を確認し、変異と多型とを区別することができる。 20

【0237】

2. 組織タイピング

本発明のPD-L2配列を用いて、微量生物試料から個体を同定することもできる。例えば、合衆国軍は、その人員の同定のために制限酵素フラグメント長遺伝子多型(RFLP)を用いることを考えている。この方法では、個人のゲノムDNAを1またはそれ以上の制限酵素で消化し、サザンブロットにおけるプローブとし、同定のためのユニークなバンドを得る。この方法は、ポジティブな同定を困難にしている、失われる、切断される、または盗用される可能性のある「ドッグ・タグ(Dog Tags)」の現在の制限に影響されない。本発明の配列は、RFLPのための追加的DNAマーカ（米国特許第5, 272, 057号に記載されている）として有用である。 30

【0238】

さらにそのうえ、本発明の配列を用いて、個体のゲノムの選択された部分の実際の塩基対-塩基DNA配列を決定するための別法を提供することができる。つまり、本明細書中に開示するPD-L2ヌクレオチド配列を用いて、配列の5'および3'末端から2つのPCRプライマーを調製することができる。次いで、これらのプライマーを用いて個体のDNAを増幅し、その後、それを配列決定することができる。

この方法で調製された、個体からの対応するDNA配列のパネルより、各個体は対立遺伝子の違いによりそのようなDNA配列のユニークなセットを有するので、ユニークに個体を同定することができる。本発明の配列を用いて、個体および組織からそのような同定配列を得ることができる。本発明のPD-L2ヌクレオチド配列は、ヒトゲノムの部分をユニークに表す。対立遺伝子の変化は、ある程度はこれらの配列のコーディング領域に生じるが、大部分は非コーディング領域に生じる。個体間の対立遺伝子の変化は、各500塩基につき約1つの頻度で起こることが見積もられる。本明細書中に開示する配列のそれぞれは、ある程度まで、個人からのDNAを同定目的のために比較することができるスタンダードとして用いることができる。より多くの遺伝子多型は非コーディング領域に生じるので、個体を識別するにはより少数の配列が必要である。それぞれが100塩基の非コーディング増幅配列を生じるおそらく10ないし1000個のプライマーのパネルを用いると、配列番号：1または4の非コーディング配列によりポジティブな個体の同定が可能となる。配列番号：3または6のごとき予想されたコーディング配列を用いる場合、ポシテ 40 50

ィブな個体の同定のためのプライマーのより適当な数は500ないし2000個であろう。

【0239】

本明細書に記載するPD-L2ヌクレオチド配列由来の試薬のパネルを用いて個体に関するユニークな同定データベースを作成する場合、それらの同じ試薬を後で用いてその個体からの組織を同定することができる。ユニークな同定データベースを用いて、生存している、または死亡した個体のポジティブな同定を、極端に少量の組織試料から行なうことができる。

【0240】

3. 法生物学 (forensic biology) における部分PD-L2配列の使用
DNAに基づく同定法は、法生物学にも用いることができる。法生物学は、犯罪現場で見出される生物学的証拠の遺伝学的分類を、例えば犯罪の犯人をポジティブに同定するための手段として用いている科学分野である。そのような同定を行うのに、PCR技術を用いて、犯罪現場で見出される組織、例えば髪もしくは皮膚、または体液、例えば血液、唾液、または精液などの非常に少量の生物学的試料から採取されたDNA配列を増幅することができる。次いで、増幅された配列をスタンダードと比較することができ、これにより生物試料の起源を同定することが可能となる。

【0241】

本発明の配列を用いて、ヒトゲノムの特定の位置に標的され、例えば別の「同定マーカー」(即ち、特定の個体にユニークな他のDNA配列)を提供することによりDNAに基づく法的同定の確実性を高めることができるポリヌクレオチド試薬、例えばPCRプライマーを提供することができる。前記のように、実際の塩基配列情報を、制限酵素により作成されたフラグメントにより形成されるパターンに確実に代わるものとして同定に用いることができる。遺伝子多型の大部分は非コーディング領域に生じるので、配列番号:1または配列番号:4の非コーディング領域に標的化される配列がこの使用のために特に適当であり、この方法を用いて、個体を区別することがいっそう容易になる。ポリヌクレオチド試薬の例は、PD-L2ヌクレオチド配列またはその部分、例えば、少なくとも20塩基、好ましくは少なくとも30塩基の長さを有する配列番号:1または4の非コーディング領域由来のフラグメントを包含する。

【0242】

本明細書中に開示するPD-L2ヌクレオチド配列をさらに用いて、特定組織、例えば脳組織を同定するために、例えばin situハイブリダイゼーション法で用いることができるポリヌクレオチド試薬、例えば標識プローブ、または標識可能なプローブを提供することができる。これは、法病理学者が未知起源の組織を与えられる場合に非常に有用であり得る。そのようなPD-L2プローブのパネルを用いて、種により、および/または器官のタイプにより組織を同定することができる。

同様に、これらの試薬、例えばPD-L2プライマーまたはプローブを用いて、コンタミネーションに関して組織培養物をスクリーニングする(即ち、培養物中の異なるタイプの細胞の混合物の存在をスクリーニングする)ことができる。

【0243】

C. 予測医学

本発明は、予後(予測)目的のために診断アッセイ、予後アッセイ、および臨床試験をモニターすることを用い、それにより個体を予防的に処置する予測医学の分野にも適する。従って、本発明の一態様は、生物学的試料(例えば、血液、血清、細胞、組織)中でのPD-L2ポリペプチドおよび/または核酸の発現ならびにPD-L2の活性を測定し、それにより、個体が異常なPD-L2の発現または活性に関連する病気または疾患に罹患しているかどうか、または疾患が進行する危険にあるかどうかを決定するための診断アッセイに関する。本発明により、個体がPD-L2ポリペプチド、核酸の発現または活性に関連する疾患を進行させる危険にあるかどうかを決定するための予後(または予測)アッセイも提供される。例えば、PD-L2遺伝子の変異を、生物試料中でアッセイすることが

できる。そのようなアッセイを予後または予測目的のために用い、それにより個体を、PD-L2ポリペプチド、核酸の発現または活性により特徴付けられる、またはそれに関連する疾患の発症前に予防的に処置することができる。

本発明の他の態様は、臨床試験で、PD-L2の発現または活性における試薬（例えば、薬剤、化合物）の影響をモニターすることに関する。

これらおよび他の試薬を、以下のセクションにさらに詳細に記載する。

【0244】

1. 診断アッセイ

PD-L2ポリペプチドまたは核酸の生物試料中での存在または不在を検出するための典型的な方法は、生物試料を試験対象から得て、次いでPD-L2ポリペプチドまたはPD-L2ポリペプチドをコードする核酸（例えば、mRNA、ゲノムDNA）を検出することができる化合物または試薬と生物試料と接触させて、生物試料中のPD-L2ポリペプチドまたは核酸の存在を検出することを特徴とする。PD-L2 mRNAまたはゲノムDNAを検出するのに好ましい試薬は、PD-L2 mRNAまたはゲノムDNAにハイブリダイズすることができる標識核酸プローブである。核酸プローブは、例えば、配列番号1、3、4または6の核酸のようなhPD-L2核酸、またはその部分、例えば少なくとも15、30、50、100、250または500ヌクレオチドの長さの、およびPD-L2 mRNAまたはゲノムDNAに対してストリンジントな条件下で特異的にハイブリダイズするのに十分なオリゴヌクレオチドであってよい。本発明の診断アッセイにおける使用に適した他のプローブを、本明細書中に記載する。

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【0245】

PD-L2ポリペプチドを検出するのに好ましい試薬は、PD-L2ポリペプチドに結合することができる抗体、好ましくは検出可能な標識を有する抗体である。抗体は、ポリクローナル、またはより好ましくはモノクローナルであってよい。インタクトな抗体、またはそのフラグメント（例えばFabまたはF(ab')₂）を用いることができる。プローブまたは抗体に関して「標識された」なる用語は、プローブまたは抗体に検出可能な物質を結合させる（即ち、物理的に結合させる）ことによりプローブまたは抗体を直接的に標識すること、ならびに、プローブまたは抗体を直接標識される他の試薬と反応させることにより間接的に標識することが含まれるものとする。間接標識の例には、蛍光標識された二次抗体を用いる一次抗体の検出、およびDNAプローブをビオチンで末端標識して、それを蛍光標識されたストレプトアビジンで検出することが含まれる。「生物学的試料」なる用語には、対象から単離された組織、細胞および生物学的液体、ならびに対象中に存在する組織、細胞および体液が含まれるものとする。即ち、本発明の検出方法を用いて生物試料においてPD-L2 mRNA、蛋白またはゲノムDNAを、インビトロならびにインビボで検出することができる。例えば、PD-L2 mRNAのインビトロでの検出法には、ノーザンハイブリダイゼーションおよびインサイチュ（*in situ*）ハイブリダイゼーションが含まれる。PD-L2ポリペプチドのインビトロでの検出法には、酵素結合免疫吸着アッセイ（ELISA）、ウェスタン・ブロット、免疫沈降および免疫蛍光法が含まれる。PD-L2ゲノムDNAのインビトロでの検出法にはサザンハイブリダイゼーションが含まれる。PD-L2ゲノムDNAの検出のためのインビトロ法には、サザンハイブリダイゼーションが含まれる。さらに、PD-L2ポリペプチドのインビボでの検出法には、標識された抗PD-L2抗体を対象に導入することが含まれる。例えば、抗体は、対象におけるその存在および位置を標準的な画像処理法で検出することができる放射活性マーカーで標識することができる。

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【0246】

1の具体例において、生物試料は試験対象からの蛋白分子を含む。別法として、生物試料には試験対象からのmRNA分子または試験対象からのゲノムDNA分子を含むことができる。好ましい生物試料は、対象から慣用的方法により単離される血清試料である。もう1つの具体例では、該方法はさらに、対照生物試料を対照の対象から得ること、対照試料をPD-L2ポリペプチド、mRNAまたはゲノムDNAを検出することができる化

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合物または試薬と接触させて、生物試料中のPD-L2ポリペプチド、mRNAまたはゲノムDNAの存在を検出すること、ついで、対照試料中のPD-L2ポリペプチド、mRNAまたはゲノムDNAの存在を、試験試料中のPD-L2ポリペプチド、mRNAまたはゲノムDNAの存在と比較することの特徴とする。

【0247】

本発明には、生物試料中のPD-L2の存在を検出するためのキットも包含される。例えば、該キットには、生物試料中のPD-L2ポリペプチドまたはmRNAを検出することができる標識化合物または試薬、試料中のPD-L2の量を測定する装置、および試料中のPD-L2の量をスタンダードと比較する装置を含めることができる。該化合物または試薬は適当な容器に封入することができる。キットには、さらに、キットをPD-L2ポリペプチドまたは核酸を検出するのに用いるための使用説明書を含めることができる。

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【0248】

2. 予後アッセイ

さらにそのうえ、本明細書記載の診断方法を用いて、異常なまたは望ましくないPD-L2発現または活性に関連した疾病または疾患を有する、あるいは発症する危険性のある対象を同定することができる。本明細書の用語「異常な」は、野生型PD-L2発現または活性から逸脱したPD-L2発現または活性を包含する。異常な発現または活性は、増加または減少した発現または活性、ならびに野生型の発達パターンの発現またはサブ細胞（subcellular）パターンの発現に従わない発現または活性を包含する。例えば、異常なPD-L2発現または活性は、PD-L2中の変異がPD-L2遺伝子の発現不足または過剰発現を引き起こす場合、ならびに変異が、無機能PD-L2ポリペプチドまたは野生型の様式で機能しないポリペプチド、例えば、PD-L2結合パートナー（例えばPD-1）と相互作用しないポリペプチド、または非PD-L2結合パートナーと相互作用するポリペプチドを生じさせる状況を包含する。本明細書の用語「望ましくない」は、免疫細胞活性化のごとき生物学的応答に関与する望ましくない現象を包含する。例えば、用語「望ましくない」は、対象において望ましくないPD-L2発現または活性を包含する。

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【0249】

上記診断アッセイまたは下記アッセイのごとき本明細書中に記載のアッセイを用いて、PD-L2活性または核酸発現の誤調節に関連した疾病、例えば、自己免疫疾患、免疫不全疾患、免疫系の癌を有する、あるいは発症する危険性のある、あるいは自発的に流産する傾向のある対象を同定することができる。別法として、予後アッセイを用いて、PD-L2活性または核酸発現の誤調節に関連した疾病、例えば、自己免疫疾患、免疫不全疾患、免疫系の癌を有する、あるいは発症する危険性のある、あるいは自発的に流産する傾向のある対象を同定することができる。かくして、本発明は、異常なPD-L2の発現または活性に関連する病気または疾患を同定するための方法であって、試験試料を対象から得て、PD-L2ポリペプチドまたは核酸（例えば、mRNA、ゲノムRNA）を検出する方法が提供し、ここで、PD-L2ポリペプチドまたは核酸の存在は、異常なPD-L2の発現または活性に関連する病気または疾患を有する、あるいはそれらを進行させる危険にある患者の診断的特徴である。本明細書中で使用する「試験試料」は、関心の持たれる対象から得られる生物試料をいう。例えば、試験試料は、生物学的液体（例えば、脳脊髄液または血清）、細胞試料、または組織から得ることができる。

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【0250】

さらにそのうえ、本明細書に記載する予後アッセイを用いて、薬剤（例えば、アゴニスト、アンタゴニスト、ペプチド模倣物、蛋白、ペプチド、核酸、小型分子または他の薬物候補）を対象に投与して、異常なまたは望ましくないPD-L2の発現または活性に関連する病気または疾患を処置することができるかどうかを決定することができる。例えば、かかる方法を用いて、自己免疫疾患、免疫不全疾患、免疫系の癌、または自発的流産傾向に対する薬剤で対象を効果的に治療しうるかどうかを決定することができる。つまり、本発明は、異常なPD-L2の発現または活性に関連する疾患のための薬剤を用いて対象を効

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果的に処置することができるかどうかを決定するための方法を提供するものであり、該方法は、試験試料を得、PD-L2ポリペプチドまたは核酸の発現または活性を検出する方法（例えば、ここで、PD-L2ポリペプチドまたは核酸の発現または活性が大きいことは、異常なPD-L2の発現または活性に関連する疾患を治療するための薬剤を投与することができる対象の診断的特徴である）である。

【0251】

本発明の方法を用いて、PD-L2遺伝子の遺伝学的変化を検出し、それにより、変化した遺伝子を有する対象がPD-L2ポリペプチド活性または核酸発現の誤調節により特徴づけられる疾患、例えば、自己免疫疾患、免疫不全疾患、免疫系の癌、または自発的流産傾向の危険性があるかどうかを決定することができる。好ましい具体例において、該方法は、対象からの細胞の試料において、PD-L2ポリペプチドをコードしている遺伝子の完全性に影響を与える少なくとも一つの変化により特徴付けられる遺伝学的変化の存在または不在、またはPD-L2遺伝子の誤発現を検出することを特徴とする。例えば、そのような遺伝学的変化は、1) PD-L2遺伝子からの1またはそれ以上のヌクレオチドの欠失；2) PD-L2遺伝子への1またはそれ以上のヌクレオチドの付加；3) PD-L2遺伝子の1またはそれ以上のヌクレオチドの置換；4) PD-L2遺伝子の染色体転位；5) PD-L2遺伝子のメッセンジャーRNA転写のレベルでの変化；6) PD-L2遺伝子、例えばゲノムDNAのメチル化パターンの異常な変化；7) PD-L2遺伝子のメッセンジャーRNA転写物の非野生型スプライシングパターンの存在；8) PD-L2ポリペプチドの非野生型レベル；9) PD-L2遺伝子の対立遺伝子欠失、および10) PD-L2ポリペプチドの翻訳後の不適当な修飾、の少なくとも一つの存在を確認することにより検出することができる。本明細書に記載するように、PD-L2遺伝子の変化を検出するのに用いることができる、当該分野で公知の多数のアッセイ法が存在する。好ましい生物試料は、対象から慣用的手段により単離された組織または血清試料である。

【0252】

ある具体例において、変化の検出には、ポリメラーゼ連鎖反応（PCR）（例えば、U. S. 特許第4,683,195および4,683,202を参照されたい）、例えばアンカーPCRまたはRACE・PCRにおける、あるいは、ライゲーション連鎖反応（LCR）（例えば、Landegran et al. (1988) Science 241: 1077-1080; and Nakazawa et al. (1994) Proc. Natl. Acad. Sci. USA 91: 360-364参照）におけるプローブ／プライマーの使用が含まれ、後者はPD-L2遺伝子における点突然変異を検出するのに特に有用であり得る（Abravaya et al. (1995) Nucleic Acids Res. 23: 675-682を参照されたい）。この方法は、対象から細胞の試料を収集すること、試料の細胞から核酸（例えば、ゲノム、mRNAまたは両方）を単離すること、PD-L2遺伝子（存在する場合）のハイブリダイゼーションおよび増幅が生じる条件下で、PD-L2遺伝子に特異的にハイブリダイズする1またはそれ以上のプライマーと核酸試料を接触させること、次いで増幅産物の存在または不存在を検出すること、あるいは増幅産物のサイズを検出し、および対照試料と長さを比較すること、を特徴とする。PCRおよび／またはLCRは、本明細書に記載する変異を検出するのに用いられる方法のいずれかと組み合わせて予備的増幅ステップとして用いることが望ましいと考えてもよい。

【0253】

別の増幅法には、自己保存配列複製（Guatelli, J. C. et al., (1990) Proc. Natl. Acad. Sci. USA 87: 1874-1878）、転写増幅システム（Kwoh, D. Y. et al. (1989) Proc. Natl. Acad. Sci. USA 86: 1173-1177）、Q-ベータレプリカーゼ（Lizardi, P. M. et al. (1988) Bio-Technology 6: 1197）またはいずれかの他の核酸増幅法が含まれ、この後、当業者に周知の方法を用いる増幅分子の検出を行う。これらの検出スキームは、核酸分子

が非常に少数で存在している場合に核酸分子を検出するのに特に有用である。

【0254】

別の具体例において、試料細胞からのPD-L2遺伝子における変異は、制限酵素開裂パターンの変化により同定することができる。例えば、試料および対照DNAを単離し、増幅し（所望により）、1またはそれ以上の制限エンドヌクレアーゼで消化し、フラグメントの長さのサイズをゲル電気泳動により決定し、そして比較する。フラグメントの長さのサイズに関する試料および対照DNA間の違いが、試料DNAにおける変異を示す。さらに、配列特異的リボザイムを使用して（例えば、U. S. 特許第5, 498, 531号を参照されたい）、リボザイム開裂部位の発生または消失により特異的変異の存在を評価することができる。

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【0255】

他の具体例において、PD-L2の遺伝的変異は、試料および対照の核酸、例えばDNAまたはRNAを、数百または数千のオリゴヌクレオチドプローブを含む高密度アレイにハイブリダイゼーションさせることにより同定することができる（Cronin, M. T. et al. (1996) Human Mutation 7: 244-255; Kozal, M. J. et al. (1996) Nature Medicine 2: 753-759）。例えば、PD-L2の遺伝的変異は、Cronin, M. T. et al（既出）に開示されているような光により生成する（light-generated）DNAプローブを含む二次元アレイ中にて同定することができ、プローブの第一ハイブリダイゼーションアレイを用いて、試料および対照中のDNAの長い鎖を探索し、配列がオーバーラップしているプローブの線状アレイ（array）を作成することにより配列間の塩基変化を同定することができる。この工程により点突然変異の同定が可能となる。この工程を行った後に、検出されたすべての変種または変異に相補的な、小さな、特異化されたプローブアレイを用いることにより特異的変異の特定を可能とする二次ハイブリダイゼーションアレイを用いる。各変異アレイはパラレルなプローブのセットから構成され、一方は野生型遺伝子に相補的であり、もう一方は、変異遺伝子に相補的である。

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【0256】

さらにもう一つの具体例において、当該分野で公知の種々の配列決定反応のいずれかを用いてPD-L2遺伝子を直接配列決定し、試料PD-L2の配列を対応する野生型（対照）の配列と比較することにより変異を検出することができる。配列決定反応の例には、Maxam and Gilbert ((1977) Proc. Natl. Acad. Sci. USA 74: 560) またはSanger ((1977) Proc. Natl. Acad. Sci. USA 74: 5463) により開発された方法に基づくものが含まれる。診断アッセイ ((1995) Biotechniques 19: 448) を行う場合、質量スペクトル分析（例えば、PCT国際公開NO. WO94/16101; Cohen et al. (1996) Adv. Chromatogr. 36: 127-162; およびGriffin et al. (1993) Appl. Biochem. Biotechnol. 38: 147-159を参照されたい）を含む種々の自動配列決定法のいずれかを用いることができることも考えられる。

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【0257】

PD-L2遺伝子の変異を検出するための他の方法には、開裂試薬からの保護を用いてRNA/RNAまたはRNA/DNAヘテロ二本鎖におけるミスマッチ塩基対を検出する方法が含まれる（Myers et al. (1985) Science 230: 1242）。一般に、「ミスマッチ開裂」の当該分野における方法は、野生型PD-L2配列を含む（標識された）RNAまたはDNAを、組織試料から得られる潜在的に変異したRNAまたはDNAとハイブリダイズさせることにより形成されるヘテロ二本鎖を提供することで始まる。二本鎖の分子を、対照および試料鎖間の塩基対ミスマッチのために存在しているであろう二本鎖分子の一本鎖領域を開裂する薬剤を用いて処理する。例えば、RNA/DNA二本鎖はRNaseで処理することができ、およびDNA/DNAハイブリッドはS1ヌクレアーゼで処理してミスマッチ領域を酵素消化することができる。他の具体例

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では、ミスマッチ領域を消化するために、DNA/DNAまたはRNA/DNA二本鎖のいずれかをヒドロキシルアミンまたは四酸化オスミウム、およびピペリジンで処理することができる。ミスマッチ領域の消化後、生じた物質を変性ポリアクリルアミドゲル上でサイズ分けし、変異の部位を決定する。例えば、Cotton et al. (1988) Proc. Natl. Acad. Sci. USA 85: 4397; Saleeb et al. (1992) Methods Enzymol. 217: 286-295を参照されたい。好ましい具体例において、対照DNAまたはRNAを、検出のために標識することができる。

【0258】

さらにもう1つの具体例において、ミスマッチ開裂反応は、二本鎖DNAにおけるミスマッチ塩基対を認識する1またはそれ以上の蛋白（いわゆる「DNAミスマッチ修復」酵素）を、細胞の試料から得られたPD-L2における点突然変異を検出およびマッピングするために規定されたシステムにおいて利用する。例えば、イー・コリのmutY酵素はG/AミスマッチでAを開裂し、ヒラ細胞由来のチミジンDNAグリコシラーゼはG/TミスマッチでTを開裂する（Hsu et al. (1994) Carcinogenesis 15: 1657-1662）。典型的な具体例に従い、PD-L2配列、例えば野生型PD-L2配列に基づくプローブを、試験細胞からのcDNAまたは他のDNA産物にハイブリダイズさせる。二本鎖をDNAミスマッチ修復酵素で処理し、そして開裂産物を、それが存在する場合には、電気泳動プロトコールなどから検出することができる。例えば、U. S. 特許第5, 459, 039号を参照されたい。

【0259】

他の具体例において、電気泳動の移動度の変化を用いてPD-L2遺伝子における変異を同定する。例えば、一本鎖構造遺伝子多型(SSCP)を用いて変異型および野生型核酸間の電気泳動の移動度の違いを検出してもよい（Orita et al. (1989) Proc. Natl. Acad. Sci. USA: 86: 2766; Cotton (1993) Mutat. Res. 285: 125-144; およびHayashi (1992) Genet. Anal. Tech. Appl. 9: 73-79）。試料および対照のPD-L2核酸の一本鎖DNAフラグメントを変性させ、次いで復元させる。一本鎖核酸の二次構造は、配列によって異なり、電気泳動の移動度に関して結果的に生じる変化から、わずか一つの塩基変化も検出することができる。DNAフラグメントは標識プローブを用いて標識または検出することができる。アッセイの感度は、(DNAよりも)二次構造が配列の変化に対してよりセンシティブなRNAを用いることにより高めることができる。好ましい具体例では、目的の方法は、ヘテロ二本鎖の分析を利用して、電気泳動の移動度の変化に基づいて二本鎖のヘテロ二本鎖分子を分離するものである（Keen et al. (1991) Trends Genet 7: 5）。

【0260】

さらにもう1つの具体例において、変性剤の勾配を含むポリアクリルアミドゲル中での変異型または野生型フラグメントの移動を、変性勾配ゲル電気泳動(DGGE)を用いてアッセイする（Myers et al. (1985) Nature 313: 495）。DGGEを分析法として用いる場合、例えば約40bpの高融点のGCに富むDNAのGCクランプをPCRにより追加することによりDNAを修飾して、DNAが完全に変性しないことを保証する。さらなる具体例では、温度勾配を変性勾配の代わりに用いて、対照と試料DNAの移動度の違いを同定する（Rosenbaum and Reissner (1987) Biophys. Chem. 265: 12753）。

【0261】

点突然変異を検出するための他の方法の例には、選択的オリゴヌクレオチドハイブリダイゼーション、選択的増幅または選択的プライマー伸長が包含されるが、これらに限らない。例えば、知られた変異が中心に位置するオリゴヌクレオチドプライマーを調製し、次いで完全なマッチが認められる場合にのみハイブリダイゼーションを許容する条件下で標的DNAにハイブリダイズさせることができる（Saiki et al. (1986) N

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ature 324: 164); Saiki et al. (1989) Proc. Natl. Acad. Sci. USA 86: 6230)。オリゴヌクレオチドがハイブリダイジング膜に結合し、そして標識された標的DNAとハイブリダイズする場合、そのような対立遺伝子特異的オリゴヌクレオチドは、PCR増幅された標的DNAまたは多くの異なる変異体にハイブリダイズする。

【0262】

別法として、選択的PCR増幅に依存する対立遺伝子特異的増幅法を、本発明と組み合わせ用いてよい。特異的増幅のためのプライマーとして用いられるオリゴヌクレオチドは、興味ある変異を分子の中心に有していてもよく（その結果、増幅はディファレンシャルハイブリダイゼーションに依存する）(Gibbs et al. (1989) Nucleic Acids Res. 17: 2437-2448)、あるいは、適当な条件下でミスマッチによりポリメラーゼの伸張が妨げられ、または減じられ得る場合には、ある種のプライマーの3'末端に興味ある変異を有していてもよい(Prossner et al. (1993) Tibtech 11: 238)。さらに、新規な制限酵素部位を変異の領域に導入して、切断に基づいて検出を行うことが望ましい可能性もある(Gaspardini et al. (1992) mol. Cell Probes 6:1)。ある具体例において、増幅用Taqリガーゼを用いて増幅を行ってもよいことが予想される(Barany (1991) Proc. Natl. Acad. Sci. USA 88: 189)。そのような場合、ライゲーションは、5'配列の3'末端に完全なマッチ(match)が存在する場合にのみ起こり、増幅の存在または不在を調べることでにより特定部位での知られた変異の存在を検出することができる。

【0263】

例えば、本明細書に記載する少なくとも一つのプローブ核酸または抗体試薬を含む予め包装された診断用キットであって、例えばPD-L2遺伝子に関与する疾患または病気の兆候または家族歴を示している患者を診断するために臨床環境で簡便に用いることができる診断用キットを利用することにより、本明細書に記載した方法を行うことができる。さらに、PD-L2を発現するあらゆる細胞タイプまたは組織を本明細書に記載する予後アッセイに用いてよい。

【0264】

3. 臨床試験の間の効果のモニタリング

PD-L2ポリペプチドの発現または活性に対する作用剤（例えば、薬剤）の影響（例えば、細胞増殖および／または移動のモジュレーション）のモニタリングを、基本的な薬剤スクリーニングのみならず臨床試験に適用することができる。例えば、本明細書記載のスクリーニングアッセイにより決定された作用剤の、PD-L2遺伝子発現、ポリペプチドレベルを増大させ、あるいはPD-L2活性をアップレギュレーションする有効性を、PD-L2遺伝子発現、ポリペプチドレベルの低下、あるいはPD-L2活性のダウンレギュレーションを示す対象の臨床試験においてモニターすることができる。別法として、スクリーニングアッセイにより決定された作用剤の、PD-L2遺伝子発現、ポリペプチドレベルを減少させ、あるいはPD-L2活性をダウンレギュレーションする有効性を、PD-L2遺伝子発現、ポリペプチドレベル、またはPD-L2活性の上昇を示す対象の臨床試験においてモニターすることができる。かかる臨床試験において、PD-L2遺伝子、および好ましくは例えばPD-L2関連疾患に関連する他の遺伝子の発現または活性を、特定細胞の表現型の「リードアウト(read out)」またはマーカーとして用いることができる。

【0265】

例えば、そして限定するものではないが、PD-L2活性をモジュレーションする作用剤（例えば、化合物、薬剤または小型分子）（例えば、本明細書記載のスクリーニングアッセイにおいて同定される）での処理により細胞においてモジュレーションされるPD-L2などの遺伝子を同定することができる。かくして、例えば臨床試験において、PD-L2関連疾患（例えば、誤調節されたPD-1活性により特徴づけられる）に対する作用剤

の影響を研究するために、細胞を単離し、RNAを調製し、PD-L2およびPD-L2関連疾患に關与する他の遺伝子の発現レベルをそれぞれ分析することができる。本明細書記載のノーザンブロット分析またはRT-PCRにより、あるいは別法として産生ポリペプチド量を測定することにより、本明細書に記載の方法の1つにより、あるいはPD-L2または他の遺伝子の活性のレベルを測定することにより、遺伝子発現のレベル（例えば、遺伝子発現パターン）を定量することができる。このようにして、遺伝子発現パターンは、作用剤に対する細胞の生理学的応答を示すマーカーとして役立ちうる。したがって、個体を作用剤で治療する前および治療中の種々に時点においてこの応答状態を調べてもよい。

【0266】

好ましい具体例において、本発明は、作用剤（例えば、本明細書記載のスクリーニングアッセイにより同定されたアゴニスト、アンタゴニスト、ペプチド模倣物、ポリペプチド、ペプチド、核酸、小型分子、または他の薬剤候補）での対象の治療の有効性をモニタリングする方法を提供し、該方法は、（i）作用剤の投与の前に対象から投与前試料を得る；（ii）投与前試料中のPD-L2ポリペプチド、mRNA、またはゲノムDNAの発現レベルを調べる；（iii）対象から1またはそれ以上の投与後試料を得る；（iv）PD-L2ポリペプチド、mRNA、またはゲノムDNAの発現または活性のレベルを調べる；（v）投与前試料中のPD-L2ポリペプチド、mRNA、またはゲノムDNAの発現または活性のレベルと、投与後試料中のPD-L2ポリペプチド、mRNA、またはゲノムDNAの発現または活性のレベルを比較する；次いで（vi）適宜、対象に対する作用剤の投与を変更する工程を含む。例えば、作用剤の投与増加は、PD-L2の発現または活性を検出レベルよりも高いレベルにまで上昇させる、すなわち、作用剤の有効性を高めるために望ましい。あるいはまた、作用剤の投与減少は、PD-L2の発現または活性を検出レベルよりも低いレベルにまで低下させる、すなわち、作用剤の有効性を低くするために望ましい。かかる具体例によれば、観察可能な表現型応答の不存在下においてさえも、PD-L2活性または発現を作用剤の有効性のインジケータとして用いることができる。

【0267】

D. 治療方法：

本発明は、不十分または過剰なPD-L2蛋白の産生あるいはPD-L2野生型蛋白と比較して低いまたは異常な活性を有するPD-L2蛋白形態の産生により特徴付けられる疾病の危険性のある（感受性のある）対象の予防的ならびに治療的処置方法を提供する。そのうえ、本発明の抗-PD-L2抗体を用いてPD-L2蛋白を検出し単離し、PD-L2蛋白の生体利用性を調節し、例えばPD-L2とPD-1との相互作用をモジュレーションすることによりPD-L2活性をモジュレーションすることができる。

【0268】

1. 予防方法

1の態様において、本発明は、PD-L2ポリペプチドあるいはPD-L2ポリペプチド発現または少なくとも1つのPD-L2活性をモジュレーションする薬剤を対象に投与することによる、対象において異常なPD-L2発現または活性に関連する疾患または病状を防ぐ方法を提供する。異常なPD-L2発現または活性により誘発されるかまたはそれに起因する疾患の危険がある対象は、例えばここに記載されている診断または予後アッセイのいずれかまたは組み合わせにより確認され得る。予防薬の投与は、疾患または障害を阻止するかまたは他の場合その進行を遅らせるように、PD-L2の異常を特徴とする徴候が現れる前に行うことができる。PD-L2の異常または症状のタイプにより、例えば、PD-L2ポリペプチド、PD-L2アゴニストまたはPD-L2アンタゴニスト薬剤を、対象処置のために使用することができる。適当な薬剤は、臨床的徴候に基いて決定することができる、例えば本明細書に記載されているスクリーニングアッセイを用いて確認することができる。

【0269】

2. 治療方法

本発明のもう1つの態様は、治療目的でPD-L2の発現または活性またはその本来の結合パートナー、例えばPD-1との相互作用をモジュレーションする方法に関する。PD-L2は、活性化免疫細胞の同時刺激および増殖を阻害し、PD-1を介して阻害性シグナルを免疫細胞に伝達することが示されている。したがって、PD-L2の活性および／または発現、ならびにPD-L2とPD-1との間の相互作用をモジュレーションして、免疫応答をモジュレーションすることができる。PD-L2は阻害性受容体に結合するので、PD-L2活性のアップレギュレーションは免疫応答のダウンレギュレーションを引き起こし、PD-L2活性のダウンレギュレーションは免疫応答のアップレギュレーションを引き起こす。好ましい具体例において、PD-L2は阻害性受容体に結合する。特に好ましい具体例において、PD-L2はPD-1に結合する。 10

【0270】

本発明のモジュレーション方法は、PD-L2ポリペプチドまたは細胞に結合したPD-L2ポリペプチドの1またはそれ以上の活性をモジュレーションする作用剤と細胞とを接触させることを含む。作用剤は、例えば、PD-L2の発現または活性をモジュレーションするもの、および／またはPD-L2とその本来の結合パートナーとの相互作用をモジュレーションするものである。好ましい具体例において、結合パートナーはPD-1である。PD-L2ポリペプチド活性をモジュレーションする作用剤は本明細書に記載の作用剤、例えば、核酸またはポリペプチド、PD-L2ポリペプチドの本来の結合パートナー（例えば、PD-1）、PD-L2抗体、PD-L2アゴニストまたはアンタゴニスト、PD-L2アゴニストまたはアンタゴニストのペプチド模倣物、あるいは他の小型分子でありうる。可溶性形態のPD-L2を用いて、PD-1のその本来の結合パートナーまたはリガンドへの結合を妨害してもよい。 20

【0271】

PD-L2の発現をモジュレーションする作用剤は、例えば、アンチセンス核酸分子、三重鎖オリゴヌクレオチド、リボザイム、またはPD-L2ポリペプチド発現のための組み換えベクターである。例えば、PD-L2翻訳開始部位周辺領域に相補的なオリゴヌクレオチドを合成することができる。典型的には200 μg/mlの1またはそれ以上のアンチセンスオリゴヌクレオチドを細胞培地に添加することができ、あるいは1またはそれ以上のアンチセンスオリゴヌクレオチドを患者に投与してPD-L2ポリペプチドの合成を防止することができる。アンチセンスオリゴヌクレオチドは細胞により摂取され、PD-L2 mRNAとハイブリダイゼーションし、翻訳を妨害する。別法として、二本鎖DNAに結合して三重鎖構築物を構成し、DNAの巻き戻しおよび転写を妨害するオリゴヌクレオチドを用いることもできる。いずれにせよ結果として、PD-L2ポリペプチドの合成がブロックされる。PD-L2発現がモジュレーションされる場合、かかるモジュレーションはPD-L2遺伝子のノックアウト以外の手段により引き起こされる。 30

【0272】

作用剤が細胞におけるPD-L2量を制御するという事実により、発現をモジュレーションする作用剤はまた細胞におけるPD-L2活性の全量をモジュレーションする。1の具体例において、PD-L2をモジュレーションする作用剤は、1またはそれ以上のPD-L2活性を刺激する。かかる刺激剤の例は、活性PD-L2ポリペプチドおよび細胞に導入されたPD-L2をコードする核酸分子を包含する。もう1つの具体例において、作用剤は1またはそれ以上のPD-L2活性を阻害する。好ましい具体例において、作用剤はPD-L2とその本来の結合パートナーとの間の相互作用を阻害または促進する。特に好ましい具体例において、結合パートナーはPD-1である。かかる阻害剤の例は、アンチセンスPD-L2核酸分子、抗PD-L2抗体、PD-L2阻害剤、および主題のスクリーニングアッセイにおいて同定される化合物を包含する。さらに好ましい具体例において、阻害剤は抗PD-L2抗体と抗PD-1抗体の組み合わせである。 40

【0273】

これらのモジュレーション方法をインビトロで行なうことができ（例えば、細胞を作用剤 50

と接触させることにより)、あるいはまた、インビボで作用剤と細胞を接触させることにより行なうこともできる(例えば、作用剤を対象に投与することにより)。そのようなものとして、本発明は、PD-L2ポリペプチドのアップレギュレーションまたはダウンレギュレーションから利益を受けるであろう症状または疾病、例えば、PD-L2ポリペプチドまたは核酸分子の望ましくない、不十分な、あるいは異常な発現または活性により特徴付けられる疾病にかかっている個体の治療方法を提供する。1の具体例において、該方法は、PD-L2発現または活性をモジュレーション(例えば、アップレギュレーションまたはダウンレギュレーション)する作用剤(例えば、本明細書記載のスクリーニングアッセイにより同定される作用剤)または作用剤の組み合わせを投与することを含む。もう1つの具体例において、該方法は、PD-L2ポリペプチドまたは核酸分子を治療薬として投与して、低下した、異常な、あるいは望ましくないPD-L2発現または活性を補償することを含む。

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【0274】

PD-L2活性の刺激は、PD-L2が異常にダウンレギュレーションされる状況、および/または増大したPD-L2活性が有益な効果をもたらす可能性のある状況において望ましい。同様に、PD-L2活性の阻害は、PD-L2が異常にアップレギュレーションされる状況、および/または減少したPD-L2活性が有益な効果をもたらす可能性のある状況において望ましい。

【0275】

PD-L2をダウンレギュレーションするのに使用される典型的な作用剤(すなわち、PD-L2アンタゴニスト)は、例えば、アンチセンス核酸、PD-L2を認識しブロックする抗体、PD-L2を認識しブロックする抗体とPD-1を認識しブロックする抗体との組み合わせ、ならびにPD-L2と免疫細胞上のその本来の結合パートナーとの相互作用をブロックする化合物(例えば、可溶性、1価のPD-L2分子;抗原提示細胞上のFc受容体に結合しない可溶性形態のPD-L2;可溶性形態のPD-L2結合パートナー;または主題のスクリーニングアッセイにおいて同定される化合物)を包含する。好ましい具体例において、結合パートナーはPD-1である。PD-L2をダウンレギュレーションするのに使用される典型的な作用剤(すなわち、PD-L2アゴニスト)は、例えば、PD-L2ポリペプチドをコードする核酸分子、多価形態のPD-L2、PD-L2の発現を増加させる化合物、PD-L2とその本来の結合パートナー(例えば、PD-1)との相互作用を促進する化合物、ならびにPD-L2を発現する細胞を包含する。

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【0276】

3. 免疫応答のダウンレギュレーション

PD-L2ポリペプチドの阻害的機能をアップレギュレーションすることにより免疫応答をダウンレギュレーションする本発明の具体例が多く存在する。ダウンレギュレーションは、すでに進行している免疫応答を阻害または抑制する形態であってもよく、あるいは免疫応答の誘導を妨害することを含むものであってもよい。免疫細胞応答をダウンレギュレーションすることにより、あるいは免疫細胞における特異的アネルギーを誘導することにより、あるいはそれら両方により、活性化された免疫細胞の機能を阻害することができる。

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【0277】

例えば、PD-L2が阻害性受容体、例えば、PD-1に結合する具体例において、阻害性受容体に結合するPD-L2の形態、例えば、細胞上の多価PD-L2を用いて免疫応答をダウンレギュレーションすることができる。同様に、さらなる作用剤、例えば、PD-L1とPD-1との相互作用をブロックする作用剤を用いることにより、PD-L2-PD-1相互作用を促進し、さらに免疫応答をダウンレギュレーションすることもできる。

【0278】

1の具体例において、本発明は、PD-L2活性を刺激するために用いられる活性化抗体は二特異的(bispecific)抗体である。例えば、かかる抗体は、PD-L2結

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合部位および免疫細胞、例えばT細胞、B細胞またはミエロイド細胞上の細胞表面受容体を標的とするもう1つの結合部位を含むものであってよい。1の具体例において、かかる抗体は、PD-L2結合部位のほかに、B細胞抗原受容体、T細胞抗原受容体、またはFc受容体に結合する結合部位をさらに含んでいて、特定の細胞集団に対する分子を標的とするものであってもよい。二特異的抗体に対するこの第2の抗原の選択は、阻害されるべき細胞集団の選択に柔軟性を与える。

【0279】

PD-L2活性を促進する作用剤、あるいはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を促進する作用剤（例えば、PD-L2活性化抗体またはPD-L2活性化小型分子）を、免疫細胞増殖および／またはエフェクター機能を抑制する能力、あるいはインビトロアッセイに付け加えられる場合にはアネルギー誘導能により同定することができる。例えば、活性化受容体を介してシグナルトランスダクションを刺激する作用剤の存在下において細胞を培養することができる。多くの当該分野で認識された細胞活性化のリードアウトを用いて、活性化剤の存在下において例えば細胞増殖またはエフェクター機能（例えば、抗体産生、サイトカイン産生、ファゴサイトーシス）を測定することができる。この活性をブロックする試験作用剤の能力を、測定すべき増殖またはエフェクター機能の低下に対する作用剤の影響能を測定することにより決定することができる。1の具体例において、低い抗原濃度において、PD-L2-PD-1相互作用は強力なB7-CD28シグナルを阻害する。もう1つの具体例において、高い抗原濃度において、PD-L2-PD-1相互作用はサイトカイン産生を減少させるが、T細胞増殖を阻害しない。したがって、異なった抗原濃度においてサイトカイン産生および／または増殖を測定することにより、活性をブロックする試験化合物の能力を決定することができる。

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【0280】

本発明の1の具体例において、抗原とPD-L2アゴニストとの同時投与により特異的抗原に対する寛容が誘導される。例えば、特定のポリペプチドに対して寛容を誘導することができる。1の具体例において、免疫応答が望ましくないアレルゲンまたは外来ポリペプチドに対する免疫応答を抑制することができる。例えば、因子V I I Iを受け取った患者は高い頻度でこの凝血因子に対する抗体を産生する。PD-L2活性、またはその本来の結合パートナー、例えばPD-1との相互作用を刺激する作用剤と組換え因子V I I Iとの同時投与（または例えば架橋による因子V I I IへのPD-L2の物理的結合）は免疫応答のダウンレギュレーションを引き起こす可能性がある。

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【0281】

1の具体例において、PD-L2アゴニストおよび免疫細胞上の同時刺激受容体の活性をブロックする別の作用剤を用いて免疫応答をダウンレギュレーションすることができる。典型的な分子は下記のを包含する：アゴニスト形態の他のPD-1リガンド（例えば、PD-L1）、可溶性形態のCTLA-4、抗-B7-1抗体、抗-B7-2抗体、またはそれらの組み合わせ。あるいはまた、2つの別個のペプチド（例えば、PD-L2ポリペプチドとブロック形態のB7-2および／またはB7-1）、または抗体の組み合わせ（例えば、PD-L2ポリペプチドに対する活性化抗体とブロック抗-B7-2および／またはB7-1モノクローナル抗体）を1つの組成物にまとめて、あるいは別個に投与（同時投与または逐次投与）して、対象における免疫細胞により媒介される免疫応答をダウンレギュレーションすることができる。さらにそのうえ、PD-L2ポリペプチド活性を有する治療的に活性のある量の1またはそれ以上のペプチドを、B7-1および／またはB7-2活性を有する1またはそれ以上のポリペプチドと一緒に、他のダウンレギュレーション薬剤とともに使用して免疫応答に影響を及ぼすことができる。他の免疫モジュレーション薬剤の例は、同時刺激シグナルをブロックする抗体（例えば、CD28またはICOSに対するもの）、CTLA4を介して阻害性シグナルを活性化させる抗体、および／または他の免疫細胞マーカーに対する抗体（CD40、CD40リガンド、またはサイトカインに対するもの）、融合蛋白（例えば、CTLA4-FcまたはPD-1-Fc）、ならびに免疫抑制剤（例えば、ラパマイシン、サイクロスポリンA、またはF

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K 5 0 6) を包含する。

【0 2 8 2】

P D - L 2 ポリペプチドは、細胞の破壊により免疫細胞機能をブロックする治療薬の構築においても有用でありうる。例えば、P D - L 2 ポリペプチドの部分をトキシンに連結して、結合すべき細胞の破壊の引き金を引くことのできる細胞毒性作用剤を作成することができる。

【0 2 8 3】

細胞毒性作用剤を作成するために、当該分野で知られた方法、例えば、架橋または組換え D N A 法により本発明のポリペプチドをトキシンに連結あるいは作動可能に結合させてもよい。一般的には、免疫トキシンの調製は当該分野においてよく知られている（例えば、
10 米国特許第 4 3 4 0 5 3 5 号および欧州特許第 4 4 1 6 7 号、両方を参照により本明細書に一体化させる）。多くのタイプのジスルフィド結合含有リンカーが知られており、それらをうまく用いてトキシン部分とポリペプチドとを抱合させることができる。1 の具体例において、インビボにおいてより高い安定性があり、それゆえ作用部位における結合の前のトキシン放出を防止するので、立体的に「隠れた」ジスルフィド結合含有リンカーが好ましい。

【0 2 8 4】

広範なトキシンが当該分野で知られており、それらを本発明のポリペプチドまたは抗体に抱合させてもよい。例は下記のものを含む：多くの有用な植物、真菌または細菌由来のトキシン。それらは、例えば、以下のものを含む：種々の A 鎖トキシン、特にリシン A 鎖；サポニンまたはゲロニンのごときリボソーム不活性化蛋白；アルファールサルシン；アスペルギリン；レストリクトシン；および胎盤リボヌクレアーゼのごときリボヌクレアーゼ、血管形成剤、ジフテリアトキシン、あるいはシュードモナス外毒素。本発明における使用に好ましいトキシン部分は、炭水化物残基を修飾または除去するように処理されたトキシン A 鎖、脱糖鎖 A 鎖（米国特許第 5 7 7 6 4 2 7 号）である。
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【0 2 8 5】

かかる細胞毒性作用剤の 1 またはそれ以上の組み合わせ（後、P D - L 2 - リシン単独または P D - L 1 - リシンとの組み合わせ）を患者に輸液することは、特に、活性化免疫細胞が多量の P D - L 2 結合パートナー、例えば P D - 1 を産生するという事実を考慮すれば、免疫細胞の死滅を引き起こしうる。例えば、P D - 1 は活性化リンパ球の表面上で誘導されるので、P D - L 2 ポリペプチドを用いて、F c - R 依存的機構によりあるいは P D - L 2 への細胞毒性薬剤（例えば、リシン、サポニン、またはカリヘアミシン）を抱合させることによる剥奪により、これらの特定細胞の枯渇を標的とすることができる。もう 1 つの具体例において、トキシンを抗 - P D - L 2 抗体に抱合させて、P D - L 2 を発現する抗原提示細胞の死滅を標的とすることができる。さらなる具体例において、P D - L 2 抗体 - トキシンは二特異的抗体でありうる。かかる二特異的抗体は、例えば、特定タイプの細胞上、例えば、B リンパ球、単球、樹状細胞、またはランゲルハンス細胞上にのみ見出されるマーカーを用いて、特定の細胞集団を標的化するのに有用である。
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【0 2 8 6】

P D - L 2 活性または P D - L 2 - P D - 1 相互作用の活性化による免疫応答のダウンレギュレーション（かくして P D - 1 の負のシグナリング機能を刺激する）は、免疫応答、例えば、in situでの組織、皮膚および器官移植、対宿主移植片疾患（G V H D）、またはアレルギー、または全身性エリトマトーデスおよび多発性硬化症のごとき自己免疫疾患における免疫応答のダウンレギュレーションに有用である。典型的には、組織移植において、移植片拒絶反応は、免疫細胞がそれを外来物と認識しすることから生じ、次いで、移植片を破壊する免疫反応が起こる。P D - L 2 の活性または免疫細胞上の本来の結合パートナー（例えば P D - 1）と P D - L 2 との相互作用を促進する分子（例えば、可溶性、多価形態の P D - L 2 ポリペプチド）の投与は、移植の前の時点で、単独あるいは別のダウンレギュレーション作用剤と一緒に、同時刺激シグナルの発生を阻害する。そのうえ、P D - L 2 活性または P D - L 2 - P D - 1 相互作用の促進（よって、P
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D-1の阻害性シグナル)は免疫細胞に拮抗するに十分であり得、そのことにより対象に寛容が誘導される。PD-1により媒介される阻害性シグナルを促進することによる長期寛容の誘導は、これらの活性化剤の繰り返し投与の必要性を回避しうる。

【0287】

十分な免疫抑制または寛容を対象中で達成するには、他の分子の同時刺激機能をブロックすることが望ましいかもしれない。例えば、移植前の時点で、これらの抗原の各々の活性を有する可溶性形態のペプチドの組み合わせ、あるいはこれらの抗原に対するブロッキング抗体を投与することにより(別個にあるいは1の組成物として)、B7-1およびB7-2の機能をブロックすることが望ましいかもしれない。別法として、PD-L2の阻害活性を促進し、B7-1および/またはB7-2の同時刺激活性を阻害することが望ましいかもしれない。本発明のダウンレギュレーション法と一緒に使用されうる他のダウンレギュレーション作用剤は、例えば、CTLA4を介して阻害性シグナルを伝達する作用剤、可溶性形態のCTLA4、CTLA4を介して阻害性シグナルを活性化する抗体、他の免疫細胞マーカーに対するブロッキング抗体、または可溶性形態の他の受容体リガンドペアー(例えば、CD40とCD40リガンドとの間の相互作用を破壊する作用剤(例、抗CD40リガンド抗体))、サイトカインに対する抗体、または免疫抑制剤を包含する。

【0288】

例えば、PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を活性化させること(かくして該結合パートナーの阻害性機能)は、自己免疫疾患の治療において有用でありうる。多くの自己免疫疾患は、自己組織に対し上反応性があり、疾病の病理に関与するサイトカインおよび自己抗体の産生を促進する免疫細胞の不適切な活性化の結果である。自己反応性免疫細胞の活性化を防止することは疾病の徴候を減少または除去しうる。PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を活性化させる作用剤の投与は、疾病からの長期寛解を誘導しうる自己反応性免疫細胞の抗原特異的寛容を誘導しうる。さらに、B7分子と同時刺激受容体との受容体-リガンド相互作用を破壊することにより免疫細胞の同時刺激をブロックする作用剤の同時投与は、免疫細胞活性化において有用であり得、その結果、疾病プロセスに関与しうる自己抗体またはサイトカインの産生が防止される。自己免疫疾患を防止または改善することにおける薬剤の有効性を、ヒト自己免疫疾患に関する十分に特徴付けられた多くの動物モデルを用いて調べることができる。例は、ネズミ実験自己免疫脳炎、MRL/lpr/lprマウスまたはNZBハイブリッドマウスにおける全身性エリトマトーデス、ネズミ自己免疫コラーゲン関節炎、NODマウスおよびBBラットにおける真性糖尿病、ならびにネズミ実験重症筋無力症(Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856参照)を包含する。

【0289】

免疫細胞活性化の阻害は、例えば、IgE産生を阻害することにより、アレルギーおよびアレルギー性反応の治療において有用である。PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を促進する作用剤をアレルギーの対象に投与して、対象における免疫細胞により媒介されるアレルギー性応答を阻害することができる。PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を刺激することを、適当なMHC分子と一緒にアレルゲンに曝露することにより行なうことができる。アレルゲンの侵入経路および肥満細胞または好塩基球上のIgE沈着パターンにより、アレルギー性反応は全身的または局所的な性質であってよい。かくして、PD-L2活性またはPD-L2-PD-1相互作用を促進する作用剤の投与により、免疫細胞により媒介されるアレルギー性応答を全身的または局所的に阻害することができる。

【0290】

PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用の刺激による免疫細胞活性化の阻害は、免疫細胞の病原体感染(ウイルスまたは細菌

による)において治療的に重要でもある。例えば、後天性免疫不全症候群(AIDS)において、免疫細胞活性化によりウイルス複製が刺激される。PD-L2活性またはPD-L2-PD-1相互作用の刺激はウイルス複製を阻害する可能性があり、そのことによりAIDSの経過が改善されうる。

【0291】

PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用の刺激による免疫応答のダウンレギュレーションは、妊娠の維持を促進することにおいても有用でありうる。通常には、PD-L2は胎盤栄養芽胞、すなわち母体と胎児との間の界面を形成する層において高発現され、胎児の母体拒絶反応を防止する役割を果たしている。胚または胎児の免疫学的拒絶反応により自発的流産の危険のあるメス(例えば、

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【0292】

PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用の刺激による免疫応答のダウンレギュレーションは、自己由来の組織の自己免疫攻撃の治療にも有用でありうる。例えば、通常には、PD-L2は心臓で高発現され、心臓を自己免疫攻撃から防御する。このことは、Balb/c PD-1ノックアウトマウスが激しい自己免疫攻撃により心臓に血栓を生じるという事実により明らかである。かくして、自己免疫攻撃により引き起こされあるいは悪化させられる症状(好ましくは例において、心臓疾患、心筋梗塞またはアテローム性動脈硬化)が、PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用の増加により改善または軽快されうる。それゆえ、自己免疫疾患のごとき自己免疫攻撃により悪化させられる症状(ならびに心臓疾患、心筋梗塞またはアテローム性動脈硬化のごとき症状)を、PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を刺激することによりモジュレーションすることは本発明の範囲内である。

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【0293】

さらなる具体例において、本明細書記載のいずれかの方法を実施する場合、PD-L2およびPD-L1の両方の活性を刺激することにより免疫応答をダウンレギュレーションすることは本発明の範囲内である。PD-L1はさらなるPD-1リガンドであり、同時係属の米国出願第09/644934号;国際公開第WO01/14557号;Dong, H. et al. (1999) Nat. Med. 5: 1365-1369;およびFreeman, G. J. et al. (2000) J. Exp. Med. 192: 1027-1034に記載されており、それぞれの内容を参照により本明細書に一体化させる。さらなる具体例において、本明細書記載のいずれかの方法を実施する場合、1またはそれ以上のさらなる薬剤を投与することにより免疫応答をダウンレギュレーションすることは本発明の範囲内である。例えば、免疫応答をダウンレギュレーションすることが知られている他の作用剤を、PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を刺激する作用剤と一緒に使用することができる。

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【0294】

4. 免疫応答のアップレギュレーション

PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用の阻害は、免疫応答をアップレギュレーションする手段として、治療においても有用である。免疫応答のアップレギュレーションは、存在している免疫応答を高めるかまたは初期免疫応答を誘導する形態であり得る。例えば、PD-L2活性またはPD-L2-PD-1相互作用の阻害により免疫応答を促進することは、微生物、例えば細菌、ウイルス、または寄生体の感染の場合において、あるいは免疫抑制の場合において有用でありうる。例えば、1の具体例において、PD-L2活性またはPD-L2-PD-1相互作用を阻害する作用剤、例えば、PD-L2に対する非活性化抗体(すなわち、ブロッキング抗

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体)、PD-L2およびPD-L1に対する非活性化抗体の組み合わせ、あるいは可溶性形態のPD-L2は、より迅速かつ完全なウイルス、細菌または寄生体のクリアランスを生じさせる抗体および細胞により媒介される応答のアプレギュレーションが有益である場合に、*in situ*において治療的に有用である。これらの症状は、ウイルス性皮膚疾患、例えばヘルペスまたは帯状疱疹を包含し、それらの場合において、かかる作用剤を局所的に皮膚にデリバリーすることができる。さらに、インフルエンザ、通常のカゼ、および脳炎のごとき全身性ウイルス疾患を、かかる作用剤を全身投与することにより改善することができる。特定の例において、免疫応答をアプレギュレーションする他の作用剤、例えば、同時刺激受容体を介してシグナルを伝達するB7ファミリーのメンバーの形態の他の作用剤をさらに投与して免疫応答をさらに改善することが望ましいかもしれない。

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【0295】

別法として、免疫細胞を患者から取り、PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を阻害する作用剤と免疫細胞をインビトロで接触させ、次いで、インビトロで刺激された免疫細胞を患者中に再導入することにより、感染患者における免疫応答を促進することができる。もう1つの具体例において、免疫応答を促進する方法は、感染細胞、例えばウイルス感染細胞を患者から単離し、本来の結合パートナー、例えばPD-1に結合できない形態のPD-2をコードする核酸分子でそれらの感染細胞をトランスフェクションして、細胞がその表面にPD-L2分子の全体または一部を発現するようにし、次いで、トランスフェクションされた細胞を患者に再導入することを含む。トランスフェクションされた細胞は阻害性シグナルを妨害し、そのことによりインビボで免疫細胞を活性化しうる可能性がある。

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【0296】

PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を阻害する作用剤を、種々のポリペプチド、例えば、病原体由来のポリペプチドに対するワクチン中に予防的に使用することができる。適当なアジュバント中のPD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を阻害する作用剤とともにウイルスポリペプチドを接種することにより、病原体、例えば、ウイルスに対する免疫を誘導することができる。別法として、病原性抗原およびPD-L2-PD-1相互作用をブロックする形態のPD-L2の両方をコードする遺伝子を含むベクターを接種に使用することもできる。例えば、注射（例えば、筋肉内、経皮注射、あるいは粒子を皮膚に注入するための特殊な加速装置または圧縮空気を用いる遺伝子銃でのDNA被覆金粒子の表皮中への注入）により核酸ワクチンを投与することができる。別法として、非侵襲的手段により核酸ワクチンを投与することもできる。例えば、純粋または脂質処方されたDNAを呼吸器系にデリバリーし、あるいは他の場所、例えば、DNAの経口デリバリーによりパイエル板を標的とすることができる (Schubert (1997) Proc. Natl. Acad. Sci. USA 94:961)。弱毒化微生物を用いて粘膜表面にデリバリーすることができる (Sizemore et al (1995) Science 270:29)。

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【0297】

もう1つの具体例において、ワクチン中の抗原は自己抗原である。かかるワクチンは生物における寛容のモジュレーションにおいて有用である。自己抗原およびPD-L2活性またはPD-L2とその本来の結合パートナー（例えばPD-1）との相互作用をブロックする作用剤での免疫は、寛容を破壊（すなわち、自己抗原に対する寛容を妨害）する可能性がある。かかるワクチンは、アラム (alum) のときアジュバントまたはサイトカイン（例えば、GM-CSF、IL-12、B7-1、またはB7-2）を含んでいてもよい。

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【0298】

1の具体例において、例えば、PD-L2ポリペプチドまたはブロッキング抗体およびMHCクラスIα鎖ポリペプチドおよびβ2ミクログロブリンを同時発現するようにトランスフェクションされた細胞により、PD-L2活性またはPD-L2とその本来の結合パ

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ートナー、例えばPD-1との相互作用を阻害する作用剤をクラスI MHCポリペプチドとともに投与して、T細胞を活性化させ、感染に対する免疫を生じさせることができる。例えば、ワクチンが有用であるウイルス病原体は以下のものを包含する：B型肝炎、C型肝炎、Epstein-Barrウイルス、サイトメガロウイルス、HIV-1、HIV-2、結核、マラリアおよび住血吸虫。

【0299】

もう1つの適用例において、PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用の阻害は、腫瘍免疫の治療において有用でありうる。腫瘍細胞（例えば、肉腫、黒色腫、リンパ腫、白血球、神経芽腫、または癌腫）を、PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を阻害する核酸分子でトランスフェクションすることができる。これらの分子は、例えば、PD-L2に対してアンチセンスである核酸分子であってもよく、あるいは非活性化抗PD-L2抗体または抗PD-L2および抗PD-1抗体の組み合わせをコードするものであってもよい。これらの分子は抗PD-L2抗体および／または抗PD-1抗体の可変領域であってもよい。所望ならば、同時刺激を活性化させる他のポリペプチド（例えば、B7-1またはB7-2）で腫瘍細胞をトランスフェクションすることもできる。トランスフェクションされた腫瘍細胞を患者に戻し、そのことによりPD-L2活性またはPD-L2-PD-1相互作用の阻害（例えば、局所的阻害）が起こる。別法として、遺伝子治療法を用いて標的腫瘍細胞をインビボでトランスフェクションすることもできる。

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【0300】

PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を阻害する作用剤で患者を処置することによって、PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を阻害することにより、腫瘍細胞に対する免疫応答の刺激を行なうことができる。かかる作用剤の好ましい例は、例えば、アンチセンス核酸分子、PD-L2を認識しブロックする抗体、PD-L2を認識しブロックする抗体とPD-L1を認識しブロックする抗体との組み合わせ、ならびに免疫細胞上の本来の結合パートナーとPD-L2との相互作用をブロックする化合物（例えば、可溶性の1価PD-L2分子；抗原提示細胞上のFc受容体に結合しない可溶性形態のPD-L2分子；可溶性形態のPD-L2結合パートナー；および主題スクリーニングにおいて同定される化合物）を包含する。最も好ましい具体例において、PD-L2結合パートナーはPD-1である。

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【0301】

さらに、MHCクラスIまたはMHCクラスII分子を欠くか、または十分な量のMHCクラスIまたはMHCクラスII分子を発現し得ない腫瘍細胞は、MHCクラスIα鎖蛋白およびβ₂ミクログロブリン蛋白またはMHCクラスIIα鎖蛋白およびMHCクラスIIβ鎖蛋白の全部または一部（例えば、細胞質ドメイン末端切断部分）をコードする核酸でトランスフェクションされることにより、細胞表面でMHCクラスIまたはMHCクラスII蛋白を発現し得る。PD-L2阻害性ポリペプチドまたはアンチセンス核酸と共に適当なクラスIまたはクラスII MHCを発現させると、トランスフェクション腫瘍細胞に対してT細胞媒介免疫応答が誘導される。所望により、MHCクラスII関連蛋白、例えば不変鎖の発現をブロックするアンチセンス構築物をコードする遺伝子はまた、PD-L2ポリペプチドをコードするDNAと共にトランスフェクションされることにより、腫瘍関連抗原の提示を促進し、腫瘍特異的免疫性を誘導し得る。B7陰性ネズミ腫瘍細胞によるB7-1の発現は、腫瘍拒絶および長期間防御を伴うT細胞により媒介される特異的免疫を誘導することにより、マウスにおける腫瘍攻撃を行うことが示された（Chen, L. ら（1992）Cell 71, 1093-1102、Townsend, S. E. および Allison, J. P. （1993）Science 259, 368-370、Baskar, S. ら（1993）Proc. Natl. Acad. Sci. 90, 5687-5690）。かくして、ヒト対象における免疫細胞により媒介される免疫応答

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の誘導は、対象において腫瘍特異的寛容を打ち負かすのに十分であり得る。

【0302】

もう1つの具体例において、PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用の阻害により免疫応答を刺激して、すでに存在している寛容を克服することができる。例えば、対象が有意な免疫応答をマウントできない抗原、例えば、腫瘍特異的抗原に対する免疫応答を、PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を阻害する作用剤を投与することにより誘導することができる。PD-1アンタゴニストをアジュバントとして用いて、能動免疫のプロセスにおいて外来抗原に対する応答を増強することができる。

【0303】

1の具体例において、免疫細胞を対象から入手し、PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を阻害する作用剤の存在下、エクスピボで培養してT細胞集団を増大させる。さらなる具体例において、その後、免疫細胞を対象に投与する。例えば、当該分野において知られているように、T細胞に一次活性化シグナルおよび同時刺激シグナルを提供することにより、T細胞をインビトロで刺激して増殖させることができる。また、様々な形態のPD-L2ポリペプチドを用いてT細胞の増殖を同時刺激することもできる。1の具体例において、PCT出願第WO94/29436号記載の方法に従い免疫細胞をエクスピボで培養する。共刺激分子は、可溶性であってもよく、細胞膜に結合されるかまたは固体表面、例えばビーズに結合され得る。

【0304】

さらなる具体例において、本明細書記載のいずれかの方法を実施する場合、1またはそれ以上のさらなる薬剤を投与することにより免疫応答をアップレギュレーションすることは本発明の範囲内である。例えば、サイトカイン、アジュバント、または刺激性形態の同時刺激分子またはそれらのリガンドのごとき免疫応答を刺激することが知られている他の作用剤を、PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用を阻害する作用剤と一緒に使用することができる。例えば、PD-L1を阻害する作用剤（例えば、PD-L1に対するブロック抗体）をPD-L2を阻害する作用剤（例えば、PD-L2に対するブロック抗体）と一緒に使用することができる。PD-L1は同時係属米国出願第09/644934号；国際公開第WO01/14557号；Dong, H. et al. (1999) Nat. Med. 5:1365-1369；およびFreeman, G. J. et al. (2000) J. Exp. Med. 192:1027-1034（各内容を参照により本明細書に一体化させる）に記載されている、さらなるPD-1リガンドである。

【0305】

E. PD-L2活性またはPD-L2-PD-1相互作用のモジュレーションによりモジュレーションされるサイトカインの同定

本明細書記載のPD-L2分子を用いて、PD-L2活性またはPD-L2とその本来の結合パートナー、例えばPD-1との相互作用のモジュレーションに応答した免疫細胞により産生されるサイトカイン、あるいは産生がそのような免疫細胞中で促進されるサイトカインを同定することができる。T細胞は1次活性化シグナルで最適以下にインビトロ刺激され得、例えば、T細胞は、ホルボールエステル、抗CD3抗体または好ましくはMHCクラスII分子を随伴した抗原で刺激されることができ、刺激性形態のGL50抗原により同時刺激シグナルを与えられることができ、例えばGL50ポリペプチドをコードしその表面でペプチドを発現させる核酸でトランスフェクションされた細胞により、または可溶性、刺激性の形態のペプチドにより、同時刺激シグナルを与えられることができる。次いで、細胞を、PD-L2発現細胞と接触させ、そして／あるいはPD-L2-PD-1相互作用を阻害する作用剤（例えば、PD-L2に対する抗体、またはPD-L2に対する抗体とPD-L1に対する抗体との組み合わせ）で処理することができる。培地中に放出された知られたサイトカインを、ELISAにより、あるいはサイトカインをブロックして免疫細胞の増殖または該サイトカインにより誘導される他の細胞タイプの増殖を阻

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害する抗体の能力により同定することができる。例えば、I L - 4 E L I S A キットは I L - 7 ブロッキング抗体の場合と同様ジェンザイム（ケンブリッジ、マサチューセッツ）から入手できる。I L - 9 および I L - 1 2 に対するブロッキング抗体は、ジェネティクス・インスティテュート（ケンブリッジ、マサチューセッツ）から入手できる。次いで、サイトカインのプロファイルに基づいて P D - L 2 活性または P D - L 2 と P D - 1 との相互作用の刺激またはブロッキングの効果を調べることができる。

【0306】

上記のインビトロでの免疫細胞同時刺激アッセイを、P D - L 2 活性または P D - L 2 - P D - 1 相互作用のモジュレーションによりモジュレーションされうる新規サイトカインを同定する方法に使用することができる。例えば、C D 2 8 / C T L A 4 経路の刺激が I L - 2 分泌を促進すると思われる場合、I C O S 経路の刺激は I L - 1 0 分泌を促進すると思われる（H u t t l o f f ら、1 9 9、N a t u r e 3 9 7 : 2 6 3）。同時刺激により誘導される特定の活性、例えば免疫細胞増殖を既知サイトカインに対するブロッキング抗体の添加により阻害できない場合、その活性は未知サイトカインの作用から生じるものである可能性がある。同時刺激後、慣用的方法によりこのサイトカインを培地から精製し、免疫細胞増殖を誘導する活性によりその活性を測定することができる。

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【0307】

寛容の誘導においてある一定の役割を演じ得るサイトカインを同定するために、上記のインビトロ T 細胞共刺激性アッセイを使用することができる。この場合、T 細胞は、1 次活性化シグナルを与えられ、選択されたサイトカインと接触させられるが、共刺激性シグナルは与えられない。免疫細胞を洗浄し、休止させた後、細胞を 1 次活性化シグナルおよび共刺激性シグナルの両方により再攻撃する。免疫細胞が応答（例、サイトカインを増殖または生産）しない場合、それらは寛容にされており、サイトカインは寛容の誘導を妨害していない。しかしながら、免疫細胞が応答する場合、寛容の誘導はサイトカインにより妨害されている。寛容の誘導を妨害し得るそれらのサイトカイン類は、移植レシピエントまたは自己免疫疾患対象において寛容を誘導するより有効な手段として B リンパ球抗原をブロックする試薬と共にインビボでのブロックに関して標的化され得る。例えば、P D - L 2 活性または P D - L 2 - P D - 1 相互作用を促進する作用剤とともにサイトカインブロッキング抗体を投与することができる。

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【0308】

本発明を以下の実施例によりさらに説明するが、実施例は限定的なものとして解釈されるべきではない。本出願中に引用される全文献、特許および公開された特許出願の内容ならびに図および配列表を参照により本明細書に一体化させる。

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【0309】

実施例

下記の材料および方法は実施例 1 ～ 9 に使用された。

【0310】

材料および方法

マウス

H o f f m a n - L a R o c h e, N u t l e y, N J により、I A^d に関連して O V A ペプチド（3 2 3 - 3 3 9）に特異的な T C R 導入遺伝子（D O 1 1. 1 0）が提供された。T a c o n i c F a r m s（G e r m a n t o w n, N Y）から B a l b / c マウスを得た。すべてのマウスは生後 6 ～ 1 2 週目のものを用いた。協会のガイドラインに従ってマウスを世話した。

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【0311】

分子クローニング

P D - L 1 に対して 3 8 % のアミノ酸同一性を有するものとして同定された G e n b a n k 受託番号 A F 1 4 2 7 8 0（本明細書において、マウス P D - L 2 ともいう）をクエリー配列として、ヒト P D - L 1 蛋白配列を用いて、C e n t e r f o r B i o t e c h n o l o g y I n f o r m a t i o n（N C B I）の非重複データベースの B L A S T

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検索を行なった。PD-L1は、同時係属の米国出願第09/644,934号；国際公開第WO 01/14557号；Dong, H. et al. (1999) Nat. Med. 5:1365-1369；およびFreeman, G. J. et al. (2000) J. Exp. Med. 192:1027-1034（各内容を参照により本明細書に一体化させる）に記載されているPD-1リガンドである。5'-dGtcgaccaccatgctgctcctgctgccgata-3'（配列番号：7）および5'-dGTCGACTCACTAGATCCTCTTTCTCTGGATTATCAC-3'（配列番号：8）をプライマーとして用いるPCRによりAF142780コーディング領域を増幅し、pEF6（Invitrogen, Carlsbad, CA）中にクローン化した。NCBI ESTデータベースの検索により、ネズミPD-L2に対して相同性がある2つのヒト胎児心臓EST（GenBank受託番号AA247117およびAA247128）が同定された。5'-dGTACATAAATAGAGCATGGCAGCA-3'（配列番号：9）および5'-dCCACCTTTTGCAAACTGGCTGT-3'（配列番号：10）をプライマーとして用いて101bpの領域を増幅した。PCR生成物をビオチン標識し、pAXEFベクター中のヒト胎盤cDNAライブラリー（Freeman, G. J. et al. (2000) J. Exp. Med. 192:1027-1034）からCloncapture（Clontech, Palo Alto, CA）から完全長cDNAを単離するために使用した。完全長ヒトPD-L2 cDNA配列はGenBankに寄託されている（受託番号が付与される）。

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【0312】

融合蛋白および細胞トランスフェクション

Ig融合蛋白は、ネズミIg γ 2aのヒンジ-CH2-CH3ドメインに連結された受容体の完全な細胞外領域からなっており（Fc受容体および補体結合をブロックする4つの点突然変異を伴う）、その結果、Ig(γ 2a)融合物が得られる（Duncan, A. R. et al. (1988) Nature 332:563-564；Morgan, A. et al. (1995) Immunology 86:319-324）。対照Igは、ネズミIg γ 2aに連結したオンコスタチン-Mリーダー配列からなる。これらの組換え蛋白は安定にトランスフェクションされたCHO細胞系において産生され、プロテインA-セファロースを用いて馴らし培地から精製された。pEF6中のネズミPD-L2 cDNAをScaIにより線状とし、ホスホグリセラートキナーゼ遺伝子プロモーターの制御下にあるプロマイシン耐性遺伝子を含むプラスミド構築物とともにCHO-I-A^dまたはCHO-I-A^d. mB7-2細胞中に同時エレクトロポレーションした。pAXEF中のヒトPD-L2 cDNAをApaIで線状にし、ホスホグリセラートキナーゼ遺伝子プロモーターの制御下にあるプロマイシン耐性遺伝子とともにCHO-K1細胞中に同時トランスフェクションした。10 μ g/mlプロマイシン中でトランスフェクション体を選択し、hPDI-Igで染色し、ソーティングし、次いで、限界希釈法によりクローン化した。

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【0313】

ノーザンブロット分析

製造者の指示に従って、マウスおよびヒトの多数の組織のノーザンブロット（Clontech, Palo Alto, CA）を、QuikHyb（Stratagene, La Jolla, CA）中の³²P-dCTP放射性標識cDNAプローブでプローブした。ヒトPD-L2プローブは、コーディング領域および3' UTR配列を含む1.2 kbのXbaIフラグメントからなっていた。マウスPD-L2プローブは、コーディング領域を含む444 kbのKpnI/EcoRV cDNAフラグメントからなっていた。2X SSC, 0.1% SDS中室温で、次いで、0.1X SSC, 0.1% SDS中、65℃で、ブロットを室温で2回洗浄し、そしてラジオオートグラフィーにより試験した。

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【0314】

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フローサイトメトリー

PD-L2を検出するために、 5×10^4 個のトランスフェクションされた細胞を、 $5 \mu\text{g}/\text{ml}$ のヒトPD-1 Ig (hPD-1. Ig) (Genetics Institute, Cambridge, MA) とともにインキュベーションし、ヤギ抗マウス IgG2a-フィコエリスリン (PE) (Southern Biotechnology Associates Inc, Birmingham, AL) で発色させた。さらに、 $5 \mu\text{g}/\text{ml}$ の抗-IA^d-PEまたはB7.2-PE (Pharmingen, San Diego, CA) で別々に染色した。

CD4⁺ T細胞をビオチン化抗-PD-1 (またはビオチン化抗-CD28 (Pharmingen) もしくはビオチン化抗-CD25 (Pharmingen) とともにインキュベーションし、ストレプトアビジン-PE (Pharmingen) で染色した。Pharmingenからすべてのイソタイプ対照を得た。各工程後、PBS/1% BSA/0.02% アジ化ナトリウムで細胞を3回洗浄した。最終インキュベーション後、細胞を1%パラホルムアルデヒドで固定した。FACSCalibur (Becton Dickinson, Mountain View, CA) にて1万のイベントを分析した。すべてのイソタイプ対照をPharmingenから得た。

【0315】

T細胞の活性化

活性化された抗原に特異的なT細胞を得るために、DO11.10マウスから脾臓細胞を調製し、Tris-NH₄Clで処理して赤血球を枯渇させた。10% FCS (Sigma, St Louis, MO), 2 mM L-グルタミン, 100 U/ml ペニシリン, 100 mg/ml ストレプトマイシン, 250 ng/ml アンホテリシン B, 10 mM Hepes, 50 pM 2-ME (すべてLife Technologiesから得た) および 15 mg/ml of ゲンタマイシン (BioWhittaker, Walkersville, MD) を補足したRPMI 1640 (Life Technologies, Grand Island, NY) 中で、 $1 \mu\text{g}/\text{mg}$ のOVAペプチド (Analytical Biotechnology Services, Boston, MA) とともに細胞を72時間培養した。磁気活性化細胞ソーティング分離カラム (Miltenyi Biotec, Auburn, CA) を用いるポジティブセクションによりCD4⁺ T細胞を精製して、98%よりも高純度とした。再刺激の前に細胞を一晩休止させた。

【0316】

T細胞のビーズ刺激

抗-CD Ab (2C11; Pharmingen, La Jolla, CA)、mPD-L2. Igおよび対照Igをポリウレタン被覆トシル-活性化Dynabeads (Dyna1, Lake Success, NY) に共有結合させた。一定の最適以下の抗-CD3 Ab濃度 (全結合蛋白の60%) およびmPD-L2. Igまたは対照Ig (全結合蛋白の40%) を用いてビーズを調製した。ビーズは、ビーズ 10^7 個あたり $5 \mu\text{g}$ の蛋白結合能を有する。細胞エンリッチメントカラム (R & D Systems, Minneapolis, MN) を用いるネガティブセクションによりBalb/cリンパ節からT細胞を精製した。示された被覆トシルビーズ: 細胞比を2:1として、96ウェルプレート中でウェル1個あたり $0.5 \sim 1 \times 10^5$ 個のT細胞を培養した。72時間の培養中最後の10時間における [³H] -チミジン取りこみにより増殖を測定した。

【0317】

T細胞のCHO細胞刺激

$50 \mu\text{g}/\text{ml}$ のマイトマイシンC (Bristol Laboratories, Princeton, NJ) とともに37℃で16時間インキュベーションすることにより、トランスフェクションされたCHO細胞の増殖を阻害した。インキュベーション期間の終わりに、PBS中の10mM EDTAとともに細胞を収穫し、2回洗浄し、氷上で1

時間放置した。次いで、細胞を3回洗浄し、培地に再懸濁した。 10^5 個のすでに活性化されている $CD4^+$ T細胞を、種々の濃度のOVAペプチドおよび 10^4 個のマイトマイシン処理されたCHOトランスフェクション対とともに培養した。増殖をアッセイするために、培養物を48時間インキュベーションし、 $1\mu Ci/\text{ウェル}$ の $[^3H]$ チミジン (New England Nuclear, Boston, MA) で少なくとも6時間のインキュベーション期間の間パルスした。

【0318】

サイトカインELISA

培養後種々の時点で上清の一部を取った。Pharmingenから得たmAbsおよび組換えサイトカイン標準を用いてIL-2、IL-4、IFN- γ およびIL-10レベルを分析した。検出限界は以下のおり：IL-2： 20 pg/ml ；IL-4： 40 pg/ml ；IFN- γ ： 100 pg/ml ；およびIL-10： 200 pg/ml 。

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【0319】

RNAase保護アッセイ (RPA)

種々のCHO細胞トランスフェクション体および $0.01\mu\text{g/ml}$ OVAペプチドで $CD4^+$ T細胞を再度刺激した。48時間後、細胞を取り、TRIzol^R試薬 (Life Technologies, Grand Island, NY) を用いてmRNAを単離した。製造者 (Pharmingen, San Diego, CA) の指示に従ってRiboQuantマルチポアキット mCK1を用いてRNAase保護アッセイにより $5\mu\text{g}$ のmRNAをサイトカインレベルに関して分析した。

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【0320】

細胞周期分析

上記のごとく $0.01\mu\text{g/ml}$ ペプチドおよびCHO細胞トランスフェクション体で $CD4^+$ T細胞を再刺激した。培養36時間後、細胞を回収し、抗-CD4-FITCで染色した。細胞をPBSで洗浄し、氷上で70%エタノールで1時間固定し、次いで、 $10\mu\text{g/ml}$ RNase (Sigma, St. Louis, MO) および $50\mu\text{g/ml}$ ヨウ化プロピジウム (Sigma) を含有するPBSに再懸濁した。染色から1時間以内にFACSalyzerを用いて分析を行なった。

【0321】

実施例1：ヒトおよびマウスのPD-L2 cDNAの同定および特徴付け

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この実施例において、ヒトPD-L2およびマウスPD-L2をコードする遺伝子の同定および特徴付けを説明する。

【0322】

ヒトPD-L2 cDNAの単離

本発明は、少なくとも一部には、本明細書でヒトPD-L2と称する新規ポリペプチドをコードするヒト遺伝子の発見に基づく。ヒトPD-L2 cDNAはヒト胎盤cDNAライブラリーから、ヒトEST AA247117をプローブとして用いて単離された。ヒトPD-L2の全配列を決定し、ヒト「PD-L2」と呼ばれる読み取り枠を含むことがわかった。

ヒトPD-L2をコードするヌクレオチド配列を図1および配列番号：1に示す。この核酸によりコードされるポリペプチドは約273個のアミノ酸を含み、図1および配列番号：2に示すアミノ酸配列を有する。配列番号：1のコーディング領域 (読み取り枠) を配列番号：3に示す。

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マウスPD-L2をコードするヌクレオチド配列を図2および配列番号：4に示す。この核酸によりコードされるポリペプチドは約247個のアミノ酸を含み、図2および配列番号：5に示すアミノ酸配列を有する。配列番号：4のコーディング領域 (読み取り枠) を配列番号：6に示す。

【0323】

ヒトおよびマウスのPD-L2分子の分析

シグナルペプチドの存在に関して他のB7ファミリーのメンバーと比較することによりヒ

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トおよびマウスのPD-L2の各アミノ酸配列を分析した。これらの分析により、ネイティブなヒトPD-L2（配列番号：2）の残基ほぼ1～19にアミノ酸配列中のシグナルペプチドドメインが同定された（図3）。これらの分析により、ネイティブなマウスPD-L2（配列番号：5）の残基ほぼ1～19にアミノ酸配列中のシグナルペプチドドメインがさらに同定された（図3）。

また、IgVドメインの存在に関して他のB7ファミリーのメンバーと比較することによりヒトおよびマウスのPD-L2の各アミノ酸配列を分析した。これらの分析により、ネイティブなヒトPD-L2（配列番号：2）の残基ほぼ20～120（図3）および推定成熟ポリペプチド中の残基ほぼ1～101のアミノ酸配列中にIgVドメインが同定された。これらの分析により、ネイティブなマウスPD-L2（配列番号：5）の残基ほぼ20～120（図3）および推定成熟ポリペプチド中の残基ほぼ1～101のアミノ酸配列中にIgVドメインが同定された。

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さらに、IgCドメインの存在に関して他のB7ファミリーのメンバーと比較することによりヒトおよびマウスのPD-L2の各アミノ酸配列を分析した。これらの分析により、ネイティブなヒトPD-L2（配列番号：2）の残基ほぼ121～219（図3）および推定成熟ポリペプチド中の残基ほぼ102～200のアミノ酸配列中にIgCドメインが同定された。これらの分析により、ネイティブなマウスPD-L2（配列番号：5）の残基ほぼ121～219（図3）および推定成熟ポリペプチド中の残基ほぼ102～200のアミノ酸配列中にIgCドメインが同定された。

さらに、細胞外ドメインの存在に関して他のB7ファミリーのメンバーと比較することによりヒトおよびマウスのPD-L2の各アミノ酸配列を分析した。これらの分析により、ネイティブなヒトPD-L2（配列番号：2）の残基ほぼ1～219（図1）および推定成熟ポリペプチド中の残基ほぼ1～200のアミノ酸配列中に細胞外ドメインが同定された。これらの分析により、ネイティブなマウスPD-L2（配列番号：5）の残基ほぼ1～219（図2）および推定成熟ポリペプチド中の残基ほぼ1～200のアミノ酸配列中に細胞外ドメインが同定された。

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さらに、膜貫通ドメインの存在に関して他のB7ファミリーのメンバーと比較することによりヒトおよびマウスのPD-L2の各アミノ酸配列を分析した。これらの分析により、ネイティブなヒトPD-L2（配列番号：2）の残基ほぼ220～243（図3）および推定成熟ポリペプチド中の残基ほぼ201～224のアミノ酸配列中に膜貫通ドメインが同定された。これらの分析により、ネイティブなマウスPD-L2（配列番号：5）の残基ほぼ220～242（図3）および推定成熟ポリペプチド中の残基ほぼ201～223のアミノ酸配列中に膜貫通ドメインが同定された。

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さらに、細胞質ドメインの存在に関して他のB7ファミリーのメンバーと比較することによりヒトおよびマウスのPD-L2の各アミノ酸配列を分析した。これらの分析により、ネイティブなヒトPD-L2（配列番号：2）の残基ほぼ244～273（図3）および推定成熟ポリペプチド中の残基ほぼ225～254のアミノ酸配列中に細胞質ドメインが同定された。これらの分析により、ネイティブなマウスPD-L2（配列番号：5）の残基ほぼ243～247（図3）および推定成熟ポリペプチド中の残基ほぼ224～228のアミノ酸配列中に細胞質ドメインが同定された。

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マウスPD-L2蛋白は、マウスPD-L1に対して38%のアミノ酸同一性を有する（図6参照）。マウスおよびヒトのPD-L2は70%のアミノ酸同一性を有し（図6）、ヒトおよびネズミのB7-1またはB7-2の間の46～50%よりも高い。

【0324】

実施例2：細菌細胞における組換えPD-L2ポリペプチドの発現

この実施例において、ヒトPD-L2がE. coliにおいてグルタチオン-S-トランスフェラーゼ（GST）融合蛋白として発現され、該融合蛋白が単離され特徴付けされる。特別には、PD-L2がGSTと融合され、この融合蛋白がE. coli、例えばPEB199株において発現される。PEB199株におけるGST-PD-L2融合蛋白の発現をIPTGで誘導する。誘導されたPEB199株の粗細菌溶解物から、グルタ

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チオンビーズ上のアフィニティークロマトグラフィーにより組換え融合ポリペプチドを精製する。細菌溶解物から精製されたポリペプチドのポリアクリルアミドゲル電気泳動分析を用いて、得られた融合ポリペプチドの分子量を決定する。

【0325】

実施例3：PD-1へのPD-L2の結合

AF142780、マウスPD-L2 cDNA、または対照マウスPD-1リガンドを含有する発現プラスミドでCOS細胞をトランスフェクションした。72時間後、0.5 mM EDTAを含有するPBS中、37℃で30分インキュベーションすることによりトランスフェクションしたCOS細胞を剥離させた。

種々のIg融合蛋白に結合するPD-L2発現COS細胞の能力を試験した。PD-L2でトランスフェクションされたCOS細胞によるIgG2a（対照Ig）、ICOS-IgG、およびPD-1-Igの結合についてのFACS分析は、IgG2aまたはICOS-IgGのいずれもがPD-L2または対照PD-1リガンドにより結合されないことを示した。しかしながら、PD-1-Igは、PD-L2および対照PD-1リガンドへの結合が示された（図4）。 10

ヒトPD-L2 cDNAを用いる実験により、マウスPD-L2 cDNAを用いて得られた結果と同様の結果が得られた。

CHO細胞を用いる実験も行なった。フローサイトメトリーの研究により、hPD-1-IgがPD-L2で安定にトランスフェクションされたCHO細胞を認識することが示されている（図7）。対照トランスフェクション体（CHO-IA^d、CHO-IA^d/B7.2）への結合が存在しないので、この染色は特異的である。PD-L2でトランスフェクションされたCHO細胞はCTLA4-Ig、CD28-IgまたはICOS-Igでの染色を示さなかった。IgVエクソンが欠失されているネズミPD-L2の別スライシングされた変種も単離した。この変種はPD-1-Igに結合せず、IgVドメインがPD-1へのPD-L2の結合に必要であることが示された。 20

【0326】

実施例4：PD-L2に対する全ヒト抗体の取得

この実施例において、ヒト免疫グロブリンフレームワーク遺伝子が導入されているマウスにおいてPD-L2に対する全ヒト抗体を作成する。標準的方法、例えば、Hogan et al., *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory (参照により本明細書に一体化させる)に従ってトランスジェニックマウスを作成、あるいは購入する。公表された手順 (*Teratocarcinomas and embryonic stem cells: a practical approach*, Robertson, E. J. ed., IRL Press, Washington, D. C., 1987; Zijlstra et al. (1989) *Nature* 342:435-438; and Schwartzberg et al. (1989) *Science* 246:799-803, 参照によりそれぞれを本明細書に一体化させる)に従って胚性幹細胞を処理する。Sambrook, J. et al. in *Molecular Cloning: A Laboratory Manual*, 2nd ed., 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y. (参照により本明細書に一体化させる)に従ってDNAクローニング手順を行なう。例えば製造者の指示に従ってApplied Bio Systemsオリゴヌクレオチド合成装置によりオリゴヌクレオチドを合成、あるいは購入する。 30 40

精製または組換えPD-L2あるいは少なくともPD-L2の細胞外ドメインの免疫原部分を含む融合蛋白を用いてトランスジェニックマウスを免疫する。100 µgのリン酸塩緩衝化セイライン (PBS) 中約400 µgのPD-L2を各マウスに腹腔内注射する。眼窩後部からの採血により血清試料を集める。

間接的酵素結合免疫吸着アッセイ (ELISA) を用いてPD-L2に対する抗体の反応 50

性および特異性を評価する。いくつかの免疫グロブリンスーパーファミリー分子を対照（例えば、CTLA4およびCD28）として試験して、PD-L2に対する抗体の抗体特異性を分析する。マウス免疫グロブリンに対して交差反応性を示さないヒトIgMおよびヒトIgGサブクラスに対して特異的な酵素抱合体により、PD-L2に結合するヒトフレームワーク領域を有する抗体を検出する。簡単に説明すると、PBS中5 μ g/mlのPD-L2にて、37℃で一晩ウェルを被覆することにより、PVCマイクロタイタープレートをPD-L2で被覆する。PBS, 5%血清, 0.5%ツイン-20で血清試料を希釈し、ウェル中で室温にて1時間インキュベーションし、次いで、抗-ヒトIgG FcおよびIgG F(ab')₂-セイヨウワサビペルオキシダーゼまたは抗-ヒトIgM Fc-セイヨウワサビペルオキシダーゼを同じ希釈液中でインキュベーションする。室温で1時間後、ABTS基質（Sigma, St. Louis, Mo.）の添加により酵素活性を測定し、30分後に415~490nmを読む。同じマウスからのプレー免疫血清試料中の、同じ標的抗原に対するヒト抗体力価も試験する。適切な抗体力価を有するマウスから単離された脾臓細胞を集める。脾臓細胞を適当な融合パートナー（例えば、ミエローマ細胞）と融合させてハイブリドーマを作成する。Antibodies: A Laboratory Manual, Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)（参照により本明細書に一体化させる）に従って、ハイブリドーマおよび抗体を処理する。

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【0327】

実施例5：PD-L2に対する抗体の取得

抗-ヒトPD-L2抗体

マウスのcDNA免疫により抗-DP-L2抗体を調製することもできる。抗-ヒトPD-L2抗体を製造するために、0.9%セイライン中10mMのカルジオトキシン（Sigma Chemical Company, St. Louis, MO）50 μ lを各後足の前けい骨筋に注射することによりメスのBalb/cマウス（Harlan Sprague-Dawley, Inc., Indianapolis, IN）をcDNA免疫用に調製した。5日後、0.9%セイライン中1mg/mlのpAXEF中の精製hPD-L2 cDNA 50 μ lを各マウスの再生している各前けい骨筋に注射した。cDNA免疫を2週間間隔で2回繰り返した。次いで、マウスを4週間休息させ、その後、マウス1匹あたり100 μ gのcDNAを3~4週間間隔で3回追加免疫した。融合の5日前に、融合用に選択された1匹のマウスにcDNAを腹腔内注射して追加免疫した。脾臓細胞をSP2/0ミエローマ細胞と融合させ、クローン化させ、次いで、hPD-L2-mIgG2a融合蛋白との反応性に関してハイブリドーマをELISAによりスクリーニングし、その後、hPD-L2でトランスフェクションされた300.19およびCOS細胞の細胞表面染色を行なって、未トランスフェクション細胞およびhPD-L1でトランスフェクションされた細胞との反応性の欠如を調べた。

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9種のマウス抗-ヒトPD-L2モノクローナル抗体(mAbs)を単離した。PD-1/PD-L2相互作用をブロックするこれらの抗体の能力を決定するために、抗-PD-L2 mAbsをPD-L2でトランスフェクションされた細胞とともにプレインキュベーションした。PD-L2でトランスフェクションされた細胞へのビオチン化PD-1-Igの結合を減じるmAbの能力として、相互作用の阻害を測定した。最良のmAbsは抗体24F.10C12（本明細書において10C12ともいう）および24F7G12（本明細書において7G12ともいう）であった。結果は次のとおり：

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300-hPD-L2細胞に結合した

ビオチン化hPD-1-Ig

の平均蛍光強度

mAb

7G12

10C12

陽性対照

0.110

0.109

2.57

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【0328】

抗マウスPD-L2抗体

抗マウスPD-L2抗体を製造するために、0.9%セイライン中10mMのカルジオトキシシン(Sigma Chemical Company, St. Louis, MO) 100 μ lを各後足の前けい骨筋に注射することによりメスのLewis株ラット(Harlan Sprague-Dawley, Inc., Indianapolis, IN)をcDNA免疫用に調製した。5日後、0.9%セイライン中1mg/mlのpEF6中の精製ネズミPD-L2 cDNA 100 μ lを各マウスの再生している各前けい骨筋に注射した。cDNA免疫を2~3週間間隔で3回繰り返した。次いで、 $1 \sim 5 \times 10^7$ 個のCHO-mPD-L2トランスフェクション体で4回、2~5週間間隔でラットを免疫した。融合の5日前に、融合用に選択された1匹のラットにcDNA(200 μ l)および細胞(5×10^7 個)の両方を腹腔内注射して免疫した。脾臓細胞をSP2/Oミエローマ細胞と融合させ、クローン化させ、次いで、mPD-L2でトランスフェクションされた300.19およびCOS細胞の細胞表面染色によりスクリーニングし、未トランスフェクション細胞およびmPD-L1でトランスフェクションされた細胞との反応性の欠如を調べた。mPD-L2を特異的に認識するモノクローナル抗体が産生された。

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【0329】

実施例6: PD-L2の発現

PD-L2の発現を分析するために、種々の組織および活性化抗原提示細胞におけるノーザンブロットハイブリダイゼーションによりmRNA発現を分析した。ヒトおよびネズミのPD-L2 mRNAは正常胎盤で多く発現されており、正常脾臓、リンパ節および胸腺において少なく発現されている。PD-L2はヒト心臓においても発現されているが、マウス心臓では低レベルで発現されている。PD-L2が発現される他の組織はヒト脾臓、肺および肝臓である。PD-L2 mRNAは刺激されていないヒト単球では検出されなかったが、IFN- γ 刺激によりアップレギュレーションされた。PD-L1のアップレギュレーション(Freeman, G. J. et al. (2000) J. Exp. Med. 192:1027-1034)と比較すると、PD-L2の誘導はキネティクスにおいてわずかに遅れた。ネズミ腫瘍細胞系のノーザンブロット分析により、PU5-1.8(ミエロイドリンパ腫)、RAW264.7(マクロファージリンパ腫)、R1.1(Tリンパ種)、L1210(リンパ球性白血病)、P338D1(単球/マクロファージリンパ腫)、およびP815(肥満細胞種)のごときリンパ球起源の系におけるPD-L2発現が明らかとなった。線維芽細胞系(M-MSV Balb/3T3、K-Bal b、LM)およびP19奇形癌腫細胞系においてPD-L2はあまり発現されないか、あるいは全く発現されなかった。PD-L2は肝細胞種(Hepa1-6)および神経芽腫(NB41A3)細胞系においても発現されている。

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【0330】

実施例7: PD-L2-PD-1相互作用はTCRにより媒介される応答を阻害する

T細胞活性化におけるPD-L2-PD-1経路の役割を調べるために、DynalビーズによるT細胞活性化系を用いた。Balb/cリンパ節からの精製T細胞を、抗-CD3プラス対照、IgまたはmPD-L2、Igで被覆したビーズで活性化させた。³H-チミジン取りこみにより増殖を測定した。抗-CD3プラスmPD-L2、Igで被覆したビーズで活性化されたT細胞は、抗-CD3プラス対照、Igで活性化された細胞と比較して著しい増殖の低下を示した(図8)。かくして、PD-L2-Tによる細胞上のPD-1の利用はT細胞増殖の阻害を導く。

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抗原特異的シグナルを研究するために、PD-L2およびI-A^dを同時発現するCHO細胞ならびに同様のレベルのI-A^dのみを発現するCHO細胞(図7)を、DO11.10CD4⁺T細胞を活性化させる能力に関して比較した。活性化後にPD-1がアップレギュレーションされるので、前もって活性化されたDO11.10T細胞を用いて最大PD-L2相互作用を可能ならしめた。前もって活性化されたCD4⁺T細胞を一定濃度範囲のOVA(323-339)ペプチドおよびマイチマイシンC処理CHOトランスフ

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ェクション体とともにインキュベーションした。図9からわかるように、前もって活性化されたDO11.10CD4⁺T細胞は、CHO・I-A^dにより提示されたOVAペプチドに対して中庸の増殖応答を与えた。対照的に、PD-L2存在下においてこの応答は有意に低下した。インターロイキン(IL)-4、IFN-γおよびIL-10産生もまた著しく低下した(図9)。これらの活性化条件下ではIL-2は検出されなかった。これらのデータは、PD-1-PD-L2相互作用がTCRにより媒介される増殖およびサイトカイン産生を阻害できることを示すものである。PD-1-PD-L2経路の阻害効果および非リンパ器官におけるPD-L2の発現は、自己免疫反応の制御における役割を示すものである。

【0331】

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実施例8：PD-L2はTCR-CD28シグナルを阻害しうる

最適T細胞クローン増殖にはTCRおよびCD28両方のシグナルが必要なので、TCRプラスCD28ならびにPD-L2-PD-1シグナル間の相互作用を調べた。この問題を解決するために、下記のCHO細胞を用いた：CHO・I-A^d・B7-2およびCHO・I-A^d・B7-2・PD-L2。すでにOVAで活性化されているDO11.10CD4⁺T細胞を刺激するこれらのCHOトランスフェクション体の能力を比較した。I-A^dおよびB7-2の発現はこれらのCHOトランスフェクション体上で同様に高かった(図7)。PD-1・Ig結合により測定されたPD-L2発現レベルは高かった。予想とおり、B7-2の導入は、すべての抗原濃度においてT細胞による増殖応答の増加を導き、低い抗原濃度において最も著しい刺激がみられた(図10)。CHO細胞上のPD-L2の同時発現は、低いペプチド濃度(0.01μg/mlおよび0.001μg/ml)におけるTCRおよびB7-2により媒介される増殖応答を阻害した(図10)。0.01μg/mlのペプチド濃度では、PD-L2はTCR-B7-2により媒介されるサイトカイン産生を有意に阻害し、増殖の阻害と矛盾しなかった(図11)。ごく弱い増殖の阻害が存在した0.1μg/mlのペプチド濃度では、DO11.10CD4⁺T細胞がペプチドおよびCHO・I-A^d・B7-2・PD-L2トランスフェクション体とともに培養された場合にサイトカイン産生が阻害された(図12)。それゆえ、PD-L2によるPD-1の利用はTCR-CD28により媒介されるサイトカイン産生刺激をダウンレギュレーションしうる。

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低下したサイトカイン産生が低下したmRNAレベルによるものであるかどうかを決定するために、RNAase保護アッセイによりサイトカインmRNAレベルを測定した。CHO・I-A^d・B7-2により提示された0.01μg/ml OVAペプチドでの刺激後に、インターロイキン-4、IL-10、IL-13、IL-2、IL-6およびIFN-γ mRNAは、すでに活性化されているDO11.10CD4⁺T細胞において容易に検出された。しかしながら、CHO・I-A^d・B7-2トランスフェクション体中へのPD-L2の導入は、Th1およびTh2両方のサイトカインに関してmRNAレベルを有意に低下させた。すでに活性化されているT細胞を単独で、あるいはCHO・I-A^dにより発現されるペプチドとともにインキュベーションした場合に、サイトカインmRNAの発現は最低であった。これらの結果は、抗原刺激が弱いあるいは限定的である場合におけるB7-CD28シグナルに拮抗するPD-L2-PD-1経路の能力をさらに示すものである。

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低い抗原濃度におけるPD-L2-PD-1経路の阻害能が異なる抗原濃度におけるCD28およびPD-1発現レベルの変化に関連しているかどうかを調べるために、PD-1およびCD28の表面発現を調べた。表1は、異なった活性化条件に置いた後の、DO11.10CD4⁺T細胞上のPD-1、CD28およびCD25の平均蛍光強度を示す。

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【0332】

【表1】

表1:新たに単離され、すでに活性化されているDO11.10 CD4⁺T細胞上の、ならびにCHO. 1-A^d. B7-2での再刺激中の、CD28、PD-1およびCD25のMFI

	抗体	MFI							
新たに単離された CD4 ⁺ DO11.10 T 細胞	CD28	33							
	PD-1	14							
	CD25	27							
	イソタイプ°	16							
すでに活性化されている CD4 ⁺ DO11.10 T 細胞		実験 1	実験 2						
	CD28	50	117						
	PD-1	51	51						
	CD25	373	2705						
	イソタイプ°	12	17						
再刺激された CD4 ⁺ DO11.10 T 細胞									
ペプチド濃度(μg/ml)		0.001	0.01	0.1	1.0	0.001	0.01	0.1	1.0
		実験 1	実験 2						
	CD28	77	106	123	165	77	121	338	516
	PD-1	78	56	41	39	117	92	84	55
	CD25	334	417	560	1154	2507	2602	2971	7021
	イソタイプ°	11	14						
CD4 ⁺ T細胞を、ビオチン化抗-PD-1、ビオチン化抗-CD28、ビオチン化抗-CD25、またはビオチン化イソタイプ対照とともにインキュベーションし、ストレプトアビジン-PEで発色させた。									

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【0333】

新たに単離されたDO11.10 CD4⁺T細胞はCD28を発現したが、PD-1もCD25も発現しなかった。脾臓APCsにより提示される1μg/mlのペプチドで刺激されたT細胞はCD28を中庸にアップレギュレーションし、PD-1およびCD25を強くアップレギュレーションした。すでに活性化されているDO11.10 CD4⁺T細胞をCHO. 1-A^d. B7-2により提示される種々の濃度のペプチドで再刺激した後、より高いペプチド濃度においてCD28およびCD25の発現がCD4⁺T細胞上で増大した。対照的に、PD-1発現は低いペプチド濃度で活性化されたT細胞上で最高であり、より高いペプチド濃度においては減少した。より低い抗原用量におけるより高いPD-1発現は、PD-L2-PD-1経路が弱い抗原応答を弱体化させる機構をさらに示唆する。

【0334】

実施例9: PD-1-PD-L2経路の作用機構

CTLA-4の架橋はネイティブなT細胞において細胞周期進行を阻害することが示されている(Krummel, M. F. and Allison, J. P. (1996) J. Exp. Med. 183:2533-2540; Walunas, T. L. et al. (1996) J. Exp. Med. 183:2541-2550)。アポ

トーシス中のネズミ細胞系からPD-1が単離されたので、PD-1:PD-L2経路の可能な作用機構は、プログラムされた細胞死（例えば、活性化により誘導される細胞死またはAICD）を増加させることである可能性がある。この問題に取りかかるために、DO11.10CD4⁺T細胞を0.01μg/mlのペプチドおよび種々のCHOトランスフェクション体で再刺激し、細胞周期進行を分析した。48時間後、細胞を回収し、CD4-FITCで染色し、透過性にし、次いで、ヨウ化プロピジウムとともにインキュベーションして、G₀/G₁、S/G₂およびサブ-2倍体(sub-diploid)集団を分析した。CHO-IA^dまたはCHO-IA^d/PD-L2により提示されるペプチドで再刺激されたCD4⁺T細胞はいずれも大集団のサブ-2倍体集団を有し、アポトーシスが示された(図13)。これらの結果はアネキシン染色により確認された。CHO-IA^dにより提示されるペプチド(1μg/ml)によりCD4⁺T細胞が刺激される培養物において、S/G₂期の細胞数が増加しており、細胞がサイクル中にあることが示された。IA^dトランスフェクション体中へのPD-L2の導入は、G₀/G₁期の細胞数の増加を導き、細胞周期停止が示唆された。

次いで、B7-2およびPD-L2シグナル存在下での細胞周期進行を比較した。CHO-IA^dB7-2により提示される0.01μg/mlのペプチドにより刺激されたCD4⁺T細胞は、S/G₂期の細胞数の増加およびサブ-2倍体集団数の減少を示し、細胞がサイクル中にあり、B7-CD28同時刺激によりアポトーシスからレスキューされることが示された(図14)。高いペプチド濃度におけるCHO-IA^dトランスフェクション体に関して見られるのと同様に、B7-2トランスフェクション体中へのPD-L2の導入は、G₀/G₁期の細胞数の増加およびS期の細胞数の減少を導いた。B7-2およびB7-2.PD-L2刺激T細胞間のアポトーシス細胞集団に違いはなく、PD-1架橋は細胞死の増加を導かなかったことが示された。

【0335】

実施例10:PD-1:PD-リガンド相互作用の阻害によるT細胞活性化の刺激上に示したように、PD-1を介するシグナリングは強力なTCR/CD28同時刺激シグナルにより支配されており;PD-1シグナリング経路は中庸のTCR/CD28同時刺激シグナルを阻害し、最初はT細胞増殖の減少なしにサイトカイン産生が低下する。TCR/CD28同時刺激シグナルが弱まるにつれ、PD-1経路が支配的となり、サイトカイン産生の大幅な減少が増殖の低下とともに起こる。したがって、PD-L1またはPD-L2との相互作用の阻害によりPD-1経路の阻害がT細胞活性化を促進するかどうかを調べるために、弱く機能する抗原提示細胞(APCs)(すなわち、弱いTCR/CD28同時刺激を有する抗原提示細胞)を用いて混合リンパ球反応(MLRs)を行なった。

成熟樹状細胞は強力なAPCsである。しかしながら、IL-10での処理はそれらの能力を低下させる。以前の報告は、IL-10が細胞APC能を大幅に低下させることを示しており、このことがMHC、B7-1およびB7-2の発現低下によるものであるとしている。しかしながら、本明細書の実験は、低下は中庸であり、そのうえ、IL-10処理された樹状細胞はPD-L1およびPD-L2を発現することを示すものである。

ヒト末梢血単球をIL-4およびGM-CSF中で培養することにより未成熟ミエロイド樹状細胞を単離した。IL-1β、TNF-α、IL-6およびPGE₂の炎症性カクテルへの未成熟樹状細胞の曝露は、APCsとして機能する成熟樹状細胞の発達を誘導する。しかしながら、成熟期にある炎症性サイトカインへのIL-10の添加は、1/6ないし1/3しか機能しないAPCsを生じさせる。

上で一般的に説明したようにして、PD-L1および/またはPD-L2に対する抗体、あるいは対照抗体の存在下において、IL-10処理樹状細胞をAPCsとして用いてT細胞活性化アッセイ(MLRs)を行なった。IL-10処理樹状細胞プラス同種T細胞への抗-PD-L1またはPD-L2 mAbの添加は、対照IgG処理培養物と比較した場合、3倍のT細胞増殖の増加を生じさせた(図15)。抗-PD-L1および抗-PD-L2抗体の組み合わせは、いずれかの抗体のみの場合に見られるよりも大幅な刺激の

増大を生じさせた（図16）。この刺激は、PD-1へのPD-L1および／またはPD-L2の結合により媒介される免疫阻害性シグナルのブロックから予想される結果と矛盾しない。

【0336】

均等物

当業者は、常套的な実験異常のことは行なわずに、本明細書記載の本発明の個々の具体例に対する均等物を認識し、あるいは確認することができるであろう。かかる均等物は請求の範囲により包含されるものである。

【図面の簡単な説明】

【図1】図1は、ヒトPD-L2のcDNA配列および推定アミノ酸配列を示す。ヌクレオチド配列は配列番号：1の核酸1～1223に対応する。アミノ酸配列は配列番号：2のアミノ酸1～273に対応する。ヒトPD-L2遺伝子の5'または3'非翻訳領域を伴わないコーディング領域を配列番号：3に示す。 10

【図2】図2は、マウスPD-L2のcDNA配列および推定アミノ酸配列を示す。ヌクレオチド配列は配列番号：4の核酸1～1655に対応する。アミノ酸配列は配列番号：5のアミノ酸1～247に対応する。マウスPD-L2遺伝子の5'または3'非翻訳領域を伴わないコーディング領域を配列番号：6に示す。

【図3】図3は、ヒトおよびマウスPD-L2ポリペプチドのアミノ酸配列を示し（それぞれ配列番号：2および配列番号：5）、シグナルペプチド、IgV、IgC、細胞外、膜貫通、および細胞質ドメインを示す。 20

【図4】図4は、マウスPD-L2または対照マウスPD-1リガンドでトランスフェクションされたCOS細胞へのIgG2a（対照Ig）、ICOS-IgG、および対照PD-1-Igの結合のFACS分析結果を示す。

【図5】図5は、マウスおよびヒトPD-L2ポリペプチドのアミノ酸配列（それぞれ配列番号：5および配列番号：2）の並置比較を示す。2つの配列間の同一アミノ酸が示されている。

【図6】図6は、マウスPD-L2（配列番号：5）、ヒトPD-L2（配列番号：2）、マウスPD-L1（配列番号：11）、およびヒトPD-L1（配列番号：12）のアミノ酸配列の並置比較を示す。

【図7】図7はPD-L2へのPD-1の結合を示す。I-A^dのみまたはI-A^dおよびB7-2を発現するCHO細胞はマウスPD-L2でトランスフェクションされず、あるいは安定にトランスフェクションされなかった。細胞をhPD-1-Igで染色し、PE-ヤギ抗-マウスIgG2aで染色（太線）した。PE-抗-I-A^dまたはPE-抗-B7-2で（太線）別個にCHO細胞を染色した。細線はイソタイプ対照モノクローナル抗体での染色を示す。1万のイベントを分析した。 30

【図8】図8は、TCRにより媒介される応答のPD-L2-PD-1相互作用による阻害を示す。BALB/cリンパ節からの精製T細胞を、2：1のビーズ：細胞比で、抗-CD3+対照Igまたは抗-CD3+mPD-L2-Igで被覆したトシルビーズで刺激した。72時間後に増殖を測定した。これらのデータは8回以上の独立した実験の典型的なものである。 40

【図9】図9は、TCRにより媒介される応答のPD-L2-PD-1相互作用による阻害を示す。DO11.10トランスジェニックマウス由来の脾臓細胞をOVAペプチド（1μg/ml）で活性化した。CD4⁺T細胞を単離し、一晚休止させた。前もって活性化させたT細胞（10⁵個）を、CHO-I-A^dまたはCHO-I-A^d-PD-L2により提示されたペプチド（1または0.1μg/ml）で48時間再刺激した。〔³H〕チミジン取りこみを3つの同じ実験で試験した。培養開始後36時間目に上清の一部を集め、ELISAによりサイトカインを測定した。これらのデータは4回以上の独立した実験の典型的なものである。

【図10】図10は、TCRおよびCD28により媒介される応答のPD-L2-PD-1相互作用による阻害を示す。DO11.10トランスジェニックマウス由来の脾臓細胞 50

をOVAペプチド ($1 \mu\text{g}/\text{ml}$) で4日間活性化した。 $\text{CD}4^+$ T細胞を単離し、一晚休止させた。前もって活性化させたT細胞 (10^5 個) を、示されたCHOトランスフェクション体により、ペプチドの濃度を種々変更して、48時間再刺激した。 $[^3\text{H}]$ チミジン取りこみを3つの同じ実験で試験した。これらのデータは6回以上の独立した実験の典型的なものである。

【図11】図11は、TCRおよびCD28により媒介される応答のPD-L2-PD-1相互作用による阻害を示す。前もって活性化されたT細胞 (10^5 個) を、示されたCHOトランスフェクション体により提示された $0.01 \mu\text{g}/\text{ml}$ のOVAペプチドとともに培養した。培養開始後36時間目に上清の一部を集め、ELISAによりサイトカインを測定した。破線はELISAの感度を示す。

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【図12】図12は、TCRおよびCD28により媒介される応答のPD-L2-PD-1相互作用による阻害を示す。前もって活性化されたT細胞 (10^5 個) を、示されたCHOトランスフェクション体により提示された $0.1 \mu\text{g}/\text{ml}$ のOVAペプチドとともに培養した。培養開始後36時間目に上清の一部を集め、ELISAによりサイトカインを測定した。破線はELISAの感度を示す。

【図13】図13は、PD-L2-PD-1経路の使用の結果としての細胞周期停止およびアポトーシスを示す。前もって活性化されたT細胞を、OVAペプチド ($1 \mu\text{g}/\text{ml}$) および示されたCHOトランスフェクション体で再刺激した。培養36時間目に細胞を集め、抗-CD4で染色し、70%エタノール中で固定した。細胞をヨウ化プロピジウム溶液中で再懸濁した。FACSプロファイルは $\text{CD}4^+$ 集団のヨウ化プロピジウム染色である。2倍体未満 (subdiploid)、2倍体 (diploid)、および2倍体超 (supradiploid) の集団を示す。一定流速で1万のイベントを集め、分析した。これらのデータは3回以上の独立した実験の典型的なものである。

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【図14】図14は、PD-L2-PD-1経路の使用の結果としての細胞周期停止およびアポトーシスを示す。前もって活性化されたT細胞を、OVAペプチド ($0.01 \mu\text{g}/\text{ml}$) および示されたCHOトランスフェクション体で再刺激した。培養36時間目に細胞を集め、抗-CD4で染色し、70%エタノール中で固定した。細胞をヨウ化プロピジウム溶液中で再懸濁した。FACSプロファイルは $\text{CD}4^+$ 集団のヨウ化プロピジウム染色である。2倍体未満 (subdiploid)、2倍体 (diploid)、および2倍体超 (supradiploid) の集団を示す。一定流速で1万のイベントを集め、分析した。これらのデータは3回以上の独立した実験の典型的なものである。

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【図15】図15は、抗-PD-L1または抗-PD-L2抗体の存在下でのT細胞増殖の促進を示す。同種 $\text{CD}4^+$ T細胞を、IL-10処理樹状細胞による混合リンパ球反応において刺激した。

【図16】図16は、抗-PD-L1、抗-PD-L2抗体、あるいは抗-PD-L1および抗-PD-L2抗体の組み合わせの存在下でのT細胞増殖の促進を示す。同種 $\text{CD}4^+$ T細胞を、IL-10処理樹状細胞による混合リンパ球反応において刺激した。

【図 1】

FIG. 1

ヒトPD-L2ヌクレオチド配列

1 GCMAACCTTAAGCTGAATGAACAACCTTTCTCTCTTGAATATATCTTAACGCCAA
 ATTCTGAGTGTCTTTGTTACCCACCTCTCATATGTCAGCTGGAAAGAAATCTTG
 GGTGGAGCTACTGCATGTTGATGTTGTTGTTCTCTTTGGCTGTTCACTTTGG
 TGGCTACTATAAGGAATCTAAACAACAACGCAACTGTTTTGTTGTTTACTTTT
 GCATCTTTACTTGTGGAGCTGTGGAGCTCTCATATAAATACAGAACATGATCT
 TCTCTCTGTAATGTTGAGCTGGAGCTTGCAGCTTACCAGATAGCAGCTTTATTC
 ACAGTGACATCCCTAAGGAAGTCTATATATAGAGCATGGCAGCAATGTGACCTT
 GGAATGCAACTTTGACATGGAAGTCTATGTAAGCTTGGAGCAATAACAGCCAGT
 TGCAAAAGGTGGAAATGATACATCCCAACACCTGAAAGGCCACTTTTCTGGAG
 GAGCAGCTGCCCCTAGGGAAGCTCTGTTCCACATCTCAAGTCCAGTGGAGG
 CBAAGGCACTACCAATGCTATATCACTATGAGGCTGCGCTGGGACTACAAGTACC
 TGACTCTGAAAGTCAAAAGCTCTTACAGGAATAAACAACCTCACTCTAAAGGTT
 CCAGAAACAGATGAGCTAGCTCAGCTGCGAGCTTACAGTTATCTCTGCGAGA
 AGTATCTCGGCCAAGCTCAGCGTCTCTGCAACACAGCAGCTCCAGGACCCCTG
 AAGGCTCTACCAAGCTCAGCGTCTCTGCGGCTAAAGCCACCCCTGGCAGAAAC
 TTCAGCTGTGTTCTGGAATCTACGCTGAGGGAAGTCTTCTTGGCCAGCATTA
 CCTTCAAGTCAAGTGGAAACCCAGGACCACTCAACTTGGCTGCTTCACTTTTCA
 TCCCTCTCTGATCATGCTTTTCACTTTTATAGCCAGTGTATGCCCTAAGAAAA
 CAATCTGTCAAAAGCTGATTTCTTCAAAAGACACAAAAGACCTGTCAACAC
 AACAAAGGGGAAGTGAACAGTGTATCTGAACCTGTGGTCTTGGGAGCCAGGCTG
 ACTGATATGACATCTAAGAGAGCTCTGAGCTCTGAACAAGAAATGCTGGGCTG
 CAGAGCTTGCATTTGCACTTTTCAATGCTTTGGATGACCCAGCA 1223

ヒトPD-L2アミノ酸配列

1 MIFLLIMLSLELQLHQAALFTVTPKELYIEHGSNVLECNFTDGHVNLGAIT
 ASLQKRVENDTSPHRRERATLLEELPLGKASFIPIQVQRDEGQYQCIIYGVANDY
 KYLRLVKASIRKINHLKVPETDEVELTQATGTPLEAVSWPNVSPANTSHSR
 TPEGLYQVTSVLRKLPQPSRNFSFVFNWTHRELTLASIDLQSQMEPRTHPTWLLH
 IFIPSCIIAFIPIATVIALRKQLQKLYSSKDTTKRPVTTTKREVNSAI 273

【図 2】

FIG. 2

マウスPD-L2ヌクレオチド配列

1 GAATTCGGCACGAGGTCAAAATGCGCATATCTTTGTTGTCCTCTCTGTCGCCAA
 CTAGAGAGAACCACTTACGGGCTCTGTCGGGCGAGTTTGGTTGTCGGTGGAT
 TGGCTTCCAGGGAACCTGATACAGGAGCACTGTGTGCTGCTGCTTTCTGCTGCTT
 TGGCTTGGAGGAGCTGTGCTGGGCTGTATATGACACAGGACGATGCTGCTGCTG
 CCGATACCTGAACCTGTAGCTTACAACTTCTATCTGCTAGCAGCTTTATTCACCGGAC
 AGCCCTTAAAGAGTGTACACCGGTAGACGTGGGAGCAGTGTGAGCTTGGAGTGGC
 ATTTTGACCGCAGAGATGCACTGAACTGGAAGGGAAGAGCCAGTTTGGAGAGAG
 GTAGAAATGATACCTCTCTGCAAGTGAAGAGCCACCTGCTGGAGGAGCAGCT
 GCCCTTGGGAAAGGCTTTGTTCCATCTCCTAGTGTCCAGTGAAGAGTTCGGGCT
 AGTACCGTGTGCTGCTCATCTGCGGGGCCCTGGGACTACAAGTACCTGACGGGTG
 AAGTCAAAAGCTCTTACATGAGGATAGACACTAGGATCCTGGAGGTTCCAGGTAC
 AGGGAGGTGTAGCTTACCTGCGAGGCTAGAGGTATCCCTTAGCAGAGTGTCTCT
 GGCAAAATGTCACTGTCTCTGCAACACCCAGCTCAGGCTCAGGCTCAGGCTGCT
 TACCAGTGTACAGGCTTCTGCGCCTCAGGCTCAGGCTCAGGCTCAGGCTCAGGCT
 CATGTTCTGGAATGCTCAGCTGAGGAGCTGACTTCAGCATCATTTGAGCCCTGGA
 GTCGGATGGAACCAAGTCTCCAGAGCTGGGCTCCTCATGTTTCTCCCGGCC
 TGCACATGCTTTGATCTTCTGCGCATAGTGATATCAAGAGAAAGAGGATCTA
 GGGAGAGCTGTATTCAGGAAGAGTGTCTCTTCCAGATCTGGAGCTTCGGGT
 CTGGAGGTGGAAGATCTGATGGAAACCTCAAGAGACTTCTGGACTCAAGT
 GAGAACTTGCAGGAGCTGCGCACTTGCACCTTTTGAACCTTGGAGCTGAGCCAG
 GCTTCCAGAGAGAGGAGCTGTGAAGTGTGACATCTTCCCTGTGCTCAGAGCTCTCT
 GAACAGCAGAGCCCAATGAGCTTTAGACTTACCTCTGGATCTCTGAGCCAGT
 GAGGCGCTAAGGCTCTCATGACTTTCAGGCTGAGAGCAAAAGAGTGTCTCTCCG
 CCCACCCCTCCTCTCTCTTCCGAGGAGCATGTGAATTTCCAGGTATCTA
 ATAGATTGTCAATAGATTTATATAGCCCTCATTTCTCCGAGGAGTGTGAAGCT
 TCAGACAGGCTTTTCAATAAAGATCACTATGATGTTTTCAGCATCTCTAG
 AATCTGGCAGCTCTGAGTGTCTAATTAAGTGTGAAGCATTAAGCAGAGCTCAA
 GTGCCCTGTGCTGATTTGTTTCACTTTCTGTTTAAAGTGTGAGTCAACA
 GGTAAATGTGTAACCTGTGATATCACTGTTCTTGTGCTCTCTTCAACTACA
 TCTTTTAAACAAAAAAGAAAAAAGAAAAA 1655

マウスPD-L2アミノ酸配列

1 MLLLLPIILNLSLQHPVAALFTVTPKEVITVDVSSVSLCEDFDRRECTLEGIR
 ASLQKRVENDTSLQSERATLLEELPLGKALFHPISVQVRDSQYRCLVCGAANDY
 KYLTVKVKASYMRIDTRILEVPTGVEVLTCQARGYPLAEVSWQNVSPANTSHIR
 TPEGLYQVTSVLRKLPQPSRNFSFVFNWTHRELTLASIDLQSQMEPRTHPTWLLH
 VFIPACTIALIFLAIVIIQKRRI 247

【図 3】

FIG. 3

PD-L2
 ドメイン順序: シグナル、IgV、IgC、膜貫通
 細胞質
 ネズミ 247aa
 シグナル MLLLLPIILNLSLQHPVAA
 ペプチド
 IgV LFTVTPKEVITYVDVSSVSLCEDFDRRECTLEGIRASLQKRVENDTSLQ
 ERATLLEELPLGKALFHPISVQVRDSQYRCLVCGAANDYKYLTVKVK
 IgC ASYMRIDTRILEVPTGVEVLTCQARGYPLAEVSWQNVSPANTSHIR
 GLYQVTSVLRKLPQPSRNFSFVFNWTHRELTLASIDLQSQMEPRTHPTWLLH
 膜貫通 TWPLHVFIPACTIALIFLAIVII
 細胞質 QKRRI
 ヒト 273aa
 シグナル MIFLLIMLSLELQLHQAIA
 ペプチド
 IgV LFTVTPKELYIEHGSNVLECNFTDGHVNLGAITASLQKRVENDTSPH
 ERATLLEELPLGKASFIPIQVQRDEGQYQCIIYGVANDYKYLTVKVK
 IgC ASYRKINHLKVPETDEVELTQATGTPLEAVSWPNVSPANTSHSR
 GLYQVTSVLRKLPQPSRNFSFVFNWTHRELTLASIDLQSQMEPRTHPTWLLH
 膜貫通 TWLLHIFIPSCIIAFIPIATVIAL
 細胞質 RKQLQKLYSSKDTTKRPVTTTKREVNSAI

【図 4】

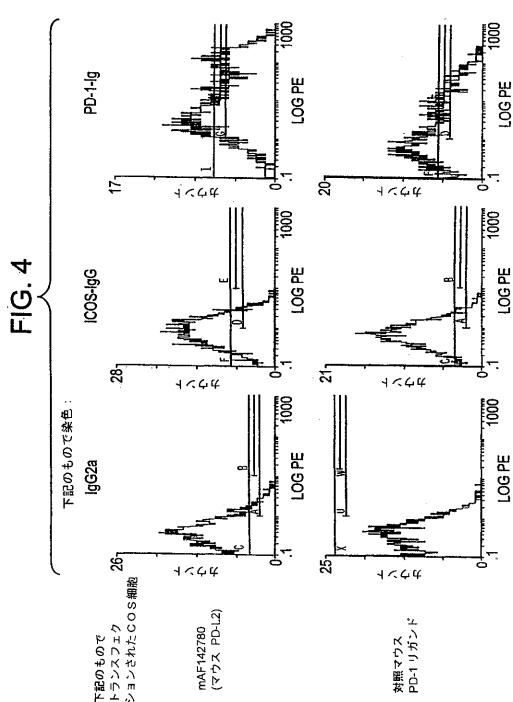


FIG. 5

【図 5】

本式ニPD-L2 対 ヒトPD-L2

70% 同一性

mPD-L2: 15 HEVAALFTVAPKEVTVVDVGSVLECDFFDRRECTELEGIRASIQKVENDTSLQSERAT 74
H +AALFTVT FRE+Y ++ GS+VLECD+FD L I ASIQKVENDTSLQSERAT 74
hPD-L2: 15 HQIAALFTVTVFRELIIIEHSGNVTLECNFDGSHVNLGATIASIQKVENDTSPHERAT 74
mPD-L2: 75 LLEEQLPLGKALFHIESVQVRDGGVRCIVICGAANDKYLTVKVKASIMRIRILEVP 134
LLEEQLPLGKA FHIP VOVRD GQY+C+I G ANDKYLTVKVKASIMRIRILEVP 134
hPD-L2: 75 LLEEQLPLGKASFHIFQVVRDEGQYQCIITGVANDKYLTVKVKASIMRIRILEVP 134
mPD-L2: 135 GTGEVLTQARGYPLAENVSWNSVPANTSHIRTPGELXQVTSVLELALPQGRNFSQMF 194
T EV+LITCOA GYPLAENVSW NSVPANTSH RTPGELXQVTSVLELALP P RNFSC+P
hPD-L2: 135 ETDEVELTQARGYPLAENVSWNSVPANTSHIRTPGELXQVTSVLELALPQGRNFSQMF 194
mPD-L2: 195 WNAMKELTSAIDPLSMPEKVPKTPHVFPACTIALIFALIVIQKRI 247
WN H+ELT A ID S+MEP+ TW LH+FE+C IA IF+A VI RK++
hPD-L2: 195 WNTVRELITASIDLSQMEPTHTLLHIFPISCIIFATFIATVIALRQL 247

FIG. 7B

【図 7 B】

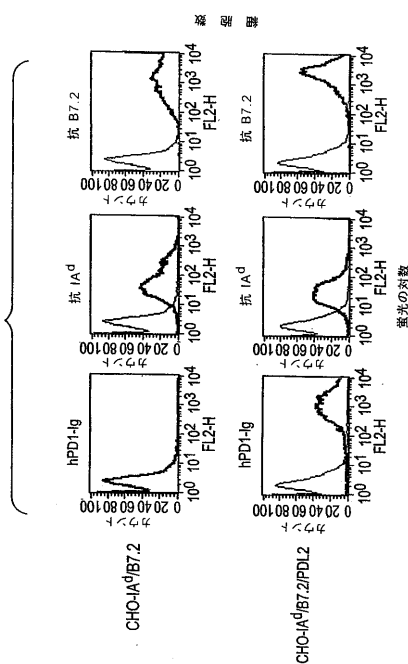
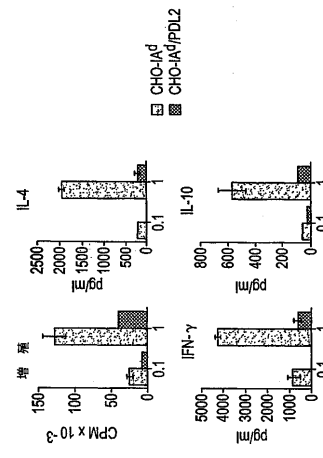


FIG. 9

【図 9】



【図 8】

FIG. 8

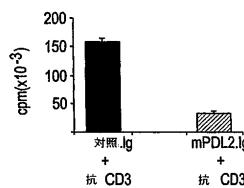
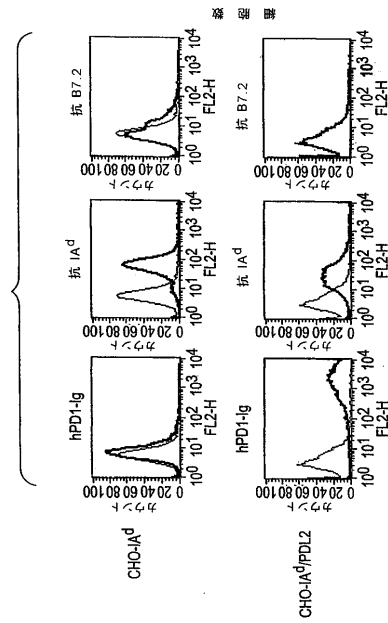


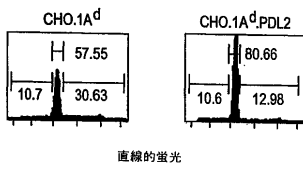
FIG. 7A

【図 7 A】



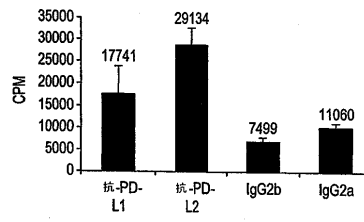
【図 13】

FIG. 13



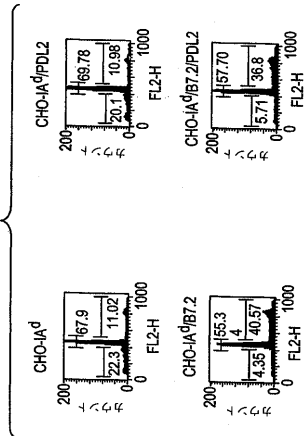
【図 15】

FIG. 15



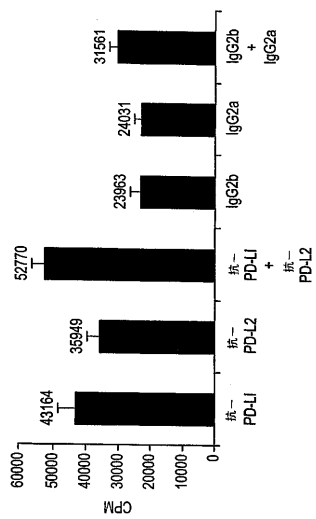
【図 14】

FIG. 14



【図 16】

FIG. 16



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(54) Title: PD-L2 MOLECULES: NOVEL PD-1 LIGANDS AND USES THEREFOR

(57) Abstract: The invention provides isolated nucleic acid molecules, designated PD-L2 nucleic acid molecules, which encode novel B7-related molecules which are ligands for PD-1. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing PD-L2 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a PD-L2 gene has been introduced or disrupted. The invention further provides isolated PD-L2 polypeptides, fusion proteins, antigenic peptides and anti-PD-L2 antibodies. The invention still further provides methods for promoting or inhibiting the interaction between PD-L2 and PD-1. Diagnostic and treatment methods utilizing compositions of the invention are also provided.

PD-L2 MOLECULES: NOVEL PD-1 LIGANDS AND USES THEREFOR**Related Applications**

5 This application claims priority to U.S. Provisional Application Serial No. 60/214,563, filed June 28, 2000; U.S. Provisional Application Serial No. 60/270,822, filed February 23, 2001; and U.S. Provisional Application Serial No. 60/271,114, filed February 23, 2001. The entire contents of each of these applications are incorporated herein by this reference.

Government Funding

10 Work described herein was supported under AI 39671, CA84500, AI41584, AI38310, and AI40614, awarded by the National Institutes of Health. The U.S. government therefore may have certain rights in this invention.

Background of the Invention

15 In order for T cells to respond to foreign polypeptides, two signals must be provided by antigen-presenting cells (APCs) to resting T lymphocytes (Jenkins, M. and Schwartz, R. (1987) *J. Exp. Med.* 165:302-319; Mueller, D.L. *et al.* (1990) *J. Immunol.* 144:3701-3709). The first signal, which confers specificity to the immune response, is transduced via the T cell receptor (TCR) following recognition of foreign antigenic peptide presented in the context of the major histocompatibility complex (MHC). The second signal, termed costimulation, induces T cells to proliferate and become functional (Lenschow *et al.* (1996) *Annu. Rev. Immunol.* 14:233). Costimulation is
25 neither antigen-specific, nor MHC-restricted, and is thought to be provided by one or more distinct cell surface molecules expressed by APCs (Jenkins, M.K. *et al.* (1988) *J. Immunol.* 140:3324-3330; Linsley, P.S. *et al.* (1991) *J. Exp. Med.* 173:721-730; Gimmi, C.D. *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:6575-6579; Young, J.W. *et al.* (1992) *J. Clin. Invest.* 90:229-237; Koulova, L. *et al.* (1991) *J. Exp. Med.* 173:759-762; Reiser, H. *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:271-275; van-Seventer, G.A. *et al.* (1990) *J. Immunol.* 144:4579-4586; LaSalle, J.M. *et al.* (1991) *J. Immunol.* 147:774-80; Dustin, M.I. *et al.* (1989) *J. Exp. Med.* 169:503; Armitage, R.J. *et al.* (1992) *Nature* 357:80-82; Liu, Y. *et al.* (1992) *J. Exp. Med.* 175:437-445).

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The CD80 (B7-1) and CD86 (B7-2) proteins, expressed on APCs, are critical costimulatory molecules (Freeman *et al.* (1991) *J. Exp. Med.* 174:625; Freeman *et al.* (1989) *J. Immunol.* 143:2714; Azuma *et al.* (1993) *Nature* 366:76; Freeman *et al.* (1993) *Science* 262:909). B7-2 appears to play a predominant role during primary immune responses, while B7-1, which is upregulated later in the course of an immune response, may be important in prolonging primary T cell responses or costimulating secondary T cell responses (Bluestone (1995) *Immunity* 2:555).

One ligand to which B7-1 and B7-2 bind, CD28, is constitutively expressed on resting T cells and increases in expression after activation. After signaling through the T cell receptor, ligation of CD28 and transduction of a costimulatory signal induces T cells to proliferate and secrete IL-2 (Linsley, P.S. *et al.* (1991) *J. Exp. Med.* 173:721-730; Gimmi, C.D. *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:6575-6579; June, C.H. *et al.* (1990) *Immunol. Today* 11:211-6; Harding, F.A. *et al.* (1992) *Nature* 356:607-609). A second ligand, termed CTLA4 (CD152) is homologous to CD28 but is not expressed on resting T cells and appears following T cell activation (Brunet, J.F. *et al.* (1987) *Nature* 328:267-270). CTLA4 appears to be critical in negative regulation of T cell responses (Waterhouse *et al.* (1995) *Science* 270:985). Blockade of CTLA4 has been found to remove inhibitory signals, while aggregation of CTLA4 has been found to provide inhibitory signals that downregulate T cell responses (Allison and Krummel (1995) *Science* 270:932). The B7 molecules have a higher affinity for CTLA4 than for CD28 (Linsley, P.S. *et al.* (1991) *J. Exp. Med.* 174:561-569) and B7-1 and B7-2 have been found to bind to distinct regions of the CTLA4 molecule and have different kinetics of binding to CTLA4 (Linsley *et al.* (1994) *Immunity* 1:793). A new molecule related to CD28 and CTLA4, ICOS, has been identified (Hutloff *et al.* (1999) *Nature* 397:263; WO 98/38216), as has its ligand, which is a new B7 family member (Aicher A. *et al.* (2000) *J. Immunol.* 164:4689-96; Mages H.W. *et al.* (2000) *Eur. J. Immunol.* 30:1040-7; Brodie D. *et al.* (2000) *Curr. Biol.* 10:333-6; Ling V. *et al.* (2000) *J. Immunol.* 164:1653-7; Yoshinaga S.K. *et al.* (1999) *Nature* 402:827-32). If T cells are only stimulated through the T cell receptor, without receiving an additional costimulatory signal, they become nonresponsive, anergic, or die, resulting in downmodulation of the immune response.

Immune cells have receptors that transmit activating signals. For example, T cells have T cell receptors and the CD3 complex, B cells have B cell receptors, and myeloid cells have Fc receptors. In addition, immune cells bear receptors that transmit

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signals that provide costimulatory signals or receptors that transmit signals that inhibit receptor-mediated signaling. For example, CD28 transmits a costimulatory signal to T cells. After ligation of the T cell receptor, ligation of CD28 results in a costimulatory signal characterized by, *e.g.*, upregulation of IL-2 α , IL-2 β , and IL-2 γ receptor, increased transcription of IL-2 messenger RNA, and increased expression of cytokine genes (including IL-2, IFN- γ , GM-CSF, and TNF- α). Transmission of a costimulatory signal allows the cell to progress through the cell cycle and, thus, increases T cell proliferation (Greenfield *et al.* (1998) *Crit. Rev. Immunol.* 18:389). Binding of a receptor on a T cell which transmits a costimulatory signal to the cell (*e.g.*, ligation of a costimulatory receptor that leads to cytokine secretion and/or proliferation of the T cell) by a costimulatory ligand results in costimulation. Thus, inhibition of an interaction between a costimulatory ligand and a receptor that transmits a costimulatory signal on immune cells results in a downmodulation of the immune response and/or specific unresponsiveness, termed immune cell anergy. Inhibition of this interaction can be accomplished using, *e.g.*, anti-CD28 Fab fragments, antibodies to B7 family molecules, or by using a soluble form of a receptor to which a B7 family member molecule can bind as a competitive inhibitor (*e.g.*, CTLA4Ig).

Inhibitory receptors that bind to costimulatory molecules have also been identified on immune cells. Activation of CTLA4, for example, transmits a negative signal to a T cell. Engagement of CTLA4 inhibits IL-2 production and can induce cell cycle arrest (Krummel and Allison (1996) *J. Exp. Med.* 183:2533). In addition, mice that lack CTLA4 develop lymphoproliferative disease (Tivol *et al.* (1995) *Immunity* 3:541; Waterhouse *et al.* (1995) *Science* 270:985). The blockade of CTLA4 with antibodies may remove an inhibitory signal, whereas aggregation of CTLA4 with antibody transmits an inhibitory signal. Therefore, depending upon the receptor to which a costimulatory molecule binds (*i.e.*, a costimulatory receptor such as CD28 or an inhibitory receptor such as CTLA4), B7 molecules including B7-4 can promote T cell costimulation or inhibition.

PD-1 is a member of the immunoglobulin family of molecules (Ishida *et al.* (1992) *EMBO J.* 11:3887; Shinohara *et al.* (1994) *Genomics* 23:704). PD-1 was previously identified using a subtraction cloning based approach designed to identify modulators of programmed cell death (Ishida *et al.* (1992) *EMBO J.* 11:3887-95; Woronicz *et al.* (1995) *Curr. Top. Microbiol. Immunol.* 200:137). PD-1 is believed to play a role in regulating lymphocyte survival, *e.g.*, during clonal selection (Honjo (1992)

Science 258:591; Agata *et al.* (1996) *Int. Immunology* 8:765; Nishimura *et al.* (1996) *Int. Immunology* 8:773). PD-1 has an extracellular region containing an immunoglobulin superfamily domain, a transmembrane domain, and an intracellular region which includes an immunoreceptor tyrosine kinase-based inhibitory motif (ITIM) (Ishida *et al.* (1992) *supra*; Shinohara *et al.* (1994) *supra*; US Patent 5,698,520). This features also define a larger family of molecules, called the immunoinhibitory receptors, which also includes gp49B, PIR-B, and the killer inhibitory receptors (KIRs) (Vivier and Daeron (1997) *Immunology Today* 18:286). It is often assumed that the tyrosyl phosphorylated ITIM motif of these receptors interacts with the SH2-domain-containing phosphatases, which leads to inhibitory signals. A subset of these immunoinhibitory receptors binds to MHC molecules, for example the KIRs, and CTLA4 binds to B7-1 and B7-2. It has been proposed that there is a phylogenetic relationship between the MHC and B7 genes (Henry *et al.* (1999) *Immunology Today* 20:285-288).

PD-1 was also implicated as a regulator of B cell responses (Nishimura (1998) *Int. Immunology* 10:1563). Unlike CTLA4, which is found only on T cells, PD-1 is also found on B cells (in response anti-IgM) and on a subset of thymocytes and myeloid cells (Agata *et al.* (1996) *supra*; Nishimura *et al.* (1996) *Int. Immunology* 8:773).

The importance of the B7:CD28/CTLA4 costimulatory pathway has been demonstrated *in vitro* and in several *in vivo* model systems. Blockade of this costimulatory pathway results in the development of antigen-specific tolerance in murine and human systems (Harding, F.A. *et al.* (1992) *Nature* 356:607-609; Lenschow, D.J. *et al.* (1992) *Science* 257:789-792; Turka, L.A. *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:11102-11105; Gimmi, C.D. *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6586-6590; Boussiotis, V. *et al.* (1993) *J. Exp. Med.* 178:1753-1763). Conversely, expression of B7 by B7-negative murine tumor cells induces T-cell mediated specific immunity accompanied by tumor rejection and long lasting protection to tumor challenge (Chen, L. *et al.* (1992) *Cell* 71:1093-1102; Townsend, S.E. and Allison, J.P. (1993) *Science* 259:368-370; Baskar, S. *et al.* (1993) *Proc. Natl. Acad. Sci* 90:5687-5690.). Therefore, manipulation of the costimulatory pathways offers great potential to stimulate or suppress immune responses in humans.

Summary of the Invention

The present invention is based, at least in part, on the discovery of novel nucleic acid molecules and polypeptides encoded by such nucleic acid molecules, referred to

herein as PD-L2 nucleic acid and polypeptide molecules, which are members of the B7 family and are ligands for PD-1. Interaction of PD-L2 with PD-1 transmits a negative signal to immune cells, downregulating immune responses. Preferred PD-L2 molecules are expressed on the surface of professional antigen presenting cells (*e.g.*, B lymphocytes, monocytes, dendritic cells, and Langerhans cells) and other antigen presenting cells (*e.g.*, keratinocytes, endothelial cells, astrocytes, fibroblasts, and oligodendrocytes), down-regulate lymphocyte activation, and/or are bound by antibodies which recognize PD-L2 molecules. The PD-L2 nucleic acid and polypeptide molecules of the present invention are useful, *e.g.*, in modulating the immune response.

Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding PD-L2 polypeptides, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of PD-L2-encoding nucleic acids.

In one embodiment, a PD-L2 nucleic acid molecule of the invention is at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the nucleotide sequence (*e.g.*, to the entire length of the nucleotide sequence) shown in SEQ ID NO:1 or 3, or a complement thereof.

In a preferred embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown in SEQ ID NO:1 or 3, or a complement thereof. In another embodiment, the nucleic acid molecule includes the nucleic acid sequence shown in SEQ ID NO:3 and nucleotides 1-273 of SEQ ID NO:1. In a further embodiment, the nucleic acid molecule includes the nucleic acid sequence shown in SEQ ID NO:3 and nucleotides 1096-1223 of SEQ ID NO:1. In another preferred embodiment, the nucleic acid molecule consists of the nucleotide sequence shown in SEQ ID NO:1 or 3.

In another embodiment, a PD-L2 nucleic acid molecule includes a nucleotide sequence encoding a polypeptide having an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2. In a preferred embodiment, a PD-L2 nucleic acid molecule includes a nucleotide sequence encoding a polypeptide having an amino acid sequence at least about 71%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the entire length of the amino acid sequence of SEQ ID NO:2.

In another preferred embodiment, an isolated nucleic acid molecule encodes the amino acid sequence of human PD-L2. In yet another preferred embodiment, the nucleic acid molecule includes a nucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO:2. In yet another preferred embodiment, the nucleic acid molecule is at least about 50, 100, 150, 200, 250, 300, 350, 400, 450, 500,

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550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more nucleotides in length. In a further preferred embodiment, the nucleic acid molecule is at least about 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more nucleotides in length and encodes a polypeptide having a PD-L2 activity (as described herein).

Another embodiment of the invention features nucleic acid molecules, preferably PD-L2 nucleic acid molecules, which specifically detect PD-L2 nucleic acid molecules relative to nucleic acid molecules encoding non-PD-L2 polypeptides. For example, in one embodiment, such a nucleic acid molecule is at least about 880, 900, 950, 1000, 1050, 1100, 1150 or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, or a complement thereof. In another embodiment, such a nucleic acid molecule is at least 20, 30, 40, 50, 100, 150, 200, 250, 300 or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising nucleotides 1-358 of SEQ ID NO:1, or a complement thereof. In a further embodiment, such a nucleic acid molecule is at least 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more nucleotides in length, includes at least 15 (*i.e.*, 15 contiguous) nucleotides of the sequence comprising nucleotides 1-358 of SEQ ID NO:1, or a complement thereof, and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, or a complement thereof.

In preferred embodiments, the nucleic acid molecules are at least about 880 nucleotides in length and hybridize under stringent conditions to the nucleotide molecule set forth in SEQ ID NO:1 (*i.e.*, to 880 contiguous nucleotides of SEQ ID NO:1), or a complement thereof. In other preferred embodiments, the nucleic acid molecules are at least about 15 nucleotides in length and hybridize under stringent conditions to nucleotides 1-358 of the nucleotide molecule set forth in SEQ ID NO:1 (*i.e.*, to 15 contiguous nucleotides of nucleotides 1-358 of SEQ ID NO:1), or a complement thereof. In further preferred embodiments, the nucleic acid molecules are at least 15 nucleotides in length, include at least 15 (*i.e.*, 15 contiguous) nucleotides of the sequence comprising nucleotides 1-358 of SEQ ID NO:1, or a complement thereof, and hybridize under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, or a complement thereof.

In still other preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the nucleic acid molecule hybridizes to a complement of a nucleic acid molecule comprising SEQ ID NO:1 or 3, or a complement thereof, under stringent conditions.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to a PD-L2 nucleic acid molecule, *e.g.*, is antisense to the coding strand of a PD-L2 nucleic acid molecule as shown in SEQ ID NO:1 or 3.

Another aspect of the invention provides a vector comprising a PD-L2 nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. In yet another embodiment, the invention provides a host cell containing a nucleic acid molecule of the invention. The invention also provides a method for producing a polypeptide, preferably a PD-L2 polypeptide, by culturing in a suitable medium, a host cell, *e.g.*, a mammalian host cell such as a non-human mammalian cell, of the invention containing a recombinant expression vector, such that the polypeptide is produced.

Another aspect of this invention features isolated or recombinant PD-L2 polypeptides (*e.g.*, proteins, polypeptides, peptides, or fragments or portions thereof). In one embodiment, an isolated PD-L2 polypeptide includes at least one or more of the following domains: a signal peptide domain, an IgV domain, an IgC domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain.

In a preferred embodiment, a PD-L2 polypeptide includes at least one or more of the following domains: a signal peptide domain, an IgV domain, an IgC domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain, and has an amino acid sequence at least about 71%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO:2. In another preferred embodiment, a PD-L2 polypeptide includes at least one or more of the following domains: a signal peptide domain, an IgV domain, an IgC domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain, and has a PD-L2 activity (as described herein).

In yet another preferred embodiment, a PD-L2 polypeptide includes at least one or more of the following domains: a signal peptide domain, an IgV domain, an IgC domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain,

and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3.

In another embodiment, the invention features fragments or portions of the polypeptide having the amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 15 amino acids (*i.e.*, contiguous amino acids) of the amino acid sequence of SEQ ID NO:2. In another embodiment, a PD-L2 polypeptide comprises or consists of the amino acid sequence of SEQ ID NO:2.

In another embodiment, the invention features a PD-L2 polypeptide which is encoded by a nucleic acid molecule consisting of a nucleotide sequence at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a nucleotide sequence of SEQ ID NO:1 or 3, or a complement thereof. This invention further features a PD-L2 polypeptide which is encoded by a nucleic acid molecule consisting of a nucleotide sequence which hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3.

The polypeptides of the present invention or portions thereof, *e.g.*, biologically active portions thereof, can be operatively linked to a non-PD-L2 polypeptide (*e.g.*, heterologous amino acid sequences) to form fusion polypeptides. The invention further features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind polypeptides of the invention, preferably PD-L2 polypeptides. In addition, the PD-L2 polypeptides (or biologically active portions thereof) or modulators of the PD-L2 molecules can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of a PD-L2 nucleic acid molecule, protein, or polypeptide in a biological sample by contacting the biological sample with an agent capable of detecting a PD-L2 nucleic acid molecule, protein, or polypeptide, such that the presence of a PD-L2 nucleic acid molecule, protein or polypeptide is detected in the biological sample.

In another aspect, the present invention provides a method for detecting the presence of PD-L2 activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of PD-L2 activity, such that the presence of PD-L2 activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating PD-L2 activity, comprising contacting a cell capable of expressing PD-L2 with an agent that modulates PD-L2 activity, such that PD-L2 activity in the cell is modulated. In one embodiment, the agent inhibits PD-L2 activity. In another embodiment, the agent stimulates PD-L2 activity. In a further embodiment, the agent interferes with or enhances the interaction between a PD-L2 polypeptide and its natural binding partner(s), *e.g.*, PD-1. In a preferred embodiment, the binding partner is PD-1. In one embodiment, the agent is an antibody that specifically binds to a PD-L2 polypeptide. In a further embodiment, the agent is a combination of an antibody that specifically binds to a PD-L2 polypeptide and an antibody that specifically binds to a PD-L1 polypeptide. In another embodiment, the agent is a peptide, peptidomimetic, or other small molecule that binds to a PD-L2 polypeptide. In yet another embodiment, the agent is another PD-1 ligand which can modulate the interaction between PD-L2 and PD-1. In still another embodiment, the agent modulates expression of PD-L2 by modulating transcription of a PD-L2 gene, translation of a PD-L2 mRNA, or post-translational modification of a PD-L2 polypeptide. In another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of a PD-L2 mRNA or a PD-L2 gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder or condition characterized by aberrant, insufficient, or unwanted PD-L2 polypeptide or nucleic acid expression or activity by administering an agent which is a PD-L2 modulator to the subject. In one embodiment, the PD-L2 modulator is a PD-L2 polypeptide. In another embodiment the PD-L2 modulator is a PD-L2 nucleic acid molecule. In a further embodiment, the PD-L2 modulator is an antibody that specifically binds to a PD-L2 polypeptide. In another embodiment, the PD-L2 modulator is a combination of an antibody that specifically binds to a PD-L2 polypeptide and an antibody that specifically binds to a PD-L1 polypeptide. In yet another embodiment, the PD-L2 modulator is a peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder or condition characterized by aberrant, insufficient, or unwanted PD-L2 polypeptide or nucleic acid expression or activity is an immune response disorder or condition that would benefit from modulation of PD-L2 activity. In another embodiment, the invention further provides treating the subject with an additional agent that modulates an immune response.

In still another embodiment, the invention provides a vaccine comprising an antigen and an agent that reduces or inhibits PD-L2 activity. In a preferred embodiment, the vaccine inhibits the interaction between PD-L2 and its natural binding partner(s). In a more preferred embodiment, the binding partner is PD-1.

5 The present invention also provides diagnostic assays for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a PD-L2 polypeptide; (ii) mis-regulation of the gene; and (iii) aberrant post-translational modification of a PD-L2 polypeptide, wherein a wild-type form of the gene encodes a polypeptide with a PD-L2 activity.

10 In another aspect the invention provides methods for identifying a compound that binds to or modulates the activity of a PD-L2 polypeptide, by providing an indicator composition comprising a PD-L2 polypeptide having PD-L2 activity, contacting the indicator composition with a test compound, and determining the effect of the test compound on PD-L2 activity in the indicator composition to identify a compound that
15 modulates the activity of a PD-L2 polypeptide.

In another aspect, this invention provides a method for modulating an immune response by modulating the interaction between PD-1 and PD-L2.

In one aspect, the invention features a method for modulating the interaction of PD-L2 with its natural binding partner(s) on an immune cell comprising contacting an
20 antigen presenting cell which expresses PD-L2 with an agent selected from the group consisting of: a form of PD-L2, a form of PD-1, or an agent that modulates the interaction of PD-L2 and its natural binding partner(s) such that the interaction of PD-L2 with its natural binding partner(s) on an immune cell is modulated. In a preferred embodiment, an agent that modulates the interaction of PD-L2 and its natural binding
25 partner(s) (e.g., PD-1) is an antibody that specifically binds to PD-L2. In another preferred embodiment, the agent is a combination of an antibody that specifically binds to PD-L2 and an antibody that specifically binds to PD-L1.

In one embodiment, the interaction of PD-L2 with its natural binding partner(s) is upregulated. In another embodiment, the interaction of PD-L2 with its natural binding
30 partner(s) is downregulated.

In one embodiment, the method further comprises contacting the immune cell or the antigen presenting cell with an additional agent that modulates an immune response.

In one embodiment, the step of contacting is performed *in vitro*. In another embodiment, the step of contacting is performed *in vivo*.

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In one embodiment, the immune cell is selected from the group consisting of: a T cell, a B cell, and a myeloid cell.

In one embodiment, the PD-L2 binding partner is PD-1.

In another aspect, the invention pertains to a method for inhibiting activation in
5 an immune cell via a non-apoptotic mechanism comprising increasing the activity or expression of PD-L2 in a cell such that immune cell activation is inhibited.

In yet another aspect, the invention pertains to a vaccine comprising an antigen and an agent that inhibits the interaction between PD-L2 and its natural binding partner(s).

10 In still another aspect, the invention pertains to a vaccine comprising an antigen and an agent that promotes the interaction between PD-L2 and its natural binding partner(s).

In one embodiment, the PD-L2 binding partner is PD-1.

In another aspect, the invention pertains to a method for treating a subject having
15 a condition that would benefit from upregulation of an immune response comprising administering an agent that inhibits the interaction between PD-L2 and its natural binding partner(s) on cells of the subject such that a condition that would benefit from upregulation of an immune response is treated.

In one embodiment, the agent comprises a blocking antibody or a small molecule
20 that binds to PD-L2 and inhibits the interaction between PD-L2 and its natural binding partner(s). In another embodiment, the agent comprises a combination of an antibody that specifically binds to PD-L2 and an antibody that specifically binds to PD-L1.

In another embodiment, the method further comprises administering a second agent that upregulates an immune response to the subject.

25 In one embodiment, the condition is selected from the group consisting of: a tumor, a pathogenic infection, or an immunosuppressive disease.

In another embodiment, the PD-L2 binding partner is PD-1.

In one aspect, the invention pertains to a method for treating a subject having a condition that would benefit from downregulation of an immune response comprising
30 administering an agent that stimulates the interaction between PD-L2 and its natural binding partner(s) on cells of the subject such that a condition that would benefit from downregulation of an immune response is treated.

In one embodiment agent comprises an antibody or a small molecule that stimulates the interaction between PD-L2 and its natural binding partner(s).

In another embodiment, the method further comprises administering a second agent that downregulates an immune response to the subject.

In another embodiment, the condition is selected from the group consisting of: a transplant, an allergy, and an autoimmune disorder.

5 In one embodiment, the PD-L2 binding partner is PD-1.

In another aspect, the invention pertains to a cell-based assay for screening for compounds which modulate the activity of PD-L2 comprising contacting a cell expressing a PD-L2 target molecule with a test compound and determining the ability of the test compound to modulate the activity of the PD-L2 target molecule

10 In still another aspect, the invention pertains to a cell-free assay for screening for compounds which modulate the binding of PD-L2 to a target molecule comprising contacting a PD-L2 polypeptide or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the PD-L2 polypeptide or biologically active portion thereof.

15 In another embodiment, the invention pertains to a method of identifying a compound which modulates T cell activation at a first and second antigen concentration comprising contacting a T cell expressing a PD-L2 target molecule with a test compound at a first antigen concentration, determining the ability of the test compound to modulate T cell proliferation or cytokine production at the first antigen concentration, 20 contacting a T cell expressing a PD-L2 target molecule with the test compound at a second antigen concentration, and determining the ability of the test compound to modulate T cell proliferation or cytokine production at the second antigen concentration, thereby identifying a compound which modulates T cell activation at a first and second antigen concentration.

25 In one embodiment, the PD-L2 target molecule is PD-1.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

30 *Figure 1* depicts the cDNA sequence and predicted amino acid sequence of human PD-L2. The nucleotide sequence corresponds to nucleic acids 1-1223 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1-273 of SEQ ID NO:2. The coding region without the 5' or 3' untranslated regions of the human PD-L2 gene is shown in SEQ ID NO:3.

Figure 2 depicts the cDNA sequence and amino acid sequence of mouse PD-L2. The nucleotide sequence corresponds to nucleic acids 1-1655 of SEQ ID NO:4. The amino acid sequence corresponds to amino acids 1-247 of SEQ ID NO:5. The coding region without the 5' or 3' untranslated region of the mouse PD-L2 gene is shown in

5 SEQ ID NO:6.

Figure 3 depicts the amino acid sequences of the human and mouse PD-L2 polypeptides (SEQ ID NO:2 and SEQ ID NO:5, respectively) and illustrates the signal peptide, IgV, IgC, extracellular, transmembrane, and cytoplasmic domains.

Figure 4 illustrates the results of FACS analysis of the binding of IgG2a (control Ig), ICOS-IgG, and control PD-1-Ig to COS cells transfected with mouse PD-L2 or with a control mouse PD-1 ligand.

Figure 5 depicts an alignment of the amino acid sequences of the mouse and human PD-L2 polypeptides (SEQ ID NO:5 and SEQ ID NO:2, respectively). Identical amino acids are illustrated between the two sequences.

15 *Figure 6* depicts an alignment of the amino acid sequences of the mouse PD-L2 (SEQ ID NO:5), human PD-L2 (SEQ ID NO:2), mouse PD-L1 (SEQ ID NO:11), and human PD-L1 (SEQ ID NO:12).

Figure 7 depicts the binding of PD-1 to PD-L2. CHO cells expressing I-A^d alone or I-A^d and B7-2 were either not transfected or stably transfected with mouse PD-L2. Cells were stained with hPD-1-Ig and stained with PE-goat anti-mouse IgG2a (thick line). CHO cells were stained separately using PE-anti-I-A^d or PE-anti-B7-2 (thick line). The thin lines indicate staining with isotype control monoclonal antibody. Ten-thousand events were analyzed.

Figure 8 depicts inhibition of TCR-mediated responses by PD-L2-PD-1 interaction. Purified T cells from BALB/c lymph nodes were stimulated at a 2:1 bead:cell ratio with tosyl beads coated with anti-CD3 + control Ig or anti-CD3 + mPD-L2-Ig. Proliferation was measured after 72 hours. These data are representative of more than eight independent experiments.

30 *Figure 9* depicts inhibition of TCR-mediated responses by PD-L2-PD-1 interaction. Splenocytes from DO11.10 transgenic mice were activated with OVA peptide (1 µg/ml). CD4⁺ T cells were isolated and rested overnight. Previously activated T cells (10⁵) were restimulated with peptide (1 or 0.1 µg/ml) presented by CHO-I-A^d or CHO-I-A^d-PD-L2 for 48 hours. [³H]thymidine incorporation was measured in triplicate. Aliquots of supernatants were collected at 36 hours after

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initiation of cultures and cytokines measured by ELISA. These data are representative of four independent experiments.

Figure 10 depicts inhibition of TCR and CD28 mediated responses by PD-L2-PD-1 interaction. Splenocytes from DO11.10 transgenic mice were activated with OVA peptide (1 µg/ml) for 4 days. CD4⁺ T cells were isolated and rested overnight. Previously activated T cells (10⁵) were restimulated, with varying peptide concentrations, by the indicated CHO transfectants for 48 hours. [³H]thymidine incorporation was measured in triplicate. These data are representative of six independent experiments.

Figure 11 depicts inhibition of TCR and CD28 mediated responses by PD-L2-PD-1 interaction. Previously activated T cells (10⁵) were cultured with 0.01 µg/ml OVA peptide presented by the indicated CHO transfectants. Aliquots of supernatants were collected at 36 hours after initiation of cultures and cytokines measured by ELISA. The broken line indicates the sensitivity of the ELISA.

Figure 12 depicts inhibition of TCR and CD28 mediated responses by PD-L2-PD-1 interaction. Previously activated T cells (10⁵) were cultured with 0.1 µg/ml OVA peptide presented by the indicated CHO transfectants. Aliquots of supernatants were collected at 36 hours after initiation of cultures and cytokines measured by ELISA. The broken line indicates the sensitivity of the ELISA.

Figure 13 depicts cell cycle arrest and apoptosis as a result of the engagement of the PD-L2-PD-1 pathway. Previously activated T cells were restimulated with OVA peptide (1 µg/ml) and the indicated CHO transfectants. Cells were collected after 36 hours of culture, stained with anti-CD4 and fixed in 70% ethanol. Cells were resuspended in propidium iodide solution. FACS profiles are propidium iodide staining of the CD4⁺ population. Subdiploid, diploid, and supradiploid populations are indicated. Ten-thousand events were collected and analyzed at a constant flow rate. These data are representative of three independent experiments.

Figure 14 depicts cell cycle arrest and apoptosis as a result of the engagement of the PD-L2-PD-1 pathway. Previously activated T cells were restimulated with OVA peptide (0.01 µg/ml) and the indicated CHO transfectants. Cells were collected after 36 hours of culture, stained with anti-CD4 and fixed in 70% ethanol. Cells were resuspended in propidium iodide solution. FACS profiles are propidium iodide staining of the CD4⁺ population. Subdiploid, diploid, and supradiploid populations are

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indicated. Ten-thousand events were collected and analyzed at a constant flow rate. These data are representative of three independent experiments.

Figure 15 depicts the enhancement of T cell proliferation in the presence of anti-PD-L1 or anti PD-L2 antibodies. Allogeneic CD4+ T cells were stimulated in a mixed lymphocyte reaction by IL-10 treated dendritic cells.

Figure 16 depicts the enhancement of T cell proliferation in the presence of anti-PD-L1, anti PD-L2 antibodies, or a combination of anti-PD-L1 and anti-PD-L2 antibodies. Allogeneic CD4+ T cells were stimulated in a mixed lymphocyte reaction by IL-10 treated dendritic cells.

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Detailed Description of the Invention

In addition to the previously characterized B lymphocyte activation antigens, *e.g.*, B7-1 and B7-2, there are other antigens on the surface of antigen-presenting cells (*e.g.*, B cells, monocytes, dendritic cell, Langerhans cells, keratinocytes, endothelial cells, astrocytes, fibroblasts, and oligodendrocytes) which modulate the activation of B cells, T cells, and other immune cells. The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as PD-L2 polypeptides, which bind to the PD-1 receptor and down-regulate the activation of these immune cells and/or to downregulate immune responses. These novel molecules play a role in the modulating the immune response.

The instant discovery that PD-L2 binds to PD-1 places PD-L2 in a family of inhibitory ligands, and sequence analysis places PD-L2 in the B7 family. While engagement of a costimulatory receptor results in a costimulatory signal in an immune cell, engagement of an inhibitory receptor, *e.g.*, CTLA4 or PD-1 (for example by crosslinking or by aggregation), leads to the transmission of an inhibitory signal in an immune cell resulting in downmodulation of immune cell responses and/or in immune cell anergy. While transmission of an inhibitory signal leads to downmodulation in immune cell responses (and a resulting downmodulation in the overall immune response), the prevention of an inhibitory signal (*e.g.*, by using a non-activating antibody against PD-1) in immune cells leads to upmodulation of immune cell responses (and a resulting upmodulation of an immune response).

PD-L2 has homology to PD-L1, a previously described ligand for PD-1 (see the co-pending U.S. Application Serial No. 09/644,934; International Publication WO 01/14557; Dong, H. *et al.* (1999) *Nat. Med.* 5:1365-1369; and Freeman, G.J. *et al.*

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(2000) *J. Exp. Med.* 192:1027-1034 the contents of each of which are incorporated herein by reference).

The instant invention makes available agents useful for modulating the activity and/or expression of PD-L2; agents for modulating the interaction between PD-L2 and its natural binding partner(s) (*e.g.*, PD-1), and agents for modulating the immune response via modulation of the interaction between PD-L2 and its natural binding partner(s) (*e.g.*, PD-1). Exemplary modulatory agents for use in these methods are described further as follows.

10 Definitions

As used herein, the term "immune cell" includes cells that are of hematopoietic origin and that play a role in the immune response. Immune cells include lymphocytes, such as B cells and T cells; natural killer cells; and myeloid cells, such as monocytes, macrophages, eosinophils, mast cells, basophils, and granulocytes.

15 As used herein, the term "T cell" includes CD4+ T cells and CD8+ T cells. The term T cell also includes both T helper 1 type T cells and T helper 2 type T cells. The term "antigen presenting cell" includes professional antigen presenting cells (*e.g.*, B lymphocytes, monocytes, dendritic cells, and Langerhans cells) as well as other antigen presenting cells (*e.g.*, keratinocytes, endothelial cells, astrocytes, fibroblasts, and oligodendrocytes).

As used herein, the term "immune response" includes T cell-mediated and/or B cell-mediated immune responses that are influenced by modulation of T cell costimulation. Exemplary immune responses include B cell responses (*e.g.*, antibody production) T cell responses (*e.g.*, cytokine production, and cellular cytotoxicity) and activation of cytokine responsive cells, *e.g.*, macrophages. As used herein, the term "downmodulation" with reference to the immune response includes a diminution in any one or more immune responses, while the term "upmodulation" with reference to the immune response includes an increase in any one or more immune responses. It will be understood that upmodulation of one type of immune response may lead to a corresponding downmodulation in another type of immune response. For example, upmodulation of the production of certain cytokines (*e.g.*, IL-10) can lead to downmodulation of cellular immune responses.

As used herein, the term "costimulatory receptor" includes receptors which transmit a costimulatory signal to an immune cell, *e.g.*, CD28 or ICOS. As used herein, the term "inhibitory receptors" includes receptors which transmit a negative signal to an immune cell (*e.g.*, CTLA4 or PD-1).

5 As used herein, the term "costimulate", with reference to activated immune cells, includes the ability of a costimulatory molecule to provide a second, non-activating, receptor-mediated signal (a "costimulatory signal") that induces proliferation or effector function. For example, a costimulatory signal can result in cytokine secretion, *e.g.*, in a T cell that has received a T cell-receptor-mediated signal. Immune cells that have
10 received a cell receptor-mediated signal, *e.g.*, via an activating receptor, are referred to herein as "activated immune cells."

An inhibitory signal as transduced by an inhibitory receptor can occur even if a costimulatory receptor (such as CD28 or ICOS) is not present on the immune cell and, thus, is not simply a function of competition between inhibitory receptors and
15 costimulatory receptors for binding of costimulatory molecules (Fallarino *et al.* (1998) *J. Exp. Med.* 188:205). Transmission of an inhibitory signal to an immune cell can result in unresponsiveness, anergy or programmed cell death in the immune cell. Preferably, transmission of an inhibitory signal operates through a mechanism that does not involve apoptosis. As used herein the term "apoptosis" includes programmed cell
20 death which can be characterized using techniques which are known in the art. Apoptotic cell death can be characterized, *e.g.*, by cell shrinkage, membrane blebbing, and chromatin condensation culminating in cell fragmentation. Cells undergoing apoptosis also display a characteristic pattern of internucleosomal DNA cleavage.

Depending upon the form of the PD-L2 molecule that binds to a receptor, a
25 signal can be either transmitted (*e.g.*, by a multivalent form of a PD-L2 molecule that results in crosslinking of the receptor or by a soluble form of PD-L2 that binds to Fc receptors on antigen presenting cells) or inhibited (*e.g.*, by a soluble, monovalent form of a PD-L2 molecule or a soluble form of PD-L2 that is altered using methods known in the art such that it does not bind to Fc receptors on antigen presenting cells), *e.g.*, by
30 competing with activating forms of PD-L2 molecules for binding to the receptor. However, there are instances in which a soluble molecule can be stimulatory. The effects of the various modulatory agents can be easily demonstrated using routine screening assays as described herein.

As used herein, the term "activating receptor" includes immune cell receptors that bind antigen, complexed antigen (*e.g.*, in the context of MHC molecules), or antibodies. Such activating receptors include T cell receptors (TCRs), B cell receptors (BCRs), cytokine receptors, LPS receptors, complement receptors, and Fc receptors.

For example, T cell receptors are present on T cells and are associated with CD3 molecules. T cell receptors are stimulated by antigen in the context of MHC molecules (as well as by polyclonal T cell activating reagents). T cell activation via the TCR results in numerous changes, *e.g.*, protein phosphorylation, membrane lipid changes, ion fluxes, cyclic nucleotide alterations, RNA transcription changes, protein synthesis changes, and cell volume changes.

The term "B cell receptor" (BCR) as used herein includes the complex between membrane Ig (mIg) and other transmembrane polypeptides (*e.g.*, Ig α and Ig β) found on B cells. The signal transduction function of mIg is triggered by crosslinking of receptor molecules by oligomeric or multimeric antigens. B cells can also be activated by anti-immunoglobulin antibodies. Upon BCR activation, numerous changes occur in B cells, including tyrosine phosphorylation.

The term "Fc receptor" (FcRs) include cell surface receptors for the Fc portion of immunoglobulin molecules (Igs). Fc receptors are found on many cells which participate in immune responses. Among the human FcRs that have been identified so far are those which recognize IgG (designated Fc γ R), IgE (Fc ϵ R1), IgA (Fc α R), and polymerized IgM/A (Fc μ α R). FcRs are found in the following cell types: Fc ϵ R I (mast cells), Fc ϵ R II (many leukocytes), Fc α R (neutrophils), and Fc μ α R (glandular epithelium, hepatocytes) (Hogg, N. (1988) *Immunol. Today* 9:185-86). The widely studied Fc γ Rs are central in cellular immune defenses, and are responsible for stimulating the release of mediators of inflammation and hydrolytic enzymes involved in the pathogenesis of autoimmune disease (Unkeless, J.C. (1988) *Annu. Rev. Immunol.* 6:251-87). The Fc γ Rs provide a crucial link between effector cells and the lymphocytes that secrete Ig, since the macrophage/monocyte, polymorphonuclear leukocyte, and natural killer (NK) cell Fc γ Rs confer an element of specific recognition mediated by IgG. Human leukocytes have at least three different receptors for IgG: hFc γ RI (found on monocytes/macrophages), hFc γ RII (on monocytes, neutrophils, eosinophils, platelets, possibly B cells, and the K562 cell line), and Fc γ III (on NK cells, neutrophils, eosinophils, and macrophages).

With respect to T cells, transmission of a costimulatory signal to a T cell involves a signaling pathway that is not inhibited by cyclosporin A. In addition, a costimulatory signal can induce cytokine secretion (*e.g.*, IL-2 and/or IL-10) in a T cell and/or can prevent the induction of unresponsiveness to antigen, the induction of anergy, or the induction of cell death in the T cell.

As used herein, the term "inhibitory signal" refers to a signal transmitted via an inhibitory receptor (*e.g.*, CTLA4 or PD-1) molecule on an immune cell. Such a signal antagonizes a signal via an activating receptor (*e.g.*, via a TCR, CD3, BCR, or Fc molecule) and can result, *e.g.*, in inhibition of: second messenger generation; proliferation; or effector function in the immune cell, *e.g.*, reduced phagocytosis, antibody production, or cellular cytotoxicity, or the failure of the immune cell to produce mediators (such as cytokines (*e.g.*, IL-2) and/or mediators of allergic responses); or the development of anergy.

As used herein, the term "unresponsiveness" includes refractivity of immune cells to stimulation, *e.g.*, stimulation via an activating receptor or a cytokine. Unresponsiveness can occur, *e.g.*, because of exposure to immunosuppressants or high doses of antigen. As used herein, the term "anergy" or "tolerance" includes refractivity to activating receptor-mediated stimulation. Such refractivity is generally antigen-specific and persists after exposure to the tolerizing antigen has ceased. For example, anergy in T cells (as opposed to unresponsiveness) is characterized by lack of cytokine production, *e.g.*, IL-2. T cell anergy occurs when T cells are exposed to antigen and receive a first signal (a T cell receptor or CD-3 mediated signal) in the absence of a second signal (a costimulatory signal). Under these conditions, reexposure of the cells to the same antigen (even if reexposure occurs in the presence of a costimulatory molecule) results in failure to produce cytokines and, thus, failure to proliferate. Anergic T cells can, however, mount responses to unrelated antigens and can proliferate if cultured with cytokines (*e.g.*, IL-2). For example, T cell anergy can also be observed by the lack of IL-2 production by T lymphocytes as measured by ELISA or by a proliferation assay using an indicator cell line. Alternatively, a reporter gene construct can be used. For example, anergic T cells fail to initiate IL-2 gene transcription induced by a heterologous promoter under the control of the 5' IL-2 gene enhancer or by a multimer of the AP1 sequence that can be found within the enhancer (Kang *et al.* (1992) *Science* 257:1134).

Modulation of a costimulatory signal results in modulation of effector function of an immune cell. Thus, the term "PD-L2 activity" includes the ability of a PD-L2 polypeptide to bind its natural binding partner(s), *e.g.*, PD-1, the ability to modulate immune cell costimulatory or inhibitory signals, and the ability to modulate the immune response.

With respect to PD-1, the term "activity" includes the ability of a PD-1 polypeptide to modulate an inhibitory signal in an activated immune cell, *e.g.*, by engaging a natural ligand on an antigen presenting cell. PD-1 transmits an inhibitory signal to an immune cell in a manner similar to CTLA4. Modulation of an inhibitory signal in an immune cell results in modulation of proliferation of and/or cytokine secretion by an immune cell. PD-1 can also modulate a costimulatory signal by competing with a costimulatory receptor for binding of its natural ligand(s). Thus, the term "PD-1 activity" includes the ability of a PD-1 polypeptide to bind its natural ligand(s), the ability to modulate immune cell costimulatory or inhibitory signals, and the ability to modulate the immune response.

As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

As used herein, an "antisense" nucleic acid molecule comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule, complementary to an mRNA sequence or complementary to the coding strand of a gene. Accordingly, an antisense nucleic acid molecule can hydrogen bond to a sense nucleic acid molecule.

As used herein, the term "coding region" refers to regions of a nucleotide sequence comprising codons which are translated into amino acid residues, whereas the term "noncoding region" refers to regions of a nucleotide sequence that are not translated into amino acids (*e.g.*, 5' and 3' untranslated regions).

As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid molecule to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are

introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" or simply "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

As used herein, the term "host cell" is intended to refer to a cell into which a nucleic acid molecule of the invention, such as a recombinant expression vector of the invention, has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It should be understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

As used herein, a "transgenic animal" refers to a non-human animal, preferably a mammal, more preferably a mouse, in which one or more of the cells of the animal includes a "transgene". The term "transgene" refers to exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, for example directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal.

As used herein, a "homologous recombinant animal" refers to a type of transgenic non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

As used herein, an "isolated protein" refers to a protein that is substantially free of other proteins, cellular material and culture medium when isolated from cells or produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized.

5 An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the PD-L2 protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of PD-L2 protein in which
10 the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of PD-L2 protein having less than about 30% (by dry weight) of non- PD-L2 protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non- PD-L2 protein, still more
15 preferably less than about 10% of non- PD-L2 protein, and most preferably less than about 5% non- PD-L2 protein. When the PD-L2 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein
20 preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of PD-L2 protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals"
25 includes preparations of PD-L2 protein having less than about 30% (by dry weight) of chemical precursors or non- PD-L2 chemicals, more preferably less than about 20% chemical precursors or non- PD-L2 chemicals, still more preferably less than about 10% chemical precursors or non- PD-L2 chemicals, and most preferably less than about 5% chemical precursors or non- PD-L2 chemicals.

30 The term "antibody", as used herein, includes an "antigen-binding portion" of an antibody (or simply "antibody portion"), as well as whole antibody molecules. The term "antigen-binding portion", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, PD-L2). It has been shown that the antigen-binding function of an antibody can be performed by

fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; (v) a dAb fragment (Ward *et al.* (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Osbourn *et al.* 1998 *Nat. Biotechnol.* 16:778). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Any VH and VL sequences of specific scFv can be linked to human immunoglobulin constant region cDNA or genomic sequences, in order to generate expression vectors encoding complete IgG molecules or other isotypes. VH and VL can also be used in the generation of Fab, Fv, or other fragments of immunoglobulins using either protein chemistry or recombinant DNA technology. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P. *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J. *et al.* (1994) *Structure* 2:1121-1123).

Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecules, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S.M. *et al.* (1995) *Hum. Antibodies Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S.M. *et al.* (1994) *Mol. Immunol.* 31:1047-1058). Antibody portions, such as Fab and F(ab')₂ fragments,

can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

5 Antibodies may be polyclonal or monoclonal; xenogeneic, allogeneic, or syngeneic; or modified forms thereof, *e.g.*, humanized, chimeric, etc. Preferably, antibodies of the invention bind specifically or substantially specifically to PD-L2 molecules. The terms "monoclonal antibodies" and "monoclonal antibody composition", as used herein, refer to a population of antibody molecules that contain
10 only one species of an antigen binding site capable of immunoreacting with a particular epitope of an antigen, whereas the term "polyclonal antibodies" and "polyclonal antibody composition" refer to a population of antibody molecules that contain multiple species of antigen binding sites capable of interacting with a particular antigen. A monoclonal antibody composition, typically displays a single binding affinity for a
15 particular antigen with which it immunoreacts.

 The term "humanized antibody", as used herein, is intended to include antibodies made by a non-human cell having variable and constant regions which have been altered to more closely resemble antibodies that would be made by a human cell. For example, by altering the non-human antibody amino acid sequence to incorporate amino acids
20 found in human germline immunoglobulin sequences. The humanized antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs. The term "humanized antibody", as used herein, also includes antibodies in which CDR
25 sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

 An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds PD-L2 is substantially free of antibodies that
30 specifically bind antigens other than PD-L2). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

PD-L2 Nucleic Acid and Polypeptide Molecules

The term "family" when referring to the polypeptide and nucleic acid molecules of the invention is intended to mean two or more polypeptide or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first polypeptide of human origin, as well as other, distinct polypeptides of human origin or alternatively, can contain homologues of non-human origin, *e.g.*, monkey polypeptides. Members of a family may also have common functional characteristics.

For example, the family of PD-L2 polypeptides of the present invention preferably comprises least one "signal peptide domain". As used herein, a "signal sequence" or "signal peptide" includes a peptide containing about 15 or more amino acids which occurs at the N-terminus of secretory and membrane bound polypeptides and which contains a large number of hydrophobic amino acid residues. For example, a signal sequence contains at least about 10-30 amino acid residues, preferably about 15-25 amino acid residues, more preferably about 18-20 amino acid residues, and even more preferably about 19 amino acid residues, and has at least about 35-65%, preferably about 38-50%, and more preferably about 40-45% hydrophobic amino acid residues (*e.g.*, Valine, Leucine, Isoleucine or Phenylalanine). Such a "signal sequence", also referred to in the art as a "signal peptide", serves to direct a polypeptide containing such a sequence to a lipid bilayer, and is cleaved in secreted and membrane bound polypeptides. A signal sequence was identified in the amino acid sequence of native human PD-L2 at about amino acids 1-19 of SEQ ID NO:2 (Figure 3). A signal sequence was also identified in the amino acid sequence of native mouse PD-L2 at about amino acids 1-19 of SEQ ID NO:5 (Figure 3).

In another embodiment of the invention, a PD-L2 polypeptide of the present invention is identified based on the presence of a "transmembrane domain". As used herein, the term "transmembrane domain" includes an amino acid sequence of about 15 amino acid residues in length which spans the plasma membrane. More preferably, a transmembrane domain includes about at least 20, 25, 30, 35, 40, or 45 amino acid residues and spans the plasma membrane. Transmembrane domains are rich in hydrophobic residues, and typically have an alpha-helical structure. In a preferred embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a

transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, tyrosines, or tryptophans. Transmembrane domains are described in, for example, Zagotta, W.N. *et al.* (1996) *Annu. Rev. Neurosci.* 19:235-263, the contents of which are incorporated herein by reference. Amino acid residues 220-243 of the native human PD-L2 polypeptide, and amino acid residues 201-243 of the predicted mature polypeptide, are predicted to comprise transmembrane domains (see Figure 3). Amino acid residues 220-242 of the native mouse PD-L2 polypeptide, and amino acid residues 201-223 of the predicted mature polypeptide, are predicted to comprise transmembrane domains (see Figure 3). Accordingly, PD-L2 polypeptides having at least 71-80%, or more preferably about 80-90% homology with a transmembrane domain of human PD-L2 are within the scope of the invention.

In another embodiment, a PD-L2 molecule of the present invention is identified based on the presence of an "IgC domain" or an "IgV domain" in the polypeptide or corresponding nucleic acid molecule. As used herein, IgV and IgC domains are recognized in the art as Ig superfamily member domains. These domains correspond to structural units that have distinct folding patterns called Ig folds. Ig folds are comprised of a sandwich of two β sheets, each consisting of antiparallel β strands of 5-10 amino acids with a conserved disulfide bond between the two sheets in most, but not all, domains. IgC domains of Ig, TCR, and MHC molecules share the same types of sequence patterns and are called the C1 set within the Ig superfamily. Other IgC domains fall within other sets. IgV domains also share sequence patterns and are called V set domains. IgV domains are longer than C-domains and form an additional pair of β strands. Amino acid residues 20-120 of the native human PD-L2 polypeptide, and amino acid residues 1-101 of the predicted mature polypeptide, are predicted to comprise IgV domains (see Figure 3). Amino acid residues 20-120 of the native mouse PD-L2 polypeptide, and amino acid residues 1-101 of the predicted mature polypeptide, are also predicted to comprise IgV domains (see Figure 3). Amino acid residues 121-219 of the native human PD-L2 polypeptide, and amino acid residues 102-200 of the predicted mature polypeptide, are predicted to comprise IgC domains (see Figure 3). Amino acid residues 121-219 of the native mouse PD-L2 polypeptide, and amino acid residues 102-200 of the predicted mature polypeptide, are also predicted to comprise IgC domains (see Figure 3). In a preferred embodiment, the presence of an IgV domain is required for binding of PD-L2 to its natural binding partner, *e.g.*, PD-1.

In another embodiment, a PD-L2 molecule of the present invention is identified based on the presence of a "extracellular domain" in the polypeptide or corresponding nucleic acid molecule. As used herein, the term "extracellular domain" represents the N-terminal amino acids which extend as a tail from the surface of a cell. An
 5 extracellular domain of the present invention includes an IgV domain and an IgC domain, and may include a signal peptide domain. Amino acid residues 1-219 of the native human PD-L2 polypeptide, and amino acid residues 1-200 of the predicted mature polypeptide, are predicted to comprise extracellular domains (see Figure 3). Amino acid residues 1-219 of the native mouse PD-L2 polypeptide, and amino acid
 10 residues 1-200 of the predicted mature polypeptide, are also predicted to comprise extracellular domains (see Figure 3).

In still another embodiment, a PD-L2 molecule of the present invention is identified based on the presence of a "cytoplasmic domain" in the polypeptide or corresponding nucleic acid molecule. As used herein, the term "cytoplasmic domain"
 15 represents the C-terminal amino acids which extend as a tail into the cytoplasm of a cell. Amino acid residues 244-273 of the native human PD-L2 polypeptide, and amino acid residues 225-273 of the predicted mature polypeptide, are predicted to comprise cytoplasmic domains (see Figure 3). Amino acid residues 243-247 of the native mouse PD-L2 polypeptide, and amino acid residues 224-228 of the predicted mature
 20 polypeptide, are also predicted to comprise cytoplasmic domains.

In a preferred embodiment, the PD-L2 molecules of the invention include at least one or more of the following domains: a signal peptide domain, an IgV domain, an IgC domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain.

Isolated polypeptides of the present invention, preferably PD-L2 polypeptides,
 25 have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2, or are encoded by a nucleotide sequence sufficiently identical to SEQ ID NO:1 or 3. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid
 30 residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30%, 40%, or 50% homology, preferably 60% homology, more preferably 70%-80%, and even more

preferably 90-95% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently identical. Furthermore, amino acid or nucleotide sequences which share at least 30%, 40%, or 50%, preferably 60%, more preferably 70-80%, or 90-95%
 5 homology and share a common functional activity are defined herein as sufficiently identical.

As used interchangeably herein, "PD-L2 activity", "biological activity of PD-L2" or "functional activity of PD-L2", refers to an activity exerted by a PD-L2 protein, polypeptide or nucleic acid molecule on a PD-L2-responsive cell or tissue, or on a PD-L2 polypeptide binding partner, as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, a PD-L2 activity is a direct activity, such as an association with a PD-L2 binding partner. As used herein, a "target molecule" or "binding partner" is a molecule with which a PD-L2 polypeptide binds or interacts in nature, such that PD-L2-mediated function is achieved. In an exemplary embodiment, a
 15 PD-L2 target molecule is the receptor PD-1. Alternatively, a PD-L2 activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the PD-L2 polypeptide with its natural binding partner, *e.g.*, PD-1. The biological activities of PD-L2 are described herein. For example, the PD-L2 polypeptides of the present invention can have one or more of the following activities: 1) bind to and/or modulate
 20 the activity of the receptor PD-1 or other PD-L2 natural binding partners, 2) modulate intra- or intercellular signaling, 3) modulate activation of immune cells, *e.g.*, T lymphocytes, and 4) modulate the immune response of an organism, *e.g.*, a mouse or human organism.

Accordingly, another embodiment of the invention features isolated PD-L2 proteins and polypeptides having a PD-L2 activity. Other preferred polypeptides are PD-L2 polypeptides having one or more of the following domains: a signal peptide domain, an IgV domain, an IgC domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain, and, preferably, a PD-L2 activity.

Additional preferred PD-L2 polypeptides have at least one extracellular domain,
 30 and one or more of a signal peptide domain, an IgV domain, an IgC domain, an transmembrane domain, and a cytoplasmic domain, and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising a complement of the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6.

The nucleotide sequence of the isolated human PD-L2 cDNA and the predicted amino acid sequence of the human PD-L2 polypeptide are shown in Figure 1 and in SEQ ID NO:1 and 2, respectively. The nucleotide sequence of the isolated mouse PD-L2 cDNA and the amino acid sequence of the mouse PD-L2 polypeptide are shown in Figure 2 and in SEQ ID NO:4 and 5, respectively.

The human PD-L2 gene, which is approximately 1223 nucleotides in length, encodes a polypeptide having a molecular weight of approximately 30.0 kD and which is approximately 273 amino acid residues in length. The mouse PD-L2 gene, which is approximately 1655 nucleotides in length, encodes a polypeptide having a molecular weight of approximately 27.2 kD and which is approximately 247 amino acid residues in length.

Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode PD-L2 polypeptides or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify PD-L2-encoding nucleic acid molecules (*e.g.*, PD-L2 mRNA) and fragments for use as PCR primers for the amplification or mutation of PD-L2 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid molecule is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid molecule) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated PD-L2 nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb,

2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid molecule is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium, when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1,3,4, or 6, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of SEQ ID NO:1, 3, 4, or 6 as a hybridization probe, PD-L2 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J. *et al. Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989*).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, 3, 4, or 6 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, 3, 4, or 6.

A nucleic acid molecule of the invention can be amplified using cDNA, mRNA or, alternatively, genomic DNA as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid molecule so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to PD-L2 nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6. This cDNA may comprise sequences encoding the human PD-L2 polypeptide (*i.e.*, "the coding region", from nucleotides 274-1092), as well as 5' untranslated sequences (nucleotides 1-273) and 3' untranslated sequences (nucleotides 1093-1223) of SEQ ID NO:1. Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1 (*i.e.*, nucleotides 274-1092, corresponding to SEQ ID NO:3). Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention comprises SEQ ID NO:3 and nucleotides 1-274 of SEQ ID NO:1. In yet another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:3 and nucleotides 1093-1223 of SEQ ID

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NO:1. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:1 or SEQ ID NO:3. In still another embodiment, the nucleic acid molecule can comprise the coding region of SEQ ID NO:1 (*e.g.*, nucleotides 274-1092, corresponding to SEQ ID NO:3), as well as a stop codon (*e.g.*,
 5 nucleotides 1093-1095 of SEQ ID NO:1).

This cDNA may also comprise sequences encoding the mouse PD-L2 polypeptide (*i.e.*, the coding region, from nucleotides 210-950), as well as 5' untranslated sequences (nucleotides 1-209) and 3' untranslated sequences (nucleotides 951-1655) of SEQ ID NO:4. Alternatively, the nucleic acid molecule can comprise only
 10 the coding region of SEQ ID NO:4 (*i.e.*, nucleotides 210-950, corresponding to SEQ ID NO:6). Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention comprises SEQ ID NO:6 and nucleotides 1-210 of SEQ ID NO:4. In yet another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:6 and nucleotides 951-1655 of SEQ ID NO:4. In yet another embodiment, the nucleic acid
 15 molecule consists of the nucleotide sequence set forth as SEQ ID NO:4 or SEQ ID NO:6. In still another embodiment, the nucleic acid molecule can comprise the coding region of SEQ ID NO:4 (*e.g.*, nucleotides 210-950, corresponding to SEQ ID NO:4), as well as a stop codon (*e.g.*, nucleotides 951-953 of SEQ ID NO:4).

In another preferred embodiment, an isolated nucleic acid molecule of the
 20 invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6 such that it can hybridize to the
 25 nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, respectively, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical
 30 to the entire length of the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, or a portion of any of these nucleotide sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, 3, 4, or 6, for example, a fragment which can be used as a probe or primer or a fragment which encodes a portion

of a PD-L2 polypeptide, *e.g.*, a biologically active portion of a PD-L2 polypeptide. The nucleotide sequences determined from the cloning of the human PD-L2 gene allow for the generation of probes and primers designed for use in identifying and/or cloning other PD-L2 family members, as well as PD-L2 homologues from other species. The

- 5 probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, 3, 4, or 6; of an anti-sense sequence of SEQ ID NO:1, 3, 4,
10 or 6; or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, 3, 4, or 6.

- In one embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is greater than about 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-
15 1150 or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, 3, 4, or 6, or the complement thereof. In a further embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is greater than about 880-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1150 or more nucleotides in length and hybridizes
20 under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1 or 3, or the complement thereof. In yet another embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is greater than 50-100, 100-150, 150-200, 200-250, 250-300 or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising nucleotides 1-
25 358 of SEQ ID NO:1, or a complement thereof. In yet a further embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is greater than about 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 850-900, 900-950, 950-1000-1050-1100, 1100-1150 or more nucleotides in length, includes at least about
30 15 (*i.e.*, 15 contiguous) nucleotides of the sequence comprising nucleotides 1-358 of SEQ ID NO:1, or a complement thereof, and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, or a complement thereof.

Probes based on the PD-L2 nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous polypeptides. In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme

5 co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a PD-L2 polypeptide, such as by measuring a level of a PD-L2-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting PD-L2 mRNA levels or determining whether a genomic PD-L2 gene has been mutated or deleted.

10 A nucleic acid fragment encoding a "biologically active portion of a PD-L2 polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6 which encodes a polypeptide having a PD-L2 biological activity (*e.g.*, the ability to bind to its natural binding partner(s) (*e.g.*, PD-1), and/or modulate immune cell activity), expressing the encoded portion of the PD-L2 polypeptide (*e.g.*, by

15 recombinant expression *in vitro*) and assessing the activity of the encoded portion of the PD-L2 polypeptide.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6 due to degeneracy of the genetic code and thus encode the same PD-L2 polypeptides as those encoded by the nucleotide

20 sequence shown in SEQ ID NO:1, 3, 4, or 6. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a polypeptide having an amino acid sequence shown in SEQ ID NO:2 or 5.

In addition to the PD-L2 nucleotide sequences shown in SEQ ID NO:1, 3, 4, or 6, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the PD-L2 polypeptides may exist

25 within a population (*e.g.*, the human population). Such genetic polymorphism in the PD-L2 genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a PD-L2 polypeptide,

30 preferably a mammalian PD-L2 polypeptide, and can further include non-coding regulatory sequences, and introns.

Allelic variants of human or mouse PD-L2 include both functional and non-functional PD-L2 polypeptides. Functional allelic variants are naturally occurring amino acid sequence variants of the human or mouse PD-L2 polypeptide that maintain

the ability to bind natural PD-L2 binding partner(s), *e.g.*, PD-1, and/or modulate lymphocyte activation. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2 or 5, or substitution, deletion or insertion of non-critical residues in non-critical regions of the polypeptide.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human or mouse PD-L2 polypeptide that do not have the ability to either bind natural PD-L2 binding partners, *e.g.*, PD-1, and/or modulate any of the PD-L2 activities described herein. Non-functional allelic variants will typically contain a non-conservative substitution, deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2 or 5, or a substitution, insertion or deletion in critical residues or critical regions of the polypeptide, *e.g.*, in an IgV domain.

The present invention further provides non-human, non-mouse orthologues of the human or mouse PD-L2 polypeptide. Orthologues of the human or mouse PD-L2 polypeptide are polypeptides that are isolated from non-human, non-mouse organisms and possess the same PD-1-binding activity and/or lymphocyte activation-modulating activity of the PD-L2 polypeptide. Orthologues of the human or mouse PD-L2 polypeptide can readily be identified as comprising an amino acid sequence that is substantially identical to SEQ ID NO:2 or 5.

Moreover, nucleic acid molecules encoding other PD-L2 family members and, thus, which have a nucleotide sequence which differs from the PD-L2 sequences of SEQ ID NO:1, 3, 4, or 6 are intended to be within the scope of the invention. For example, another PD-L2 cDNA can be identified based on the nucleotide sequence of mouse or human PD-L2. Moreover, nucleic acid molecules encoding PD-L2 polypeptides from different species, and which, thus, have a nucleotide sequence which differs from the PD-L2 sequences of SEQ ID NO:1, 3, 4, or 6 are intended to be within the scope of the invention. For example, a monkey PD-L2 cDNA can be identified based on the nucleotide sequence of the mouse or human PD-L2.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the PD-L2 cDNAs of the invention can be isolated based on their homology to the PD-L2 nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Nucleic acid molecules corresponding to natural

allelic variants and homologues of the PD-L2 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the PD-L2 gene.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under
 5 stringent conditions to the nucleic acid molecule comprising nucleotides 1-358 of the nucleotide sequence of SEQ ID NO:1, or nucleotides 1-85 of SEQ ID NO:3. In other embodiment, the nucleic acid is at least 880-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1150 or more nucleotides in length.

As used herein, the term "hybridizes under stringent conditions" is intended to
 10 describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to
 15 those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions
 20 includes hybridization in 4X or 6X sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70°C. A further preferred, non-limiting example of stringent hybridization conditions includes hybridization at 6X SSC at 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. A preferred, non-
 25 limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X or 6X SSC, at about 50-60°C (or alternatively hybridization in 6X
 30 SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is 0.15M NaCl, 10mM NaH₂PO₄, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the

hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, $T_m(^{\circ}\text{C}) = 2(\# \text{ of A + T bases}) + 4(\# \text{ of G + C bases})$. For hybrids between 18 and 49 base pairs in length, $T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log_{10}[\text{Na}^+]) + 0.41(\% \text{G+C}) - (600/N)$, where N is the number of bases in the hybrid, and $[\text{Na}^+]$ is the concentration of sodium ions in the hybridization buffer ($[\text{Na}^+]$ for 1xSSC = 0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (e.g., BSA or salmon or herring sperm carrier DNA), detergents (e.g., SDS), chelating agents (e.g., EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M NaH_2PO_4 , 7% SDS at about 65°C, followed by one or more washes at 0.02M NaH_2PO_4 , 1% SDS at 65°C, see e.g., Church and Gilbert (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995 (or alternatively 0.2X SSC, 1% SDS).

Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, 3, 4, or 6 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (i.e., encodes a natural polypeptide).

In addition to naturally-occurring allelic variants of the PD-L2 sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, 3, 4, or 6, thereby leading to changes in the amino acid sequence of the encoded PD-L2 polypeptides, without altering the functional ability of the PD-L2 polypeptides. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, 3, 4, or 6. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of PD-L2 (e.g., the sequence of SEQ ID NO:2 or 5) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the PD-L2 polypeptides of the present invention,

e.g., those present in an extracellular domain, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the PD-L2 polypeptides of the present invention and other members of the PD-L2 family are not likely to be amenable to alteration.

5 Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding PD-L2 polypeptides that contain changes in amino acid residues that are not essential for activity. Such PD-L2 polypeptides differ in amino acid sequence from SEQ ID NO:2 or 5, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a polypeptide, wherein the
10 polypeptide comprises an amino acid sequence at least about 71%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2 or 5.

An isolated nucleic acid molecule encoding a PD-L2 polypeptide identical to the polypeptide of SEQ ID NO:2 or 5 can be created by introducing one or more nucleotide
15 substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded polypeptide. Mutations can be introduced into SEQ ID NO:1, 3, 4, or 6 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more
20 predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar
25 side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a PD-L2 polypeptide is preferably replaced
30 with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a PD-L2 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for PD-L2 biological activity to identify mutants that retain activity. Following

mutagenesis of SEQ ID NO:1, 3, 4, or 6, the encoded polypeptide can be expressed recombinantly and the activity of the polypeptide can be determined.

In a preferred embodiment, a mutant PD-L2 polypeptide can be assayed for the ability to bind to and/or modulate the activity of a natural PD-L2 binding partner, *e.g.*, PD-1, modulate intra- or intercellular signaling, modulate activation of T lymphocytes, and/or modulate the immune response of an organism.

Yet another aspect of the invention pertains to isolated nucleic acid molecules encoding a PD-L2 fusion proteins. Such nucleic acid molecules, comprising at least a first nucleotide sequence encoding a PD-L2 protein, polypeptide or peptide operatively linked to a second nucleotide sequence encoding a non- a PD-L2 protein, polypeptide or peptide, can be prepared by standard recombinant DNA techniques.

In addition to the nucleic acid molecules encoding PD-L2 polypeptides described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a polypeptide, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire PD-L2 coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding a PD-L2. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the coding region of human PD-L2 corresponds to SEQ ID NO:3). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding PD-L2. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding human or mouse PD-L2 disclosed herein (*e.g.*, SEQ ID NO:3 or 6, respectively), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of PD-L2 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of PD-L2 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site

of PD-L2 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid molecule of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid molecule

5 (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides

10 which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-

15 methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil,

20 uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target

25 nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a PD-L2 polypeptide to thereby inhibit expression of the polypeptide, e.g., by inhibiting transcription and/or translation. The hybridization can

30 be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified

to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gautier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-*o*-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haseloff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave PD-L2 mRNA transcripts to thereby inhibit translation of PD-L2 mRNA. A ribozyme having specificity for a PD-L2-encoding nucleic acid can be designed based upon the nucleotide sequence of a PD-L2 cDNA disclosed herein (*i.e.*, SEQ ID NO:1, 3, 4, or 6). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a PD-L2-encoding mRNA. See, *e.g.*, Cech *et al.*, US Patent No. 4,987,071 and Cech *et al.*, U.S. Patent No. 5,116,742. Alternatively, PD-L2 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, PD-L2 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the PD-L2 (*e.g.*, the PD-L2 promoter and/or enhancers; *e.g.*, nucleotides 1-273 of SEQ ID NO:1 or nucleotides 1-209 of SEQ ID NO:4) to form triple helical structures that prevent transcription of the

PD-L2 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioessays* 14(12):807-15.

In yet another embodiment, the PD-L2 nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup, B. and Nielsen, P. E. (1996) *Bioorg. Med. Chem.* 4(1):5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup and Nielsen (1996) *supra* and Perry-O'Keefe *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93:14670-675.

PNAs of PD-L2 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of PD-L2 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes (*e.g.*, S1 nucleases (Hyrup and Nielsen (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup and Nielsen (1996) *supra*; Perry-O'Keefe *et al.* (1996) *supra*).

In another embodiment, PNAs of PD-L2 can be modified (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of PD-L2 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes (*e.g.*, RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds

between the nucleobases, and orientation (Hyrup and Nielsen (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup and Nielsen (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17):3357-63. For example, a DNA chain can be synthesized on a solid support using standard

5 phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a bridge between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acids Res.* 17:5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.*

10 (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5:1119-1124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating

15 transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. WO 88/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO 89/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Biotechniques*

20 6:958-976) or intercalating agents (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

Alternatively, the expression characteristics of an endogenous PD-L2 gene

25 within a cell line or microorganism may be modified by inserting a heterologous DNA regulatory element into the genome of a stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous PD-L2 gene. For example, an endogenous PD-L2 gene which is normally "transcriptionally silent", *i.e.*, a PD-L2 gene which is normally not expressed, or is expressed only at very

30 low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, a transcriptionally silent, endogenous PD-L2 gene may be activated by insertion of a promiscuous regulatory element that works across cell types.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with an endogenous PD-L2 gene, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described, *e.g.*, in Chappel, U.S. Patent No. 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991.

II. Isolated PD-L2 Polypeptides and Anti-PD-L2 Antibodies

One aspect of the invention pertains to isolated PD-L2 polypeptides, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-PD-L2 antibodies. In one embodiment, native PD-L2 polypeptides can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, PD-L2 polypeptides are produced by recombinant DNA techniques. Alternative to recombinant expression, a PD-L2 protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the PD-L2 polypeptide is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of PD-L2 polypeptide in which the polypeptide is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of PD-L2 polypeptide having less than about 30% (by dry weight) of non-PD-L2 protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-PD-L2 protein, still more preferably less than about 10% of non-PD-L2 protein, and most preferably less than about 5% non-PD-L2 protein. When the PD-L2 polypeptide or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of PD-L2 polypeptide in which the polypeptide is separated from chemical precursors or other chemicals which are involved in the synthesis of the

polypeptide. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of PD-L2 polypeptide having less than about 30% (by dry weight) of chemical precursors or non-PD-L2 chemicals, more preferably less than about 20% chemical precursors or non-PD-L2 chemicals, still more preferably less than about 10% chemical precursors or non-PD-L2 chemicals, and most preferably less than about 5% chemical precursors or non-PD-L2 chemicals.

As used herein, a "biologically active portion" of a PD-L2 polypeptide includes a fragment of a PD-L2 polypeptide which participates in an interaction between a PD-L2 molecule and a non-PD-L2 molecule, *e.g.*, a natural ligand of PD-L2, *e.g.*, PD-1.

Biologically active portions of a PD-L2 polypeptide include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the PD-L2 polypeptide, *e.g.*, the amino acid sequence shown in SEQ ID NO:2 or 5, which include fewer amino acids than the full length PD-L2 polypeptides, and exhibit at least one activity of a PD-L2 polypeptide. Typically, biologically active portions comprise a domain or motif with at least one activity of the PD-L2 polypeptide, *e.g.*, modulating PD-1 activity. A biologically active portion of a PD-L2 polypeptide can be a polypeptide which is, for example, 25, 50, 75, 100, 125, 150, 175, 200, 225 or more amino acids in length. Biologically active portions of a PD-L2 polypeptide can be used as targets for developing agents which modulate a PD-L2-mediated activity, *e.g.*, immune cell activation.

In one embodiment, a biologically active portion of a PD-L2 polypeptide comprises at least a portion of an extracellular domain. It is to be understood that a preferred biologically active portion of a PD-L2 polypeptide of the present invention may contain at least a portion of an extracellular domain (*e.g.*, comprising an IgV and/or an IgC domain), and one or more of the following domains: a signal peptide domain, a transmembrane domain, and a cytoplasmic domain. Moreover, other biologically active portions, in which other regions of the polypeptide are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native PD-L2 polypeptide.

In a preferred embodiment, the PD-L2 polypeptide has an amino acid sequence shown in SEQ ID NO:2 or 5. In other embodiments, the PD-L2 polypeptide is substantially identical to SEQ ID NO:2 or 5, and retains the functional activity of the polypeptide of SEQ ID NO:2 or 5, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above.

Accordingly, in another embodiment, the PD-L2 polypeptide is a polypeptide which comprises an amino acid sequence at least about 71%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2 or 5.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (e.g., when aligning a second sequence to the human PD-L2 amino acid sequence of SEQ ID NO:2 having 273 amino acid residues, at least 82, preferably at least 109, more preferably at least 137, even more preferably at least 164, and even more preferably at least 191, 218, 246 or more amino acid residues are aligned; when aligning a second amino acid sequence to the mouse PD-L2 amino acid sequence of SEQ ID NO:5 having 247 amino acid residues, at least 74, preferably at least 99, more preferably at least 124, even more preferably at least 148, and even more preferably at least 173, 198, 222 or more amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available online through the Genetics Computer Group), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1,

- 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available online through the Genetics Computer Group), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of Meyers, E. and Miller, W. (*Comput. Appl. Biosci.* 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0 or 2.0U), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.
- 10 The nucleic acid and polypeptide sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the
- 15 NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to PD-L2 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 100, wordlength = 3 to obtain amino acid sequences homologous to PD-L2 polypeptide molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized
- 20 as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See the internet website for the National Center for Biotechnology Information.
- The invention also provides PD-L2 chimeric or fusion proteins. As used herein,
- 25 a PD-L2 "chimeric protein" or "fusion protein" comprises a PD-L2 polypeptide operatively linked to a non-PD-L2 polypeptide. A "PD-L2 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a PD-L2 molecule, whereas a "non-PD-L2 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a polypeptide which is not substantially homologous to the
- 30 PD-L2 polypeptide, *e.g.*, a polypeptide which is different from the PD-L2 polypeptide and which is derived from the same or a different organism. Within a PD-L2 fusion protein, the PD-L2 polypeptide can correspond to all or a portion of a PD-L2 polypeptide. In a preferred embodiment, a PD-L2 fusion protein comprises at least one biologically active portion of a PD-L2 polypeptide. In another preferred embodiment, a

PD-L2 fusion protein comprises at least two domains of a PD-L2 polypeptide. Within the fusion protein, the term "operatively linked" is intended to indicate that the PD-L2 polypeptide and the non-PD-L2 polypeptide are fused in-frame to each other. The non-PD-L2 polypeptide can be fused to the N-terminus or C-terminus of the PD-L2 polypeptide and corresponds to a moiety that alters the solubility, binding affinity, stability, or valency of the PD-L2 polypeptide.

For example, in one embodiment, the fusion protein is a GST-PD-L2 fusion protein in which the PD-L2 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant PD-L2.

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In another embodiment, the fusion protein is a PD-L2 polypeptide containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of PD-L2 can be increased through use of a heterologous signal sequence.

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In a preferred embodiment, the fusion protein is an Ig-PD-L2 fusion protein in which the PD-L2 sequences are fused to a portion of an Ig molecule. The Ig portion of the fusion protein can include an immunoglobulin constant region, *e.g.*, a human C γ 1 domain or a C γ 4 domain (*e.g.*, the hinge, CH2, and CH3 regions of human IgC γ 1 or human IgC γ 4 (see, *e.g.*, Capon *et al.*, U.S. Patent No. 5,116,964; 5,580,756; 5,844,095, and the like, incorporated herein by reference). A resulting fusion protein may have altered PD-L2 solubility, binding affinity, stability and/or valency (*i.e.*, the number of binding sites per molecule) and may increase the efficiency of protein purification.

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Particularly preferred PD-L2 Ig fusion proteins include an extracellular domain portion of PD-L2 coupled to an immunoglobulin constant region (*e.g.*, the Fc region).

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The immunoglobulin constant region may contain genetic modifications which reduce or eliminate effector activity inherent in the immunoglobulin structure. For example, DNA encoding an extracellular portion of a PD-L2 polypeptide can be joined to DNA encoding the hinge, CH2, and CH3 regions of human IgG γ 1 and/or IgG γ 4 modified by site-directed mutagenesis, *e.g.*, as taught in WO 97/28267.

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The PD-L2 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The PD-L2 fusion proteins can be used to affect the bioavailability of a PD-L2 binding partner, *e.g.*, PD-1. Use of PD-L2 fusion proteins may be useful therapeutically for the treatment of conditions or disorders that would benefit from modulation of the immune response.

Moreover, the PD-L2-fusion proteins of the invention can be used as immunogens to produce anti-PD-L2 antibodies in a subject, to purify PD-L2-binding proteins, and in screening assays to identify molecules which inhibit the interaction of PD-L2 with its natural binding partner, *e.g.*, PD-1.

5 Preferably, a PD-L2 chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, 10 filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene 15 fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A PD-L2-encoding nucleic acid can be cloned into such an expression vector such that 20 the fusion moiety is linked in-frame to the PD-L2 polypeptide.

The present invention also pertains to variants of the PD-L2 polypeptides which function as either PD-L2 agonists (mimetics) or as PD-L2 antagonists. Variants of the PD-L2 polypeptides can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of a PD-L2 polypeptide. An agonist of the PD-L2 polypeptides can retain 25 substantially the same, or a subset, of the biological activities of the naturally occurring form of a PD-L2 polypeptide. An antagonist of a PD-L2 polypeptide can inhibit one or more of the activities of the naturally occurring form of the PD-L2 polypeptide by, for example, competitively modulating a PD-L2-mediated activity of a PD-L2 polypeptide. Thus, specific biological effects can be elicited by treatment with a variant of limited 30 function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the polypeptide has fewer side effects in a subject relative to treatment with the naturally occurring form of the PD-L2 polypeptide.

In one embodiment, variants of a PD-L2 polypeptide which function as either PD-L2 agonists (mimetics) or as PD-L2 antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a PD-L2 polypeptide for PD-L2 polypeptide agonist or antagonist activity. In one embodiment, a variegated library of PD-L2 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of PD-L2 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential PD-L2 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of PD-L2 sequences therein. There are a variety of methods which can be used to produce libraries of potential PD-L2 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential PD-L2 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acids Res.* 11:477).

In addition, libraries of fragments of a PD-L2 polypeptide coding sequence can be used to generate a variegated population of PD-L2 fragments for screening and subsequent selection of variants of a PD-L2 polypeptide. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a PD-L2 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the PD-L2 polypeptide.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of PD-

L2 polypeptides. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify PD-L2 variants (Arkin and Youvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delagrave *et al.* (1993) *Protein Eng.* 6(3):327-331).

In one embodiment, cell-based assays can be exploited to analyze a variegated PD-L2 library. For example, a library of expression vectors can be transfected into a cell line. The transfected cells are then contacted with PD-1-expressing cells and the effect of expression of the mutant on interaction of wild-type PD-L2 with its natural ligand(s), *e.g.*, PD-1, can be detected. Plasmid DNA can then be recovered from the cells which score for inhibition of signaling via PD-1 (leading to T cell upregulation), or alternatively, potentiation of signaling by PD-1 (leading to T cell downregulation), and the individual clones further characterized.

In addition to PD-L2 polypeptides consisting only of naturally-occurring amino acids, PD-L2 peptidomimetics are also provided. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compounds are termed "peptide mimetics" or "peptidomimetics" (Fauchere, J. (1986) *Adv. Drug Res.* 15:29; Veber and Freidinger (1985) *TINS* p.392; and Evans *et al.* (1987) *J. Med. Chem* 30:1229, which are incorporated herein by reference) and are usually developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides can be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (*i.e.*, a polypeptide that has a biological or pharmacological activity), such as human or mouse PD-L2, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: -CH₂NH-, -CH₂S-, -CH₂-CH₂-, -CH=CH- (cis and trans), -COCH₂-, -CH(OH)CH₂-, and -CH₂SO-, by methods known in the art and further described in the following references: Spatola, A.F. in *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins* Weinstein, B., ed., Marcel

- Dekker, New York, p. 267 (1983); Spatola, A.F., Vega Data (March 1983), Vol. 1, Issue 3, "Peptide Backbone Modifications"; Morley, J.S. (1980) *Trends. Pharm. Sci.* pp.463-468; Hudson, D. *et al.* (1979) *Int. J. Pept. Prot. Res.* 14:177-185 (-CH₂NH-, CH₂CH₂-); Spatola, A.F. *et al.* (1986) *Life. Sci.* 38:1243-1249 (-CH₂-S); Hann, M.M. (1982) *J. Chem. Soc. Perkin. Trans. I* 307-314 (-CH-CH-, cis and trans); Almquist, R.G. *et al.* (1980) *J. Med. Chem.* 23:1392-1398 (-COCH₂-); Jennings-White, C. *et al.* (1982) *Tetrahedron Lett.* 23:2533 (-COCH₂-); Szelke, M. *et al.*, European Patent Application No. EP 45665 (1982) CA: 97:39405 (-CH(OH)CH₂-); Holladay, M.W. *et al.* (1983) *Tetrahedron. Lett.* 24:4401-4404 (-C(OH)CH₂-); and Hruby, V.J. (1982) *Life Sci.* 31:189-199 (-CH₂-S-); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is -CH₂NH-. Such peptide mimetics may have significant advantages over polypeptide embodiments, including, for example: more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (*e.g.*, a broad-spectrum of biological activities), reduced antigenicity, and others. Labeling of peptidomimetics usually involves covalent attachment of one or more labels, directly or through a spacer (*e.g.*, an amide group), to non-interfering position(s) on the peptidomimetic that are predicted by quantitative structure-activity data and/or molecular modeling. Such non-interfering positions generally are positions that do not form direct contacts with the macromolecules(s) to which the peptidomimetic binds to produce the therapeutic effect. Derivatization (*e.g.*, labeling) of peptidomimetics should not substantially interfere with the desired biological or pharmacological activity of the peptidomimetic.

- Systematic substitution of one or more amino acids of a PD-L2 amino acid sequence with a D-amino acid of the same type (*e.g.*, D-lysine in place of L-lysine) can be used to generate more stable peptides. In addition, constrained peptides comprising a PD-L2 amino acid sequence or a substantially identical sequence variation can be generated by methods known in the art (Rizo and Gierasch (1992) *Annu. Rev. Biochem.* 61:387, incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

The amino acid sequences of the PD-L2 polypeptides identified herein will enable those of skill in the art to produce polypeptides corresponding to PD-L2 peptide sequences and sequence variants thereof. Such polypeptides can be produced in prokaryotic or eukaryotic host cells by expression of polynucleotides encoding a PD-L2

peptide sequence, frequently as part of a larger polypeptide. Alternatively, such peptides can be synthesized by chemical methods. Methods for expression of heterologous polypeptides in recombinant hosts, chemical synthesis of polypeptides, and *in vitro* translation are well known in the art and are described further in Maniatis *et al.*,
 5 *Molecular Cloning: A Laboratory Manual* (1989), 2nd Ed., Cold Spring Harbor, N.Y.; Berger and Kimmel, *Methods in Enzymology*, Volume 152, Guide to Molecular Cloning Techniques (1987), Academic Press, Inc., San Diego, Calif.; Merrifield, J. (1969) *J. Am. Chem. Soc.* 91:501; Chaiken I.M. (1981) *CRC Crit. Rev. Biochem.* 11:255; Kaiser *et al.* (1989) *Science* 243:187; Merrifield, B. (1986) *Science* 232:342; Kent, S.B.H. (1988)
 10 *Annu. Rev. Biochem.* 57:957; and Offord, R.E. (1980) *Semisynthetic Proteins*, Wiley Publishing, which are incorporated herein by reference).

Peptides can be produced, typically by direct chemical synthesis, and used *e.g.*, as agonists or antagonists of a PD-L2/PD-1 interaction. Peptides can be produced as modified peptides, with nonpeptide moieties attached by covalent linkage to the N-
 15 terminus and/or C-terminus. In certain preferred embodiments, either the carboxy-terminus or the amino-terminus, or both, are chemically modified. The most common modifications of the terminal amino and carboxyl groups are acetylation and amidation, respectively. Amino-terminal modifications such as acylation (*e.g.*, acetylation) or alkylation (*e.g.*, methylation) and carboxy-terminal modifications such as amidation, as
 20 well as other terminal modifications, including cyclization, can be incorporated into various embodiments of the invention. Certain amino-terminal and/or carboxy-terminal modifications and/or peptide extensions to the core sequence can provide advantageous physical, chemical, biochemical, and pharmacological properties, such as: enhanced stability, increased potency and/or efficacy, resistance to serum proteases, desirable
 25 pharmacokinetic properties, and others. Peptides can be used therapeutically to treat disease, *e.g.*, by altering costimulation in a patient.

An isolated PD-L2 polypeptide, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind PD-L2 using standard techniques for polyclonal and monoclonal antibody preparation. A full-length PD-L2 polypeptide can
 30 be used or, alternatively, the invention provides antigenic peptide fragments of PD-L2 for use as immunogens. In one embodiment, an antigenic peptide of PD-L2 comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 or 5 and encompasses an epitope of PD-L2 such that an antibody raised against the peptide forms a specific immune complex with the PD-L2 polypeptide. Preferably, the

antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of PD-L2 that are located in the extracellular domain of the polypeptide, *e.g.*, hydrophilic regions, as well as regions with high antigenicity.

A PD-L2 immunogen typically is used to prepare antibodies by immunizing a suitable subject (*e.g.*, rabbit, goat, mouse, or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed PD-L2 polypeptide or a chemically synthesized PD-L2 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic PD-L2 preparation induces a polyclonal anti-PD-L2 antibody response.

Accordingly, another aspect of the invention pertains to anti-PD-L2 antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as a PD-L2. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind PD-L2 molecules. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of PD-L2. A monoclonal antibody composition thus typically displays a single binding affinity for a particular PD-L2 polypeptide with which it immunoreacts.

cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497 (see also Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol. Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally Kenneth, R.H. in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); Lerner, E.A. (1981) *Yale J. Biol. Med.* 54:387-402; Geffter, M.L. *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a PD-L2 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds PD-L2.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-PD-L2 monoclonal antibody (see, *e.g.*, Galfre, G. *et al.* (1977) *Nature* 266:55052; Geffter *et al.* (1977) *supra*; Lerner (1981) *supra*; and Kenneth (1980) *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a

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monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind PD-L2, *e.g.*, using a standard ELISA assay.

Further methods for producing antibodies that bind PD-L2 are described in Examples 4 and 5.

- 5 Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-PD-L2 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with PD-L2 to thereby isolate immunoglobulin library members that bind PD-L2. Kits for generating and screening phage display libraries are commercially available
- 10 (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.*, U.S. Patent No. 5,223,409; Kang *et al.*, PCT International Publication No. WO 92/18619;
- 15 Dower *et al.*, PCT International Publication No. WO 91/17271; Winter *et al.*, PCT International Publication WO 92/20791; Markland *et al.*, PCT International Publication No. WO 92/15679; Breitling *et al.*, PCT International Publication WO 93/01288; McCafferty *et al.*, PCT International Publication No. WO 92/01047; Garrard *et al.*, PCT International Publication No. WO 92/09690; Ladner *et al.*, PCT International
- 20 Publication No. WO 90/02809; Fuchs *et al.* (1991) *Biotechnology (NY)* 9:1369-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J.* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrard *et al.* (1991) *Biotechnology (NY)*
- 25 9:1373-1377; Hoogenboom *et al.* (1991) *Nucleic Acids Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* (1990) *Nature* 348:552-554.

- Additionally, recombinant anti-PD-L2 antibodies, such as chimeric and
- 30 humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.*, International Application No. PCT/US86/02269; Akira *et al.*, European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496;

- Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, PCT International Publication No. WO 86/01533; Cabilly *et al.*, U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Cancer Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S.L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *Biotechniques* 4:214; Winter, U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyen *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

- An anti-PD-L2 antibody (*e.g.*, monoclonal antibody) can be used to isolate PD-L2 by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-PD-L2 antibody can facilitate the purification of natural PD-L2 from cells and of recombinantly produced PD-L2 expressed in host cells. Moreover, an anti-PD-L2 antibody can be used to detect PD-L2 polypeptide (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the PD-L2 polypeptide. Anti-PD-L2 antibodies can be used diagnostically to monitor polypeptide levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid molecule encoding a PD-L2 polypeptide (or a portion

thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel (1990) *Methods Enzymol.* 185:3-7. Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as

the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, PD-L2 polypeptides, mutant forms of PD-L2 polypeptides, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of PD-L2 polypeptides in prokaryotic or eukaryotic cells. For example, PD-L2 polypeptides can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors), yeast cells, or mammalian cells. Suitable host cells are discussed further in Goeddel (1990) *supra*. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of polypeptides in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino terminus of the recombinant polypeptide. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant polypeptide; 2) to increase the solubility of the recombinant polypeptide; and 3) to aid in the purification of the recombinant polypeptide by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant polypeptide to enable separation of the recombinant polypeptide from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant polypeptide.

Purified fusion proteins can be utilized in PD-L2 activity assays (*e.g.*, direct assays or competitive assays described in detail below), or to generate antibodies specific for PD-L2 polypeptides, for example. In a preferred embodiment, a PD-L2 fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated

recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.* (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.* (1990) *Methods Enzymol.* 185:60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant polypeptide expression in *E. coli* is to express the polypeptide in a host bacteria with an impaired capacity to proteolytically cleave the recombinant polypeptide (Gottesman, S. (1990) *Methods Enzymol.* 185:119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.* (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the PD-L2 expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari *et al.* (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.* (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (Invitrogen Corp, San Diego, CA).

Alternatively, PD-L2 polypeptides can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of polypeptides in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements.

For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J. *et al.*, *Molecular Cloning: A Laboratory Manual. 2nd ed.*, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example by the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to PD-L2 mRNA. Regulatory sequences operatively linked to a nucleic acid molecule cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific, or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the

regulation of gene expression using antisense genes, see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a PD-L2 nucleic acid molecule of the invention is introduced, *e.g.*, a PD-L2 nucleic acid molecule within a recombinant expression vector or a PD-L2 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a PD-L2 polypeptide can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a PD-L2 polypeptide or

can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a PD-L2 polypeptide. Accordingly, the invention further provides methods for producing a PD-L2 polypeptide using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention (into which a recombinant expression vector encoding a PD-L2 polypeptide has been introduced) in a suitable medium such that a PD-L2 polypeptide is produced. In another embodiment, the method further comprises isolating a PD-L2 polypeptide from the medium or the host cell.

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which PD-L2-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous PD-L2 sequences have been introduced into their genome or homologous recombinant animals in which endogenous PD-L2 sequences have been altered. Such animals are useful for studying the function and/or activity of a PD-L2 and for identifying and/or evaluating modulators of PD-L2 activity. As used herein, a “transgenic animal” is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a “homologous recombinant animal” is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous PD-L2 gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing a PD-L2-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a

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pseudopregnant female foster animal. The PD-L2 cDNA sequence of SEQ ID NO:1 or 4 can be introduced as a transgene into the genome of a non-human animal.

Alternatively, a nonhuman homologue of a human PD-L2 gene, such as a monkey or rat PD-L2 gene, can be used as a transgene. Alternatively, a PD-L2 gene homologue, such as another PD-L2 family member, can be isolated based on hybridization to the PD-L2 cDNA sequences of SEQ ID NO:1, 3, 4, or 6 (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a PD-L2 transgene to direct expression of a PD-L2 polypeptide to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a PD-L2 transgene in its genome and/or expression of PD-L2 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a PD-L2 polypeptide can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a PD-L2 gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the PD-L2 gene. The PD-L2 gene can be a human gene (*e.g.*, the cDNA of SEQ ID NO:1 or 3), but more preferably, is a non-human homologue of a human PD-L2 gene (*e.g.*, a cDNA isolated by stringent hybridization with the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6). For example, a mouse PD-L2 gene (*e.g.*, the cDNA of SEQ ID NO:3 or 6) can be used to construct a homologous recombination nucleic acid molecule, *e.g.*, a vector, suitable for altering an endogenous PD-L2 gene in the mouse genome. In a preferred embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous recombination, the endogenous PD-L2 gene is functionally disrupted (*i.e.*, no longer encodes a functional polypeptide; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed

such that, upon homologous recombination, the endogenous PD-L2 gene is mutated or otherwise altered but still encodes functional polypeptide (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous PD-L2 polypeptide). In the homologous recombination nucleic acid molecule, the altered

5 portion of the PD-L2 gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the PD-L2 gene to allow for homologous recombination to occur between the exogenous PD-L2 gene carried by the homologous recombination nucleic acid molecule and an endogenous PD-L2 gene in a cell, *e.g.*, an embryonic stem cell. The additional flanking PD-L2 nucleic acid sequence is of sufficient length for successful

10 homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, *e.g.*, Thomas, K.R. and Capecchi, M.R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The homologous recombination nucleic acid molecule is introduced into a cell, *e.g.*, an

15 embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced PD-L2 gene has homologously recombined with the endogenous PD-L2 gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells can then be injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, E.J., ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous

25 recombination nucleic acid molecules, *e.g.*, vectors, or homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos. WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

30 In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the

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FLP recombinase system of *S. cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected polypeptide are required. Such animals can be provided through the construction of

5 "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected polypeptide and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature*

10 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte

15 is then cultured such that it develops to the morula or blastocyte stage and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

20 IV. Pharmaceutical Compositions

The PD-L2 molecules, *e.g.*, the PD-L2 nucleic acid molecules, fragments of PD-L2 polypeptides, and anti-PD-L2 antibodies (also referred to herein as "active compounds" or "modulating agents") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically

25 comprise the nucleic acid molecule, polypeptide, or antibody and a carrier, *e.g.*, a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and

30 agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

- A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.
- Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS).
- In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, modulating agents such as a PD-L2 nucleic acid molecule, a fragment of a PD-L2 polypeptide, an anti-PD-L2 antibody, or a combination of an anti-PD-L2 antibody and an anti-PD-L1 antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active

compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention
5 enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate,
10 polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically
15 acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in US Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject
20 to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art
25 of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and
30 therapeutic effects is the therapeutic index and can be expressed as the ratio LD₅₀/ED₅₀. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity of PD-L2. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs,

organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the scope of knowledge of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram). It is

furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include

taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs
 5 or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II)
 10 (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological
 15 response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such polypeptides may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet
 20 derived growth factor, tissue plasminogen activator; or biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well
 25 known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer
 30 Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985); and Thorpe *et al.* "The Preparation And Cytotoxic

Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in US Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used
 5 as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for
 example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by
 stereotactic injection (see, *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-
 3057). The pharmaceutical preparation of the gene therapy vector can include the gene
 therapy vector in an acceptable diluent, or can comprise a slow release matrix in which
 10 the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery
 vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the
 pharmaceutical preparation can include one or more cells which produce the gene
 delivery system.

The pharmaceutical compositions can be included in a container, pack, or
 15 dispenser together with instructions for administration.

V. Uses and Methods of the Invention

The PD-L2 molecules, *e.g.*, the PD-L2 nucleic acid molecules, polypeptides,
 polypeptide homologues, and antibodies described herein can be used in one or more of
 20 the following methods: a) screening assays; b) predictive medicine (*e.g.*, diagnostic
 assays, prognostic assays, and monitoring clinical trials); and c) methods of treatment
 (*e.g.*, therapeutic and prophylactic, *e.g.*, by up- or down-modulating the immune
 response). As described herein, a PD-L2 polypeptide of the invention has one or more
 of the following activities: 1) binds to and/or modulates the activity of its natural
 25 binding partner(s), *e.g.*, PD-1, 2) modulates intra- or intercellular signaling, 3)
 modulates activation of T lymphocytes, 4) modulates the immune response of an
 organism, *e.g.*, a mammalian organism, such as a mouse or human.

The isolated nucleic acid molecules of the invention can be used, for example, to
 express PD-L2 polypeptide (*e.g.*, via a recombinant expression vector in a host cell in
 30 gene therapy applications), to detect PD-L2 mRNA (*e.g.*, in a biological sample) or a
 genetic alteration in a PD-L2 gene, and to modulate PD-L2 activity, as described further
 below. The PD-L2 polypeptides can be used to treat conditions or disorders
 characterized by insufficient or excessive production of a PD-L2 polypeptide or
 production of PD-L2 inhibitors. In addition, the PD-L2 polypeptides can be used to

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screen for naturally occurring PD-L2 binding partner(s) (in addition to PD-1), to screen for drugs or compounds which modulate PD-L2 activity, as well as to treat conditions or disorders characterized by insufficient or excessive production of PD-L2 polypeptide or production of PD-L2 polypeptide forms which have decreased, aberrant or unwanted activity compared to PD-L2 wild-type polypeptide (*e.g.*, immune system disorders such as severe combined immunodeficiency, multiple sclerosis, systemic lupus erythematosus, type I diabetes mellitus, lymphoproliferative syndrome, inflammatory bowel disease, allergies, asthma, graft-versus-host disease, and transplant rejection; immune responses to infectious pathogens such as bacteria and viruses; and immune system cancers such as lymphomas and leukemias). Moreover, the anti-PD-L2 antibodies of the invention can be used to detect and isolate PD-L2 polypeptides, regulate the bioavailability of PD-L2 polypeptides, and modulate PD-L2 activity, *e.g.*, by modulating the interaction between PD-L2 and its natural binding partner(s) (*e.g.*, PD-1).

15

A. Screening Assays:

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to PD-L2 polypeptides, have a stimulatory or inhibitory effect on, for example, PD-L2 expression or PD-L2 activity, or have a stimulatory or inhibitory effect on the interaction between PD-L2 and its natural binding partner(s), *e.g.*, PD-1.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to the PD-L2 protein or polypeptide or biologically active portion thereof, *e.g.*, modulate the ability of the PD-L2 polypeptide to interact with its natural binding partner(s). In a preferred embodiment, the binding partner is PD-1. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a PD-L2 protein or polypeptide or biologically active portion thereof (*e.g.*, cofactor or coenzyme analogs, or inhibitory molecules).

In a preferred embodiment, the invention provides assays for screening candidate or test compounds which have a stimulatory or inhibitory effect on the interaction between PD-L2 and its natural binding partner(s). In an exemplary embodiment, the binding partner is PD-1. The test compounds of the present invention can be obtained

using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994) *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP 4,099), plasmids (Cull *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:1865-1869) or phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:6378-6382; Felici (1991) *J. Mol. Biol.* 222:301-310; Ladner *supra*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a PD-L2 polypeptide or biologically active portion thereof is contacted with a test compound, and the ability of the test compound to modulate PD-L2 activity is determined. Determining the ability of the test compound to modulate PD-L2 activity can be accomplished by monitoring, for example, the ability of PD-L2 to bind to its natural binding partner(s), *e.g.*, PD-1, and modulate immune cell activity. The immune cell can be, *e.g.*, a T cell, a B cell, or a myeloid cell. Determining the ability of the test compound to modulate PD-L2 binding to PD-1 can be accomplished, for example, by coupling PD-1 with a radioisotope or enzymatic label such that binding of the PD-1 to PD-L2 can be determined by detecting the labeled PD-1 in a complex. Alternatively, PD-L2 could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate PD-L2 binding to PD-1 in a complex. Determining the ability of the test compound to bind PD-L2 can be accomplished, for example, by coupling the compound with a radioisotope or enzymatic label such that binding of the

compound to PD-L2 can be determined by detecting the labeled PD-L2 compound in a complex. For example, compounds (*e.g.*, PD-1) can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, compounds can be
5 enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (*e.g.*, PD-1) to interact with PD-L2 without the labeling of any of the
10 interactants. For example, a microphysiometer can be used to detect the interaction of a compound with PD-L2 without the labeling of either the compound or the PD-L2 (McConnell, H.M. *et al.* (1992) *Science* 257:1906-1912). As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor
15 (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and PD-L2.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a PD-L2 binding partner with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the
20 PD-L2 binding partner. In a preferred embodiment, the binding partner is PD-1. Determining the ability of the test compound to modulate the activity of a PD-L2 binding partner can be accomplished, for example, by determining the ability of the PD-L2 polypeptide to bind to or interact with the PD-L2 binding partner.

Determining the ability of the PD-L2 polypeptide, or a biologically active
25 fragment thereof, to bind to or interact with a PD-L2 binding partner, *e.g.*, PD-1, can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the PD-L2 polypeptide to bind to or interact with a PD-L2 binding partner can be accomplished by determining the activity of the binding partner. For example, the activity of the binding partner can be
30 determined by detecting induction of a cellular second messenger (*e.g.*, tyrosine kinase or phosphatase activity), detecting catalytic/enzymatic activity of an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a target-regulated cellular response. For example,

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determining the ability of the PD-L2 polypeptide to bind to or interact with a natural PD-L2 binding partner, *e.g.*, PD-1, can be accomplished by measuring the ability of a compound to modulate immune cell costimulation or inhibition in a proliferation assay, or by interfering with the ability of a PD-L2 polypeptide to bind to antibodies that recognize a portion of the PD-L2 polypeptide. In one embodiment, compounds that modulate T cell activation can be identified by determining the ability of a compound to modulate T cell proliferation or cytokine production. In a preferred embodiment, compounds that modulate T cell activation can be identified by determining the ability of a compound to modulate T cell proliferation or cytokine production at more than one antigen concentration.

In yet another embodiment, an assay of the present invention is a cell-free assay in which a PD-L2 polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the PD-L2 polypeptide or biologically active portion thereof is determined. Preferred biologically active portions of the PD-L2 polypeptides to be used in assays of the present invention include fragments which participate in interactions with non-PD-L2 molecules, *e.g.*, at least a portion of an extracellular domain which binds to a PD-L2 binding partner. In a further preferred embodiment, the binding partner is PD-1. Binding of the test compound to the PD-L2 polypeptide can be determined either directly or indirectly as described above.

In a preferred embodiment, the assay includes contacting the PD-L2 polypeptide or biologically active portion thereof with a known compound which binds PD-L2 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a PD-L2 polypeptide, wherein determining the ability of the test compound to interact with a PD-L2 polypeptide comprises determining the ability of the test compound to preferentially bind to PD-L2 or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which a PD-L2 polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the PD-L2 polypeptide or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a PD-L2 polypeptide can be accomplished, for example, by determining the ability of the PD-L2 polypeptide to bind to a PD-L2 binding partner by one of the methods described above for determining direct binding. In a preferred embodiment, the binding partner is PD-1. Determining

the ability of the PD-L2 polypeptide to bind to a PD-L2 binding partner can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA) (Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345; Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705). As used herein, "BIA" is a technology for
5 studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a PD-L2 polypeptide can be accomplished by determining the
10 ability of the PD-L2 polypeptide to further modulate the activity of a downstream effector of a PD-L2 binding partner (*e.g.*, a downstream effector of PD-1). For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

15 The cell-free assays of the present invention are amenable to use of both soluble and/or membrane-bound forms of polypeptides (*e.g.*, PD-L2 polypeptides or biologically active portions thereof, or binding partners to which PD-L2 binds, *e.g.*, PD-1). In the case of cell-free assays in which a membrane-bound form a polypeptide is used (*e.g.*, a cell-surface PD-L2), it may be desirable to utilize a solubilizing agent such
20 that the membrane-bound form of the polypeptide is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate
25 (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl-N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either PD-L2 or its binding partner to facilitate separation of complexed from uncomplexed forms of one or both of the
30 polypeptides, as well as to accommodate automation of the assay. In a preferred embodiment, the binding partner is PD-1. Binding of a test compound to a PD-L2 polypeptide, or interaction of a PD-L2 polypeptide with its binding partner in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates,

test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the polypeptides to be bound to a matrix. For example, glutathione-S-transferase/PD-L2 fusion proteins or glutathione-S-transferase/binding partner fusion proteins can be adsorbed onto
5 glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed binding partner polypeptide or PD-L2 polypeptide, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtitre
10 plate wells are washed to remove any unbound components, the matrix is immobilized in the case of beads, and complex formation is determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of PD-L2 binding or activity determined using standard techniques.

15 Other techniques for immobilizing polypeptides on matrices can also be used in the screening assays of the invention. For example, either a PD-L2 polypeptide or a PD-L2 binding partner can be immobilized utilizing conjugation of biotin and streptavidin. In a preferred embodiment, the binding partner is PD-1. Biotinylated PD-L2 polypeptide or binding partners can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals,
20 Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which are reactive with PD-L2 polypeptide or binding partners but which do not interfere with binding of the PD-L2 polypeptide to its binding partner can be derivatized to the wells of the plate, and unbound binding partner
25 or PD-L2 polypeptide is trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the PD-L2 polypeptide or binding partner, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the PD-L2 polypeptide or
30 binding partner.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a PD-L2 polypeptide can be accomplished by determining the ability of the test compound to modulate the activity of a molecule that functions downstream of PD-L2, *e.g.*, by interacting with the cytoplasmic domain of a PD-L2

binding partner, *e.g.*, PD-1. For example, levels of second messengers, the activity of the interacting molecule on an appropriate target, or the binding of the interactor to an appropriate target can be determined as previously described.

In another embodiment, modulators of PD-L2 expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of PD-L2 mRNA or polypeptide in the cell is determined. The level of expression of PD-L2 mRNA or polypeptide in the presence of the candidate compound is compared to the level of expression of PD-L2 mRNA or polypeptide in the absence of the candidate compound. The candidate compound can then be identified as a modulator of PD-L2 expression based on this comparison. For example, when expression of PD-L2 mRNA or polypeptide is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of PD-L2 mRNA or polypeptide expression. Alternatively, when expression of PD-L2 mRNA or polypeptide is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of PD-L2 mRNA or polypeptide expression. The level of PD-L2 mRNA or polypeptide expression in the cells can be determined by methods described herein for detecting PD-L2 mRNA or polypeptide.

In yet another aspect of the invention, the PD-L2 polypeptides can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other polypeptides which bind to or interact with PD-L2 ("PD-L2-binding proteins", "PD-L2 binding partners", or "PD-L2-bp") and are involved in PD-L2 activity. An example of such a binding protein is PD-1. Such PD-L2-binding proteins are also likely to be involved in the propagation of signals by the PD-L2 polypeptides or PD-L2 targets as, for example, downstream elements of a PD-L2-mediated signaling pathway. Alternatively, such PD-L2-binding polypeptides may be PD-L2 inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a PD-L2 polypeptide is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a

library of DNA sequences, that encodes an unidentified polypeptide ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" polypeptides are able to interact, *in vivo*, forming a PD-L2-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the polypeptide which interacts with the PD-L2 polypeptide.

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell-free assay, and the ability of the agent to modulate the activity of a PD-L2 polypeptide can be confirmed *in vivo*, *e.g.*, in an animal such as an animal model for cellular transformation and/or tumorigenesis.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a PD-L2 modulating agent, an antisense PD-L2 nucleic acid molecule, a PD-L2-specific antibody, or a PD-L2 binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the PD-L2 nucleotide sequences, described herein, can be used to map the location of the PD-L2 genes on a chromosome. The mapping of the PD-L2 sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, PD-L2 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the PD-L2 nucleotide sequences. Computer analysis of the PD-L2 sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the PD-L2 sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes (D'Eustachio, P. *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the PD-L2 nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a PD-L2 sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:6223-27), pre-

screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results in a reasonable amount of time. For a review of this technique, see Verma *et al.*, *Human Chromosomes: A Manual of Basic Techniques* (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data (such data are found, for example, in McKusick, V., Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987). *Nature* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the PD-L2 gene can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are

visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

5 2. Tissue Typing

 The PD-L2 sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is
10 digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in US Patent 5,272,057).

15 Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the PD-L2 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and
20 subsequently sequence it.

 Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals
25 and from tissue. The PD-L2 nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some
30 degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 or 4 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding

amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 or 6 are used, a more appropriate number of primers for positive individual identification would be 500-2000.

If a panel of reagents from PD-L2 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

10 3. Use of PD-L2 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology.

Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.*, another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 or 4 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the PD-L2 nucleotide sequences or portions thereof, *e.g.*, fragments derived from the noncoding regions of SEQ ID NO:1 or 4 having a length of at least 20 bases, preferably at least 30 bases.

The PD-L2 nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, lymphocytes. This can be very useful in cases where a forensic pathologist is presented

with a tissue of unknown origin. Panels of such PD-L2 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, PD-L2 primers or probes can be used to screen tissue culture for contamination (*i.e.*, screen for the presence of a mixture of
5 different types of cells in a culture).

C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for
10 prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining PD-L2 polypeptide and/or nucleic acid expression as well as PD-L2 activity, in the context of a biological sample (*e.g.*, blood, serum, cells, or tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at
15 risk of developing a disorder, associated with aberrant or unwanted PD-L2 expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with PD-L2 polypeptide, nucleic acid expression or activity. For example, mutations in a PD-L2 gene can be assayed in a biological sample. Such assays can be used for
20 prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with PD-L2 polypeptide, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of PD-L2 in clinical trials.

25 These and other agents are described in further detail in the following sections.

1. Diagnostic Assays

An exemplary method for detecting the presence or absence of PD-L2 polypeptide or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting PD-L2 polypeptide or nucleic acid (e.g., mRNA or genomic DNA) that encodes PD-L2 polypeptide such that the presence of PD-L2 polypeptide or nucleic acid is detected in the biological sample. A preferred agent for detecting PD-L2 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to PD-L2 mRNA or genomic DNA. The nucleic acid probe can be, for example, the PD-L2 nucleic acid set forth in SEQ ID NO:1, 3, 4, or 6, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to PD-L2 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting PD-L2 polypeptide is an antibody capable of binding to PD-L2 polypeptide, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

The term "biological sample" is intended to include tissues, cells, and biological fluids isolated from a subject, as well as tissues, cells, and fluids present within a subject. That is, the detection method of the invention can be used to detect PD-L2 mRNA, polypeptide, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of PD-L2 mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of PD-L2 polypeptide include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of PD-L2 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of PD-L2 polypeptide include introducing into a subject a labeled anti-PD-

L2 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains polypeptide molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting PD-L2 polypeptide, mRNA, or genomic DNA, such that the presence of PD-L2 polypeptide, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of PD-L2 polypeptide, mRNA or genomic DNA in the control sample with the presence of PD-L2 polypeptide, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of PD-L2 in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting PD-L2 polypeptide or mRNA in a biological sample; means for determining the amount of PD-L2 in the sample; and means for comparing the amount of PD-L2 in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect PD-L2 polypeptide or nucleic acid.

2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant or unwanted PD-L2 expression or activity. As used herein, the term "aberrant" includes a PD-L2 expression or activity which deviates from the wild type PD-L2 expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant PD-L2 expression or activity is intended to include the cases in which a mutation in the PD-L2 gene causes the PD-L2 gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional PD-L2 polypeptide or a polypeptide which does not function in a wild-type fashion, e.g., a polypeptide which does not interact with a PD-L2 binding partner (e.g., PD-1), or one

which interacts with a non-PD-L2 binding partner. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as immune cell activation. For example, the term unwanted includes a PD-L2 expression or activity which is undesirable in a subject.

5 The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in PD-L2 polypeptide activity or nucleic acid expression, such as an autoimmune disorder, an immunodeficiency disorder, an immune system cancer, or a tendency to have spontaneous abortions. Alternatively, the
10 prognostic assays can be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation of PD-L2 polypeptide activity or nucleic acid expression, such as an autoimmune disorder, and immunodeficiency disorder, an immune system cancer, or a tendency to have spontaneous abortions. Thus, the present invention provides a method for identifying a disease or disorder associated with
15 aberrant or unwanted PD-L2 expression or activity in which a test sample is obtained from a subject and PD-L2 polypeptide or nucleic acid (e.g., mRNA or genomic DNA) is detected, wherein the presence of PD-L2 polypeptide or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted PD-L2 expression or activity. As used herein, a "test sample" refers to a
20 biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., cerebrospinal fluid or serum), cell sample, or tissue.

 Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, polypeptide, peptide, nucleic acid, small molecule, or other drug
25 candidate) to treat a disease or disorder associated with aberrant or unwanted PD-L2 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for an autoimmune disorder, immunodeficiency disorder, immune system cancer, or tendency to have spontaneous abortions. Thus, the present invention provides methods for determining whether a
30 subject can be effectively treated with an agent for a disorder associated with aberrant or unwanted PD-L2 expression or activity in which a test sample is obtained and PD-L2 polypeptide or nucleic acid expression or activity is detected (e.g., wherein the abundance of PD-L2 polypeptide or nucleic acid expression or activity is diagnostic for

a subject that can be administered the agent to treat a disorder associated with aberrant or unwanted PD-L2 expression or activity).

The methods of the invention can also be used to detect genetic alterations in a PD-L2 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in PD-L2 polypeptide activity or nucleic acid expression, such as an autoimmune disorder, an immunodeficiency disorder, an immune system cancer, or a tendency to have spontaneous abortions. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one alteration affecting the integrity of a gene encoding a PD-L2 polypeptide, or the mis-expression of the PD-L2 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a PD-L2 gene, 2) an addition of one or more nucleotides to a PD-L2 gene, 3) a substitution of one or more nucleotides of a PD-L2 gene, 4) a chromosomal rearrangement of a PD-L2 gene, 5) an alteration in the level of a messenger RNA transcript of a PD-L2 gene, 6) aberrant modification of a PD-L2 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a PD-L2 gene, 8) a non-wild type level of a PD-L2 polypeptide, 9) allelic loss of a PD-L2 gene, and 10) inappropriate post-translational modification of a PD-L2 polypeptide. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in a PD-L2 gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., US Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in a PD-L2 gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a PD-L2 gene under conditions such that hybridization and amplification of the PD-L2 gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the

amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication
 5 (Guatelli, J.C. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection
 10 schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a PD-L2 gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more
 15 restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, US Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

20 In other embodiments, genetic mutations in PD-L2 can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotide probes (Cronin, M.T. *et al.* (1996) *Hum. Mutat.* 7:244-255; Kozal, M.J. *et al.* (1996) *Nat. Med.* 2:753-759). For example, genetic mutations in PD-L2 can be identified in two dimensional arrays
 25 containing light-generated DNA probes as described in Cronin *et al.* (1996) *supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential, overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array
 30 that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the PD-L2 gene and detect mutations by comparing the sequence of the sample PD-L2 with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert (1977) *Proc. Natl. Acad. Sci. USA* 74:560 or Sanger (1977) *Proc. Natl. Acad. Sci. USA* 74:5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (Naevé, C.W. (1995) *Biotechniques* 19:448-53), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the PD-L2 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes formed by hybridizing (labeled) RNA or DNA containing the wild-type PD-L2 sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digest the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:4397 and Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in PD-L2 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis*

15:1657-1662). According to an exemplary embodiment, a probe based on a PD-L2 sequence, *e.g.*, a wild-type PD-L2 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, US Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in PD-L2 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA* 86:2766; see also Cotton (1993) *Mutat. Res.* 285:125-144 and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control PD-L2 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change.

15 The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends*

20 *Genet.* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to ensure that it does

25 not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys. Chem.* 265:12753).

Examples of other techniques for detecting point mutations include, but are not

30 limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163; Saiki *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6230). Such allele specific

oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective

5 PCR amplification may be used in conjunction with the instant invention.

Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce
10 polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur
15 only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent
20 described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a PD-L2 gene.

Furthermore, any cell type or tissue in which PD-L2 is expressed may be utilized in the prognostic assays described herein.

25

3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of a PD-L2 polypeptide (*e.g.*, the modulation of cell proliferation and/or migration) can be applied not only in basic drug screening, but also in clinical trials. For example, the
30 effectiveness of an agent determined by a screening assay as described herein to increase PD-L2 gene expression, polypeptide levels, or upregulate PD-L2 activity, can be monitored in clinical trials of subjects exhibiting decreased PD-L2 gene expression, polypeptide levels, or downregulated PD-L2 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease PD-L2 gene expression,

polypeptide levels, or downregulate PD-L2 activity, can be monitored in clinical trials of subjects exhibiting increased PD-L2 gene expression, polypeptide levels, or PD-L2 activity. In such clinical trials, the expression or activity of a PD-L2 gene, and preferably, other genes that have been implicated in, for example, a PD-L2-associated disorder can be used as a "read out" or marker of the phenotype of a particular cell.

For example, and not by way of limitation, genes, including PD-L2, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates PD-L2 activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on PD-L2-associated disorders (*e.g.*, disorders characterized by dysregulated PD-1 activity), for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of PD-L2 and other genes implicated in the PD-L2-associated disorder, respectively. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of polypeptide produced, by one of the methods as described herein, or by measuring the levels of activity of PD-L2 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, polypeptide, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a PD-L2 polypeptide, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the PD-L2 polypeptide, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the PD-L2 polypeptide, mRNA, or genomic DNA in the pre-administration sample with the PD-L2 polypeptide, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of PD-L2 to higher levels than detected, *i.e.*, to increase the effectiveness of the agent.

Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of PD-L2 to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent. According to such an embodiment, PD-L2 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

D. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder characterized by insufficient or excessive production of PD-L2 protein or production of PD-L2 protein forms which have decreased or aberrant activity compared to PD-L2 wild type protein. Moreover, the anti-PD-L2 antibodies of the invention can be used to detect and isolate PD-L2 proteins, regulate the bioavailability of PD-L2 proteins, and modulate PD-L2 activity *e.g.*, by modulating the interaction of PD-L2 with PD-1.

1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted PD-L2 expression or activity, by administering to the subject a PD-L2 polypeptide or an agent which modulates PD-L2 expression or at least one PD-L2 activity. Subjects at risk for a disease or disorder which is caused or contributed to by aberrant or unwanted PD-L2 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the PD-L2 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of PD-L2 aberrancy, for example, a PD-L2 polypeptide, PD-L2 agonist or PD-L2 antagonist (*e.g.*, an anti-PD-L2 antibody or a combination of anti-PD-L2 and anti-PD-L1 antibodies) agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating PD-L2 expression or activity or interaction with its natural binding partners, *e.g.*, PD-1, for

therapeutic purposes. PD-L2 has been demonstrated to inhibit the costimulation and proliferation of activated immune cells and to transmit an inhibitory signal to immune cells via PD-1. Accordingly, the activity and/or expression of PD-L2, as well as the interaction between PD-L2 and PD-1 can be modulated in order to modulate the immune response. Because PD-L2 binds to inhibitory receptors, upregulation of PD-L2 activity results in downregulation of immune responses, whereas downregulation of PD-L2 activity results in upregulation of immune responses. In a preferred embodiment, PD-L2 binds to inhibitory receptors. In a particularly preferred embodiment, PD-L2 binds to PD-1.

Modulatory methods of the invention involve contacting a cell with a PD-L2 polypeptide or agent that modulates one or more of the activities of PD-L2 polypeptide activity associated with the cell, *e.g.*, an agent that modulates expression or activity of PD-L2 and/or modulates the interaction of PD-L2 and its natural binding partner(s). In a preferred embodiment, the binding partner is PD-1. An agent that modulates PD-L2 polypeptide activity can be an agent as described herein, such as a nucleic acid or a polypeptide, a naturally-occurring binding partner of a PD-L2 polypeptide (*e.g.*, PD-1), a PD-L2 antibody, a combination of PD-L2 and PD-L1 antibodies, a PD-L2 agonist or antagonist, a peptidomimetic of a PD-L2 agonist or antagonist, a PD-L2 peptidomimetic, or other small molecule. Soluble forms of PD-L2 may also be used to interfere with the binding of PD-1 to any of its natural binding partner(s) or ligands.

An agent that modulates the expression of PD-L2 is, *e.g.*, an antisense nucleic acid molecule, triplex oligonucleotide, ribozyme, or recombinant vector for expression of a PD-L2 polypeptide. For example, an oligonucleotide complementary to the area around a PD-L2 polypeptide translation initiation site can be synthesized. One or more antisense oligonucleotides can be added to cell media, typically at 200 µg/ml, or administered to a patient to prevent the synthesis of a PD-L2 polypeptide. The antisense oligonucleotide is taken up by cells and hybridizes to a PD-L2 mRNA to prevent translation. Alternatively, an oligonucleotide which binds double-stranded DNA to form a triplex construct to prevent DNA unwinding and transcription can be used. As a result of either, synthesis of PD-L2 polypeptide is blocked. When PD-L2 expression is modulated, preferably, such modulation occurs by a means other than by knocking out the PD-L2 gene.

Agents which modulate expression, by virtue of the fact that they control the amount of PD-L2 in a cell, also modulate the total amount of PD-L2 activity in a cell.

In one embodiment, the agent that modulates PD-L2 stimulates one or more PD-L2 activities. Examples of such stimulatory agents include active PD-L2 polypeptide and a nucleic acid molecule encoding PD-L2 that has been introduced into the cell. In another embodiment, the agent inhibits one or more PD-L2 activities. In a preferred embodiment, the agent inhibits or enhances the interaction of PD-L2 with its natural binding partner(s). In a particularly preferred embodiment, the binding partner is PD-L1. Examples of such inhibitory agents include antisense PD-L2 nucleic acid molecules, anti-PD-L2 antibodies, PD-L2 inhibitors, and compounds identified in the subject screening assays. In a further preferred embodiment, an inhibitory agent is a combination of an anti-PD-L2 antibody and an anti-PD-L1 antibody.

These modulatory methods can be performed *in vitro* (e.g., by contacting the cell with the agent) or, alternatively, by contacting an agent with cells *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a condition or disorder that would benefit from up- or down-modulation of a PD-L2 polypeptide, e.g., a disorder characterized by unwanted, insufficient, or aberrant expression or activity of a PD-L2 polypeptide or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) PD-L2 expression or activity. In another embodiment, the method involves administering a PD-L2 polypeptide or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted PD-L2 expression or activity.

Stimulation of PD-L2 activity is desirable in situations in which PD-L2 is abnormally downregulated and/or in which increased PD-L2 activity is likely to have a beneficial effect. Likewise, inhibition of PD-L2 activity is desirable in situations in which PD-L2 is abnormally upregulated and/or in which decreased PD-L2 activity is likely to have a beneficial effect.

Exemplary agents for use in downmodulating PD-L2 (i.e., PD-L2 antagonists) include, e.g., antisense nucleic acid molecules, antibodies that recognize and block PD-L2, combinations of antibodies that recognize and block PD-L2 and antibodies that recognize and block PD-L1, and compounds that block the interaction of PD-L2 with its naturally occurring binding partner(s) on an immune cell (e.g., soluble, monovalent PD-L2 molecules; soluble forms of PD-L2 molecules that do not bind Fc receptors on antigen presenting cells; soluble forms of PD-L2 binding partners; and compounds

identified in the subject screening assays). In a preferred embodiment, the binding partner is PD-1. Exemplary agents for use in upmodulating PD-L2 (*i.e.*, PD-L2 agonists) include, *e.g.*, nucleic acid molecules encoding PD-L2 polypeptides, multivalent forms of PD-L2, compounds that increase the expression of PD-L2, 5 compounds that enhance the interaction of PD-L2 with its naturally occurring binding partners (*e.g.*, PD-1) and cells that express PD-L2.

3. Downregulation of Immune Responses

There are numerous embodiments of the invention for upregulating the 10 inhibitory function of a PD-L2 polypeptide to thereby downregulate immune responses. Downregulation can be in the form of inhibiting or blocking an immune response already in progress, or may involve preventing the induction of an immune response. The functions of activated immune cells can be inhibited by downregulating immune cell responses or by inducing specific anergy in immune cells, or both.

For example, in embodiments where PD-L2 binds to an inhibitory receptor, *e.g.*, 15 PD-1, forms of PD-L2 that bind to the inhibitory receptor, *e.g.*, multivalent PD-L2 on a cell surface, can be used to downmodulate the immune response. Likewise, the PD-L2-PD-1 interaction can also be enhanced by the use of an additional agent, *e.g.*, an agent that blocks the interaction of PD-L1 with PD-1, that can further downmodulate the 20 immune response.

In one embodiment of the invention, an activating antibody used to stimulate PD-L2 activity is a bispecific antibody. For example, such an antibody can comprise a PD-L2 binding site and another binding site which targets a cell surface receptor on an immune cell, *e.g.*, a T cell, a B cell, or a myeloid cell. In one embodiment, such an 25 antibody, in addition to comprising a PD-L2 binding site, can further comprise a binding site which binds to a B cell antigen receptor, a T cell antigen receptor, or an Fc receptor, in order to target the molecule to a specific cell population. Selection of this second antigen for the bispecific antibody provides flexibility in selection of cell population to be targeted for inhibition.

30 Agents that promote a PD-L2 activity or which enhance the interaction of PD-L2 with its natural binding partners, *e.g.*, PD-1 (*e.g.*, PD-L2 activating antibodies or PD-L2 activating small molecules) can be identified by their ability to inhibit immune cell proliferation and/or effector function, or to induce anergy when added to an *in vitro* assay. For example, cells can be cultured in the presence of an agent that stimulates

signal transduction via an activating receptor. A number of art-recognized readouts of cell activation can be employed to measure, *e.g.*, cell proliferation or effector function (*e.g.*, antibody production, cytokine production, phagocytosis) in the presence of the activating agent. The ability of a test agent to block this activation can be readily

5 determined by measuring the ability of the agent to effect a decrease in proliferation or effector function being measured. In one embodiment, at low antigen concentrations, PD-L2-PD-1 interactions inhibit strong B7-CD28 signals. In another embodiment, at high antigen concentrations, PD-L2-PD-1 interactions reduce cytokine production but do not inhibit T cell proliferation. Accordingly, the ability of a test compound to block

10 activation can be determined by measuring cytokine production and/or proliferation at different concentrations of antigen.

In one embodiment of the invention, tolerance is induced against specific antigens by co-administering an antigen with a PD-L2 agonist. For example, tolerance can be induced to specific polypeptides. In one embodiment, immune responses to

15 allergens or foreign polypeptides to which an immune response is undesirable can be inhibited. For example, patients that receive Factor VIII frequently generate antibodies against this clotting factor. Co-administration of an agent that stimulates PD-L2 activity or interaction with its natural binding partner, *e.g.*, PD-1, with recombinant factor VIII (or physically linking PD-L2 to Factor VIII, *e.g.*, by cross-linking) can result in immune

20 response downmodulation.

In one embodiment, a PD-L2 agonist and another agent that can block activity of costimulatory receptors on an immune cell can be used to downmodulate immune responses. Exemplary molecules include: agonists forms of other PD-1 ligands (*e.g.*, PD-L1), soluble forms of CTLA-4, anti-B7-1 antibodies, anti-B7-2 antibodies, or

25 combinations thereof. Alternatively, two separate peptides (for example, a PD-L2 polypeptide with blocking forms of B7-2 and/or B7-1 polypeptides), or a combination of antibodies (*e.g.*, activating antibodies against a PD-L2 polypeptide with blocking anti-B7-2 and/or anti-B7-1 monoclonal antibodies) can be combined as a single composition or administered separately (simultaneously or sequentially) to downregulate immune

30 cell mediated immune responses in a subject. Furthermore, a therapeutically active amount of one or more peptides having a PD-L2 polypeptide activity, along with one or more polypeptides having B7-1 and/or B7-1 activity, can be used in conjunction with other downmodulating reagents to influence immune responses. Examples of other immunomodulating reagents include antibodies that block a costimulatory signal (*e.g.*,

against CD28 or ICOS), antibodies that activate an inhibitory signal via CTLA4, and/or antibodies against other immune cell markers (*e.g.*, against CD40, CD40 ligand, or cytokines), fusion proteins (*e.g.*, CTLA4-Fc or PD-1-Fc), and immunosuppressive drugs (*e.g.*, rapamycin, cyclosporine A, or FK506).

5 The PD-L2 polypeptides may also be useful in the construction of therapeutic agents which block immune cell function by destruction of cells. For example, portions of a PD-L2 polypeptide can be linked to a toxin to make a cytotoxic agent capable of triggering the destruction of cells to which it binds.

For making cytotoxic agents, polypeptides of the invention may be linked, or
10 operatively attached, to toxins using techniques that are known in the art, *e.g.*, via crosslinking or recombinant DNA techniques. The preparation of immunotoxins is, in general, well known in the art (see, *e.g.*, US Patent 4,340,535 and EP 44167, both incorporated herein by reference). Numerous types of disulfide bond-containing linkers are known which can successfully be employed to conjugate the toxin moiety with a
15 polypeptide. In one embodiment, linkers that contain a disulfide bond that is sterically "hindered" are to be preferred, due to their greater stability *in vivo*, thus preventing release of the toxin moiety prior to binding at the site of action.

A wide variety of toxins are known that may be conjugated to polypeptides or antibodies of the invention. Examples include: numerous useful plant-, fungus- or even
20 bacteria-derived toxins, which, by way of example, include: various A chain toxins, particularly ricin A chain; ribosome inactivating proteins such as saporin or gelonin; alpha-sarcin; aspergillin; restrictocin; and ribonucleases such as placental ribonuclease, angiogenic, diphtheria toxin, or pseudomonas exotoxin. A preferred toxin moiety for use in connection with the invention is toxin A chain which has been treated to modify
25 or remove carbohydrate residues, deglycosylated A chain. (US Patent 5,776,427).

Infusion of one or a combination of such cytotoxic agents (*e.g.*, PD-L2 ricin (alone or in combination with PD-L1-ricin), into a patient may result in the death of immune cells, particularly in light of the fact that activated immune cells that express
higher amounts of PD-L2 binding partners, *e.g.*, PD-1. For example, because PD-1 is
30 induced on the surface of activated lymphocytes, a PD-L2 polypeptide can be used to target the depletion of these specific cells by Fc-R dependent mechanisms or by ablation by conjugating a cytotoxic drug (*e.g.*, ricin, saporin, or calicheamicin) to the PD-L2 polypeptide. In one another embodiment, the toxin can be conjugated to an anti-PD-L2 antibody in order to target for death PD-L2-expressing antigen-presenting cell. In a

further embodiment, the PD-L2-antibody-toxin can be a bispecific antibody. Such bispecific antibodies are useful for targeting a specific cell population, *e.g.*, using a marker found only on a certain type of cell, *e.g.*, B lymphocytes, monocytes, dendritic cells, or Langerhans cells.

- 5 Downregulating immune responses by activating PD-L2 activity or the PD-L2-PD-1 interaction (and thus stimulating the negative signaling function of PD-1) is useful in downmodulating the immune response, *e.g.*, in situations of tissue, skin and organ transplantation, in graft-versus-host disease (GVHD), or allergies, or in autoimmune diseases such as systemic lupus erythematosus and multiple sclerosis. For example,
10 blockage of immune cell function results in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by immune cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which promotes the activity of PD-L2 or the interaction of PD-L2 with its natural binding partner(s), *e.g.*, PD-1, on
15 immune cells (such as a soluble, multimeric form of a PD-L2 polypeptide) alone or in conjunction with another downmodulatory agent prior to or at the time of transplantation can inhibit the generation of a costimulatory signal. Moreover, promotion of PD-L2 activity or PD-L2-PD-1 interaction (and thus, a PD-1 inhibitory signal) may also be sufficient to anergize the immune cells, thereby inducing tolerance in a subject.
20 Induction of long-term tolerance by promoting a PD-1-mediated inhibitory signal may avoid the necessity of repeated administration of these activating reagents.

- To achieve sufficient immunosuppression or tolerance in a subject, it may also be desirable to block the costimulatory function of other molecules. For example, it may be desirable to block the function of B7-1 and B7-2 by administering a soluble
25 form of a combination of peptides having an activity of each of these antigens or blocking antibodies against these antigens (separately or together in a single composition) prior to or at the time of transplantation. Alternatively, it may be desirable to promote inhibitory activity of PD-L2 and inhibit a costimulatory activity of B7-1 and/or B7-2. Other downmodulatory agents that can be used in connection with the
30 downmodulatory methods of the invention include, for example, agents that transmit an inhibitory signal via CTLA4, soluble forms of CTLA4, antibodies that activate an inhibitory signal via CTLA4, blocking antibodies against other immune cell markers, or soluble forms of other receptor ligand pairs (*e.g.*, agents that disrupt the interaction

between CD40 and CD40 ligand (*e.g.*, anti CD40 ligand antibodies)), antibodies against cytokines, or immunosuppressive drugs.

For example, activating PD-L2 activity or the interaction of PD-L2 with its natural binding partner(s), *e.g.*, PD-1 (and thus the inhibitory function of said binding partner(s)), may also be useful in treating autoimmune disease. Many autoimmune disorders are the result of inappropriate activation of immune cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive immune cells may reduce or eliminate disease symptoms. Administration of agents that promote activity of PD-L2 or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, may induce antigen-specific tolerance of autoreactive immune cells which could lead to long-term relief from the disease. Additionally, co-administration of agents which block costimulation of immune cells by disrupting receptor-ligand interactions of B7 molecules with costimulatory receptors may be useful in inhibiting immune cell activation to prevent production of autoantibodies or cytokines which may be involved in the disease process. The efficacy of reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythematosus in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., *Fundamental Immunology*, Raven Press, New York, 1989, pp. 840-856).

Inhibition of immune cell activation is useful therapeutically in the treatment of allergies and allergic reactions, *e.g.*, by inhibiting IgE production. An agent that promotes PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, can be administered to an allergic subject to inhibit immune cell-mediated allergic responses in the subject. Stimulation PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, can be accompanied by exposure to allergen in conjunction with appropriate MHC molecules. Allergic reactions can be systemic or local in nature, depending on the route of entry of the allergen and the pattern of deposition of IgE on mast cells or basophils. Thus, immune cell-mediated allergic responses can be inhibited locally or systemically by administration of an agent that promotes PD-L2 activity or PD-L2-PD-1 interaction.

Inhibition of immune cell activation through stimulation of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, may also be important therapeutically in pathogenic infections of immune cells (*e.g.*, by viruses or bacteria). For example, in the acquired immune deficiency syndrome (AIDS), viral replication is stimulated by immune cell activation. Stimulation of PD-L2 activity or PD-L2-PD-1 interaction may result in inhibition of viral replication and thereby ameliorate the course of AIDS.

Downregulation of an immune response via stimulation of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, may also be useful in promoting the maintenance of pregnancy. PD-L2 is normally highly expressed in placental trophoblasts, the layer of cells that forms the interface between mother and fetus and may play a role in preventing maternal rejection of the fetus. Females at risk for spontaneous abortion (*e.g.*, those identified by screening for PD-L2 activity, as described in the "Prognostic Assays" section, those who have previously had a spontaneous abortion or those who have had difficulty conceiving) because of immunologic rejection of the embryo or fetus can be treated with agents that stimulate the activity of PD-L2 or its interaction with its natural binding partner(s), *e.g.*, PD-1.

Downregulation of an immune response via stimulation of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, may also be useful in treating an autoimmune attack of autologous tissues. For example, PD-L2 is normally highly expressed in the heart and protects the heart from autoimmune attack. This is evidenced by the fact that the Balb/c PD-1 knockout mouse exhibits massive autoimmune attack on the heart with thrombosis. Thus, conditions that are caused or exacerbated by autoimmune attack (*e.g.*, in this example, heart disease, myocardial infarction or atherosclerosis) may be ameliorated or improved by increasing PD-L2 activity or PD-L2 binding to its natural binding partner, *e.g.*, PD-1. It is therefore within the scope of the invention to modulate conditions exacerbated by autoimmune attack, such as autoimmune disorders (as well as conditions such as heart disease, myocardial infarction, and atherosclerosis) by stimulating PD-L2 activity or PD-L2 interaction with PD-L1.

In an additional embodiment, in performing any of the methods described herein, it is within the scope of the invention to downregulate an immune response by stimulating the activities of both PD-L2 and PD-L1. PD-L1 is an additional PD-1 ligand which is described in the co-pending U.S. Application Serial No. 09/644,934;

International Publication WO 01/14557; Dong, H. *et al.* (1999) *Nat. Med.* 5:1365-1369; and Freeman, G.J. *et al.* (2000) *J. Exp. Med.* 192:1027-1034; the contents of each of which are incorporated herein by reference. In a further additional embodiment, in performing any of the methods described herein, it is within the scope of the invention to downregulate an immune response by administering one or more additional agents. For example, the use of other agents known to downregulate the immune response can be used in conjunction with an agent that stimulates PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1.

10 4. Upregulation of Immune Responses

Inhibition of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, as a means of upregulating immune responses is also useful in therapy. Upregulation of immune responses can be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through inhibition of PD-L2 activity or PD-L2-PD-1 interaction is useful in cases of infections with microbes, *e.g.*, bacteria, viruses, or parasites, or in cases of immunosuppression. For example, in one embodiment, an agent that inhibits PD-L2 activity or PD-L2-PD-1 interaction, *e.g.*, a non-activating antibody (*i.e.*, a blocking antibody) against PD-L2, a combination of non-activating antibodies against PD-L2 and PD-L1, or a soluble form of PD-L2, is therapeutically useful *in situations* where upregulation of antibody and cell-mediated responses, resulting in more rapid or thorough clearance of a virus, bacterium, or parasite, would be beneficial. These conditions include viral skin diseases such as Herpes or shingles, in which case such an agent can be delivered topically to the skin. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of such agents systemically. In certain instances, it may be desirable to further administer other agents that upregulate immune responses, for example, forms of B7 family members that transduce signals via costimulatory receptors, in order further augment the immune response.

Alternatively, immune responses can be enhanced in an infected patient by removing immune cells from the patient, contacting immune cells *in vitro* with an agent that inhibits the PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, and reintroducing the *in vitro*-stimulated immune cells into the patient. In another embodiment, a method of enhancing immune responses involves isolating

infected cells from a patient, *e.g.*, virally infected cells, transfecting them with a nucleic acid molecule encoding a form of PD-L2 that cannot bind its natural binding partner(s), *e.g.*, PD-1, such that the cells express all or a portion of the PD-L2 molecule on their surface, and reintroducing the transfected cells into the patient. The transfected cells
 5 may be capable of preventing an inhibitory signal to, and thereby activating, immune cells *in vivo*.

A agent that inhibits PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, can be used prophylactically in vaccines against various polypeptides, *e.g.*, polypeptides derived from pathogens. Immunity against a pathogen,
 10 *e.g.*, a virus, can be induced by vaccinating with a viral polypeptide along with an agent that inhibits PD-L2 activity or PD-L2-PD-1 interaction, in an appropriate adjuvant. Alternately, a vector comprising genes which encode for both a pathogenic antigen and a form of PD-L2 that blocks PD-L2-PD-1 interaction can be used for vaccination. Nucleic acid vaccines can be administered by a variety of means, for example, by
 15 injection (*e.g.*, intramuscular, intradermal, or the biolistic injection of DNA-coated gold particles into the epidermis with a gene gun that uses a particle accelerator or a compressed gas to inject the particles into the skin (Haynes *et al.* (1996) *J. Biotechnol.* 44:37)). Alternatively, nucleic acid vaccines can be administered by non-invasive means. For example, pure or lipid-formulated DNA can be delivered to the respiratory
 20 system or targeted elsewhere, *e.g.*, Peyer's patches by oral delivery of DNA (Schubbert (1997) *Proc. Natl. Acad. Sci. USA* 94:961). Attenuated microorganisms can be used for delivery to mucosal surfaces (Sizemore *et al.* (1995) *Science* 270:29).

In another embodiment, the antigen in the vaccine is a self-antigen. Such a vaccine is useful in the modulation of tolerance in an organism. Immunization with a
 25 self antigen and an agent that blocks PD-L2 activity or PD-L2 interaction with its natural binding partner (*e.g.*, PD-1) can break tolerance (*i.e.*, interfere with tolerance of a self antigen). Such a vaccine may also include adjuvants such as alum or cytokines (*e.g.*, GM-CSF, IL-12, B7-1, or B7-2).

In one embodiment, an agent which inhibits PD-L2 activity or PD-L2 interaction
 30 with its natural binding partner(s), *e.g.*, PD-1, can be administered with class I MHC polypeptides by, for example, a cell transfected to coexpress a PD-L2 polypeptide or blocking antibody and MHC class I α chain polypeptide and β_2 microglobulin to result in activation of T cells and provide immunity from infection. For example, viral

pathogens for which vaccines are useful include: hepatitis B, hepatitis C, Epstein-Barr virus, cytomegalovirus, HIV-1, HIV-2, tuberculosis, malaria and schistosomiasis.

In another application, inhibition of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, can be useful in the treatment of tumor immunity.

- 5 Tumor cells (*e.g.*, sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, or carcinoma) can be transfected with a nucleic acid molecule that inhibits PD-L2 activity or PD-L2-PD-1 interaction. These molecules can be, *e.g.*, nucleic acid molecules which are antisense to PD-L2, or can encode non-activating anti-PD-L2 antibodies or combinations of anti-PD-L2 and anti-PD-L1 antibodies. These molecules can also be
10 the variable region of an anti-PD-L2 antibody and/or an anti-PD-L1 antibody. If desired, the tumor cells can also be transfected with other polypeptides which activate costimulation (*e.g.*, B7-1 or B7-2). The transfected tumor cells are returned to the patient, which results in inhibition (*e.g.*, local inhibition) of PD-L2 activity or PD-L2-PD-1 interaction. Alternatively, gene therapy techniques can be used to target a tumor
15 cell for transfection *in vivo*.

- Stimulation of an immune response to tumor cells can also be achieved by inhibiting PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, by treating a patient with an agent that inhibits PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1. Preferred examples of such
20 agents include, *e.g.*, antisense nucleic acid molecules, antibodies that recognize and block PD-L2, a combination of antibodies that recognize and block PD-L2 and antibodies that recognize and block PD-L1, and compounds that block the interaction of PD-L2 with its naturally occurring binding partner(s) on an immune cell (*e.g.*, soluble, monovalent PD-L2 molecules; soluble forms of PD-L2 molecules that do not bind to Fc
25 receptors on antigen presenting cells; soluble forms of PD-L2 binding partner(s); and compounds identified in the subject screening assays). In a most preferred embodiment, the PD-L2 binding partner is PD-1.

- In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to express sufficient amounts of MHC class I or MHC class II molecules, can
30 be transfected with nucleic acid encoding all or a portion of (*e.g.*, a cytoplasmic-domain truncated portion) of an MHC class I α chain polypeptide and β_2 microglobulin polypeptide or an MHC class II α chain polypeptide and an MHC class II β chain polypeptide to thereby express MHC class I or MHC class II polypeptides on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with an

PD-L2 inhibiting polypeptide or antisense nucleic acid induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II-associated polypeptide, such as the invariant chain, can also be cotransfected with a DNA encoding a PD-L2 inhibiting polypeptide or antisense nucleic acid to promote presentation of tumor associated antigens and induce tumor specific immunity. Expression of B7-1 by B7-negative murine tumor cells has been shown to induce T cell mediated specific immunity accompanied by tumor rejection and prolonged protection to tumor challenge in mice (Chen, L. *et al.* (1992) *Cell* 71:1093-1102; Townsend, S.E. and Allison, J.P. (1993) *Science* 259:368-370; Baskar, S. *et al.* (1993) *Proc. Natl. Acad. Sci.* 90:5687-5690). Thus, the induction of an immune cell-mediated immune response in a human subject can be sufficient to overcome tumor-specific tolerance in the subject.

In another embodiment, the immune response can be stimulated by the inhibition of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, such that preexisting tolerance is overcome. For example, immune responses against antigens to which a subject cannot mount a significant immune response, *e.g.*, tumor-specific antigens, can be induced by administering an agent that inhibits the activity of PD-L2 activity or the ability of PD-L2 to bind to its natural binding partner, *e.g.*, PD-1. PD-1 antagonists can be used as adjuvants to boost responses to foreign antigens in the process of active immunization.

In one embodiment, immune cells are obtained from a subject and cultured *ex vivo* in the presence of an agent that inhibits PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, to expand the population of immune cells. In a further embodiment the immune cells are then administered to a subject. Immune cells can be stimulated to proliferate *in vitro* by, for example, providing the immune cells with a primary activation signal and a costimulatory signal, as is known in the art. Various forms of PD-L2 polypeptides or agents that inhibit PD-L2 activity or PD-L2-PD-1 and/or PD-L1/PD-1 interaction can also be used to costimulate proliferation of immune cells. In one embodiment, immune cells are cultured *ex vivo* according to the methods described in PCT Application No. WO 94/29436. The costimulatory molecule can be soluble, attached to a cell membrane or attached to a solid surface, such as a bead.

In an additional embodiment, in performing any of the methods described herein, it is within the scope of the invention to upregulate an immune response by administering one or more additional agents. For example, the use of other agents known to stimulate the immune response, such as cytokines, adjuvants, or stimulatory forms of costimulatory molecules or their ligands can be used in conjunction with an agent that inhibits PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1. For example, an agent that inhibits PD-L1 (*e.g.*, a blocking antibody against PD-L1) can be used in conjunction with an agent that inhibits PD-L2 (*e.g.*, a blocking antibody against PD-L2). PD-L1 is an additional PD-1 ligand which is described in the co-pending U.S. Application Serial No. 09/644,934; International Publication WO 01/14557; Dong, H. *et al.* (1999) *Nat. Med.* 5:1365-1369; and Freeman, G.J. *et al.* (2000) *J. Exp. Med.* 192:1027-1034; the contents of each of which are incorporated herein by reference.

15 E. Identification of Cytokines Modulated by Modulation of PD-L2 Activity or PD-L2-PD-1 Interaction

The PD-L2 molecules described herein can be used to identify cytokines which are produced by or whose production is enhanced or inhibited in immune cells in response to modulation of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1. Immune cells expressing PD-1 can be suboptimally stimulated *in vitro* with a primary activation signal, for example, T cells can be stimulated with phorbol ester, anti-CD3 antibody or preferably, antigen, in association with an MHC class II molecule, and given a costimulatory signal, *e.g.*, by a stimulatory form of B7 family antigen, for instance by a cell transfected with nucleic acid encoding a B7 polypeptide and expressing the peptide on its surface, or by a soluble, stimulatory form of the peptide. The cells can then be contacted with cells expressing PD-L2 and/or treated with agents which inhibit PD-L2-PD-1 interaction (*e.g.*, antibodies against PD-L2, or combinations of antibodies against PD-L2 and antibodies against PD-L1). Known cytokines released into the media can be identified by ELISA or by the ability of an antibody which blocks the cytokine to inhibit immune cell proliferation or proliferation of other cell types that are induced by the cytokine. For example, an IL-4 ELISA kit is available from Genzyme (Cambridge, MA), as is an IL-7 blocking antibody. Blocking antibodies against IL-9 and IL-12 are available from Genetics

Institute (Cambridge, MA). The effect of stimulating or blocking PD-L2 activity or the interaction of PD-L2 and PD-1 on the cytokine profile can then be determined.

An *in vitro* immune cell costimulation assay as described above can also be used in a method for identifying novel cytokines which can be modulated by modulation of PD-L2 activity or PD-L2-PD-1 interaction. For example, where stimulation of the CD28/CTLA4 pathway seems to enhance IL-2 secretion, stimulation of the ICOS pathway seems to enhance IL-10 secretion (Hutloff *et al.* (1999) *Nature* 397:263). If a particular activity induced upon costimulation, *e.g.*, immune cell proliferation, cannot be inhibited by addition of blocking antibodies to known cytokines, the activity may result from the action of an unknown cytokine. Following costimulation, this cytokine can be purified from the media by conventional methods and its activity measured by its ability to induce immune cell proliferation.

To identify cytokines which may play a role the induction of tolerance, an *in vitro* T cell costimulation assay as described above can be used. In this case, T cells would be given the primary activation signal and contacted with a selected cytokine, but would not be given the costimulatory signal. After washing and resting the immune cells, the cells would be rechallenged with both a primary activation signal and a costimulatory signal. If the immune cells do not respond (*e.g.*, proliferate or produce cytokines) they have become tolerized and the cytokine has not prevented the induction of tolerance. However, if the immune cells respond, induction of tolerance has been prevented by the cytokine. Those cytokines which are capable of preventing the induction of tolerance can be targeted for blockage *in vivo* in conjunction with reagents which block B lymphocyte antigens as a more efficient means to induce tolerance in transplant recipients or subjects with autoimmune diseases. For example, one could administer a cytokine blocking antibody to a subject along with an agent that promotes PD-L2 activity or PD-L2-PD-1 interaction.

This invention is further illustrated by the following examples, which should not be construed as limiting. The contents of all references, patents, and published patent applications cited throughout this application, as well as the Figures and Sequence Listing, are incorporated herein by reference.

EXAMPLES

The following Materials and Methods were used in Examples 1-9.

5 Materials and Methods

Mice

Hoffman-La Roche, Nutley, NJ, provided mice expressing a TCR transgene (DO11.10) which is specific for OVA peptide (323-339) in association with IA^d. Balb/c mice were obtained from Taconic Farms (Germantown, NY). All mice were used between the ages of 6-12 weeks. The mice were cared for in accordance with institutional guidelines.

Molecular cloning

15 A BLAST search of the non-redundant database of the National Center for Biotechnology Information (NCBI) using the human PD-L1 protein sequence as a query identified GenBank Accession No. AF142780 (also referred to herein as mouse PD-L2) as having 38% amino acid identity with PD-L1. PD-L1 is a PD-1 ligand described in the co-pending U.S. Application Serial No. 09/644,934; International Publication WO
20 01/14557; Dong, H. *et al.* (1999) *Nat. Med.* 5:1365-1369; and Freeman, G.J. *et al.* (2000) *J. Exp. Med.* 192:1027-1034; the contents of each of which are incorporated herein by reference. The AF142780 coding region was amplified by PCR using as primers 5'-dGTCGACCACCATGCTGCTCCTGCTGCCGATA-3' (SEQ ID NO:7) and 5'-dGTCGACTCACTAGATCCTCTTTCTCTGGATTATCAC-3' (SEQ ID NO:8) and
25 cloned into pEF6 (Invitrogen, Carlsbad, CA). A search of the NCBI EST database identified 2 human fetal heart ESTs (GenBank Accession Nos. AA247117 and AA247128) with homology to murine PD-L2. A 101 bp region was amplified by PCR using as primers 5'-dGTACATAATAGAGCATGGCAGCA-3' (SEQ ID NO:9) and 5'-dCCACCTTTTGCAAAGTGGCTGT-3' (SEQ ID NO:10). The PCR product was
30 biotin-labeled and used to isolate a full-length cDNA by Cloncapture (Clontech, Palo Alto, CA) from a human placenta cDNA library in the pAXEF vector (Freeman, G.J. *et al.* (2000) *J. Exp. Med.* 192:1027-1034). The full-length human PD-L2 cDNA sequence has been deposited with GenBank (accession number to be assigned).

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Fusion proteins and cell transfections

The Ig fusion proteins consist of the complete extracellular region of a receptor linked to the hinge-CH2-CH3 domains of murine Ig γ 2a (with four point mutations blocking Fc receptor and complement binding) to give Ig(γ 2a) fusions (Duncan, A.R. *et al.* (1988) *Nature* 332:563-564; Morgan, A. *et al.* (1995) *Immunology* 86:319-324). Control.Ig consists of the Oncostatin-M leader sequence linked to murine Ig γ 2a. These recombinant proteins were produced in stably transfected CHO cell lines and purified from conditioned media using protein A-Sepharose. The murine PD-L2 cDNA in pEF6 was linearized with *ScaI* and co-electroporated into CHO.I-A^d, or CHO.I-A^d.mB7-2 cells with a plasmid construct containing a puromycin resistance gene under the control of a phosphoglycerate kinase gene promoter. The human PD-L2 cDNA in pAXEF was linearized with *ApaI* and co-electroporated into CHO.K1 cells with a puromycin-resistance gene under the control of a phosphoglycerate kinase gene promoter. Transfectants were selected in 10 μ g/ml puromycin, stained with hPDI.Ig, sorted, and cloned by limiting dilution.

Northern blot analysis

Mouse and human multiple tissue northern blots (Clontech, Palo Alto, CA) were probed with ³²P-dCTP radiolabeled cDNA probes in QuikHyb (Stratagene, La Jolla, CA) according to manufacturer's instructions. The human PD-L2 probe consisted of a 1.2 kb *XbaI* fragment spanning the coding region and 3' UTR sequence. The mouse PD-L2 probe consisted of a 444 kb *KpnI/EcoRV* cDNA fragment spanning the coding region. Actin probes were supplied by Clontech. Blots were washed twice at room temperature in 2X SSC, 0.1% SDS, followed by 0.1X SSC, 0.1% SDS at 65°C, and examined by autoradiography.

Flow cytometry

For detection of PD-L2, 5x10⁴ transfected CHO cells were incubated with 5 μ g/ml of human PD-Ig (hPD-I.Ig) (Genetics Institute, Cambridge, MA) and developed with goat anti-mouse IgG2a-phycoerythrin (PE) (Southern Biotechnology Associates Inc, Birmingham, AL). In addition, cells were stained separately with 5 μ g/ml anti-IA^d-PE or B7.2-PE (Pharmingen, San Diego, CA).

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CD4⁺ T cells were incubated with biotinylated anti-PD-1 (or biotinylated anti-CD28 (Pharmingen) or biotinylated anti-CD25 (Pharmingen) and developed with streptavidin-PE (Pharmingen). All isotype controls were obtained from Pharmingen. Following each step, cells were washed three times with PBS/1% BSA/0.02% sodium azide. After the final incubation, cells were fixed with 1% paraformaldehyde. Ten thousand events were analyzed on a FACSCalibur (Becton Dickinson, Mountain View, CA). All isotype controls were all obtained from Pharmingen.

Activation of T cells

To generate activated antigen-specific T cells, splenocytes were prepared from DO11.10 mice and treated with Tris-NH₄Cl to deplete erythrocytes. Cells were cultured with 1 µg/ml of OVA peptide for 72 hours (Analytical Biotechnology Services, Boston, MA) in RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS (Sigma, St Louis, MO), 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, 250 ng/ml amphotericin B, 10 mM Hepes, 50 pM 2-ME (all from Life Technologies) and 15 mg/ml of gentamicin (BioWhittaker, Walkersville, MD). CD4⁺ T cells were purified by positive selection using magnetic-activated cell sorting separation columns (Miltenyi Biotec, Auburn, CA) with resulting purity of >98%. Cells were rested overnight before re-stimulation.

Bead Stimulation of T cells

Anti-CD3 Ab (2C11; Pharmingen, La Jolla, CA), mPD-L2.Ig, and control.Ig were covalently attached to polyurethane-coated tosyl-activated Dynabeads (DynaL, Lake Success, NY). Beads were prepared with a constant sub-optimal anti-CD3 Ab concentration (60% of the total bound protein) and mPD-L2.Ig or control Ig (40% of total bound protein). Beads have a protein binding capacity of 5 µg/10⁷ beads. T cells were purified from Balb/c lymph nodes by negative selection using cell enrichment columns (R & D Systems, Minneapolis, MN). 0.5-1x10⁵ T cells were cultured per well in 96 well plates with a 2:1 ratio of the indicated coated tosyl beads:cells. Proliferation was measured by [³H]-thymidine incorporation for the last 10 hours of a 72 hr culture.

CHO cell stimulation of T cells

Proliferation of transfected CHO cells was inhibited by incubation with 50 µg/ml of mitomycin C (Bristol Laboratories, Princeton, NJ) or 16 hours at 37°C. At the end of

the incubation period, the cells were harvested with 10 mM EDTA in PBS, washed twice and left on ice for 1 hour. The cells were subsequently washed three times and resuspended in culture medium. 10^5 previously activated $CD4^+$ T cells were cultured with varying concentrations of OVA peptide and 10^4 mitomycin C-treated CHO transfectants in 96 well plates. To assay proliferation, cultures were incubated for 48 hrs and pulsed with 1 μ Ci/well of [^{3} H] thymidine (New England Nuclear, Boston, MA) for the last 6 hours of the incubation period.

Cytokine ELISA

Aliquots of supernatants were harvested at various times after initiation of cultures. IL-2, IL-4, IFN- γ and IL-10 levels were analyzed using mAbs and recombinant cytokine standards from Pharmingen. Detection limits were as follows: IL-2: 20 pg/ml; IL-4: 40 pg/ml; IFN- γ : 100 pg/ml; and IL-10: 200 pg/ml.

RNAase protection assay (RPA)

$CD4^+$ T cells were restimulated with various CHO cell transfectants and 0.01 μ g/ml OVA peptide. After 48 hours, cells were harvested and mRNA was isolated using TRIzol[®] reagent (Life Technologies, Grand Island, NY). 5 μ g mRNA was analyzed for cytokine levels by RNAase protection assay using RiboQuant multiprobe kit mCK1 according to manufacturer's instructions (Pharmingen, San Diego, CA).

Cell Cycle Analysis

$CD4^+$ T cells were restimulated with 0.01 μ g/ml peptide and CHO transfectants as described above. After 36 hours of culture, cells were recovered and stained with anti-CD4-FITC. Cells were washed in PBS, fixed in 70% ethanol for 1 hour on ice and then resuspended in PBS containing 10 μ g/ml RNase (Sigma, St. Louis, MO) and 50 μ g/ml propidium iodide (Sigma). Analysis was performed within an hour of staining using a FACSCalibur.

EXAMPLE 1: IDENTIFICATION AND CHARACTERIZATION OF HUMAN AND MOUSE PD-L2 cDNA

In this example, the identification and characterization of the genes encoding human PD-L2 and mouse PD-L2 is described.

Isolation of the human PD-L2 cDNA

The invention is based, at least in part, on the discovery of human genes encoding novel polypeptides, referred to herein as human PD-L2. The human PD-L2 cDNA was isolated from a human placental cDNA library using as a probe the human
 5 EST AA247117. The entire sequence of human PD-L2 was determined and found to contain an open reading frame termed human "PD-L2"

The nucleotide sequence encoding the human PD-L2 is shown in Figure 1 and is set forth as SEQ ID NO:1. The polypeptide encoded by this nucleic acid comprises about 273 amino acids and has the amino acid sequence shown in Figure 1 and set forth
 10 as SEQ ID NO:2. The coding region (open reading frame) of SEQ ID NO:1 is set forth as SEQ ID NO:3.

The nucleotide sequence encoding the mouse PD-L2 is shown in Figure 2 and is set forth as SEQ ID NO:4. The polypeptide encoded by this nucleic acid comprises about 247 amino acids and has the amino acid sequence shown in Figure 2 and set forth
 15 as SEQ ID NO:5. The coding region (open reading frame) of SEQ ID NO:4 is set forth as SEQ ID NO:6.

Analysis of the Human and Mouse PD-L2 Molecules

Each of the amino acid sequences of human and mouse PD-L2 was analyzed by
 20 comparison to other B7 family members for the presence of a signal peptide. These analyses resulted in the identification of a signal peptide domain in the amino acid sequence of the native human PD-L2 (SEQ ID NO:2) at about residues 1-19 (Figure 3). These analyses further identified a signal peptide domain in the amino acid sequence of the native mouse PD-L2 (SEQ ID NO:5) at about residues 1-19 (Figure 3).

Each of the amino acid sequences of human and mouse PD-L2 was also analyzed
 25 by comparison to other B7 family members for the presence of an IgV domain. These analyses resulted in the identification of an IgV domain in the amino acid sequence of the native human PD-L2 (SEQ ID NO:2) at about residues 20-120 (Figure 3), and at about residues 1-101 in the predicted mature polypeptide. These analyses further
 30 identified an IgV domain in the amino acid sequence of the native mouse PD-L2 (SEQ ID NO:5) at about residues 20-120 (Figure 3), and at about residues 1-101 in the predicted mature polypeptide.

Additionally, each of the amino acid sequences of human and mouse PD-L2 was analyzed by comparison to other B7 family members for the presence of an IgC domain. These analyses resulted in the identification of an IgC domain in the amino acid sequence of the native human PD-L2 (SEQ ID NO:2) at about residues 121-219 (Figure 3), and at about residues 102-200 in the predicted mature polypeptide. These analyses further identified an IgC domain in the amino acid sequence of the native mouse PD-L2 (SEQ ID NO:5) at about residues 121-219 (Figure 3), and at about residues 102-200 in the predicted mature polypeptide.

Each of the amino acid sequences of human and mouse PD-L2 was further analyzed by comparison to other B7 family members for the presence of an extracellular domain. These analyses resulted in the identification of an extracellular domain in the amino acid sequence of the native human PD-L2 (SEQ ID NO:2) at about residues 1-219 (Figure 1), and at about residues 1-200 in the predicted mature polypeptide. These analyses further identified an extracellular domain in the amino acid sequence of the native mouse PD-L2 (SEQ ID NO:5) at about residues 1-219 (Figure 2), and at about residues 1-200 in the predicted mature polypeptide.

Each of the amino acid sequences of human and mouse PD-L2 was also analyzed by comparison to other B7 family members for the presence of a transmembrane domain. These analyses resulted in the identification of a transmembrane domain in the amino acid sequence of the native human PD-L2 (SEQ ID NO:2) at about residues 220-243 (Figure 3), and at about residues 201-224 in the predicted mature polypeptide. These analyses further identified a transmembrane domain in the amino acid sequence of the native mouse PD-L2 (SEQ ID NO:5) at about residues 220-242 (Figure 3), and at about residues 201-223 in the predicted mature polypeptide.

Each of the amino acid sequences of human and mouse PD-L2 was further analyzed by comparison to other B7 family members for the presence of a cytoplasmic domain. These analyses resulted in the identification of a cytoplasmic domain in the amino acid sequence of the native human PD-L2 (SEQ ID NO:2) at about residues 244-273 (Figure 3), and at about residues 225-254 in the predicted mature polypeptide. These analyses further identified a cytoplasmic domain in the amino acid sequence of the native mouse PD-L2 (SEQ ID NO:5) at about residues 243-247 (Figure 3), and at about residues 224-228 in the predicted mature polypeptide.

The mouse PD-L2 protein has 38% amino acid identity with mouse PD-L1 (see Figure 6). Mouse and human PD-L2 have 70% amino acid identity (Figure 6), which is higher than the 46-50% identity between human and murine B7-1 or B7-2.

5 **EXAMPLE 2: EXPRESSION OF RECOMBINANT PD-L2 POLYPEPTIDE IN BACTERIAL CELLS**

In this example, human PD-L2 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, PD-L2 is fused to GST and this fusion polypeptide is
 10 expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-PD-L2 fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide
 purified from the bacterial lysates, the molecular weight of the resultant fusion
 15 polypeptide is determined.

EXAMPLE 3: BINDING OF PD-L2 TO PD-1

COS cells were transfected with an expression plasmid containing AF142780, the mouse PD-L2 cDNA, or a control mouse PD-1 ligand. After 72 hours, the
 20 transfected COS cells were detached by incubation in PBS containing 0.5 mM EDTA for 30 min. at 37°C.

The ability of COS cells expressing PD-L2 to bind to various Ig fusion proteins was tested. FACS analysis of binding of IgG2a (control Ig), ICOS-IgG, and PD-1-Ig by PD-L2-transfected COS cells showed that neither IgG2a or ICOS-IgG was bound by PD-
 25 L2 or the control PD-1 ligand. PD-1-Ig, however, was shown to bind to PD-L2 and to the control PD-1 ligand (Figure 4).

Experiments using the human PD-L2 cDNA yielded results similar to those obtained using the mouse PD-L2 cDNA.

Experiments were also performed using CHO cells. Flow cytometry studies
 30 indicate that hPD-1-Ig recognizes CHO cells stably transfected with PD-L2 (Fig. 7). This staining was specific, since there was no binding to control transfectants (CHO-IA^d, CHO-IA^d/B7.2). CHO cells transfected with PD-L2 showed no staining with CTLA4-Ig, CD28-Ig or ICOS-Ig. An alternatively spliced variant of murine PD-

L2, wherein the IgV exon is deleted, was also isolated. This variant did not bind PD-1-Ig, indicating that the IgV domain is necessary for PD-L2 binding to PD-1.

EXAMPLE 4: GENERATION OF FULLY HUMAN ANTIBODIES TO PD L2

5 In this example, fully human antibodies against PD-L2 are made in mice that are transgenic for human immunoglobulin framework genes. Transgenic mice are made using standard methods, *e.g.*, according to Hogan *et al.*, *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory, which is incorporated herein by reference, or are purchased commercially. Embryonic stem cells are
10 manipulated according to published procedures (*Teratocarcinomas and embryonic stem cells: a practical approach*, Robertson, E.J. ed., IRL Press, Washington, D.C., 1987; Zijlstra *et al.* (1989) *Nature* 342:435-438; and Schwartzberg *et al.* (1989) *Science* 246:799-803, each of which is incorporated herein by reference). DNA cloning procedures are carried out according to Sambrook, J. *et al.* in *Molecular Cloning: A Laboratory Manual*, 2d ed., 1989, Cold Spring Harbor Laboratory Press, Cold Spring
15 Harbor, N.Y., which is incorporated herein by reference. Oligonucleotides are synthesized, *e.g.*, on an Applied Bio Systems oligonucleotide synthesizer according to specifications provided by the manufacturer or are purchased commercially.

Transgenic mice are immunized using a purified or recombinant PD-L2 or a
20 fusion protein comprising at least an immunogenic portion of the extracellular domain of PD-L2. Approximately four hundred µg of PD-L2 in 100 µL of phosphate buffered saline (PBS) is injected intraperitoneally into each mouse. Serum samples are collected approximately six days later by retro-orbital sinus bleeding.

Antibody reactivity and specificity for PD-L2 are assessed using an indirect
25 enzyme-linked immunosorbent assay (ELISA). Several immunoglobulin superfamily molecules are tested as controls (*e.g.*, CTLA4 and CD28) to analyze the antibody specificity of the antibody for PD-L2. Antibodies having human framework regions which bind to PD-L2 are detected by enzyme conjugates specific for human IgM and human IgG sub-classes with no cross reactivity to mouse immunoglobulin. Briefly,
30 PVC microtiter plates are coated with PD-L2 by coating wells overnight at 37°C with 5 µg/mL PD-L2 in PBS. Serum samples are diluted in PBS, 5% serum, 0.5% Tween-20 and are incubated in the wells for 1 hour at room temperature, followed by anti-human IgG Fc and IgG F(ab')-horseradish peroxidase or anti-human IgM Fc-horseradish peroxidase in the same diluent. After 1 hour at room temperature enzyme activity is

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assessed by addition of ABTS substrate (Sigma, St. Louis, Mo.) and read after 30 minutes at 415-490 nm. In pre-immunization serum samples from the same mice, titers of human antibodies to the same target antigens are also tested.

Spleen cells isolated from mice having appropriate antibody titers are harvested.

- 5 The spleen cells are fused to appropriate fusion partners (*e.g.*, myeloma cells) to make hybridomas. Hybridomas and antibodies are manipulated according to *Antibodies: A Laboratory Manual*, Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988), which is incorporated herein by reference.

10 EXAMPLE 5: GENERATION OF ANTIBODIES TO PD-L2

Anti-human PD-L2 antibodies

Anti-PD-L2 antibodies can also be prepared by cDNA immunization of mice.

- For production of anti-human PD-L2 antibodies, female Balb/c mice (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were prepared for cDNA immunization by injecting 50
 - 15 μ l of 10 mM cardiotoxin (Sigma Chemical Company, St. Louis, MO) in 0.9% saline into the tibialis anterior muscle of each hind limb. Five days later, 50 μ l of 1 mg/ml purified hPD-L2 cDNA in the pAXEF mammalian expression vector in 0.9% saline was injected into each regenerating anterior tibialis anterior muscle of each mouse. The cDNA immunization was repeated twice at 2 week intervals. The mice were then rested for
 - 20 four weeks, followed by three boosts of 100 μ g cDNA per mouse, spread 3-4 weeks apart. Five days before fusion, a mouse selected for fusion was boosted with cDNA intramuscularly. Spleen cells were fused with SP2/0 myeloma cells, cloned, and the hybridomas screened by ELISA for reactivity with hPD-L2-mIgG2a fusion protein, followed by cell surface staining of hPD-L2 transfected 300.19 and COS cells and for
 - 25 lack of reactivity with untransfected cells and hPD-L1 transfected cells.

- Nine mouse anti-human PD-L2 monoclonal antibodies (mAbs) were isolated. In order to determine the capacity of these antibodies to block the PD-1/PD-L2 interaction, the anti-PD-L2 mAbs were preincubated with PD-L2 transfected cells. Inhibition of the interaction was measured as the capacity of the mAb to reduce the binding of
 - 30 biotinylated PD-1-Ig to PD-L2 transfected cells. The best mAbs were antibodies 24F.10C12 (also referred to herein as 10C12) and 24F7G12 (also referred to herein as 7G12). The results are as follows:

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		mean fluorescence intensity of biotinylated
		hPD-1-Ig bound to 300-hPD-L2 cells
	mAb	
	7G12	0.110
	10C12	0.109
5	positive control	2.57

Anti-mouse PD-L2 antibodies

For production of anti-mouse PD-L2 antibodies, female Lewis strain rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were prepared for cDNA immunization by injecting 100 μ l of 10 mM cardiotoxin (Sigma Chemical Company, St. Louis, MO) in 0.9% saline into the tibialis anterior muscle of each hind limb. Five days later, 100 μ l of 1 mg/ml purified murine PD-L2 cDNA in the pEF6 mammalian expression vector in 0.9% saline was injected into each regenerating anterior tibialis anterior muscle of each rat. The cDNA immunization was repeated three times at 2-3 week intervals. The rats were then immunized with $1-5 \times 10^7$ CHO-mPD-L2 transfectants four times at 2-5 week intervals. Five days before fusion, a rat selected for fusion was immunized with both cDNA (200 μ g) and cells (5×10^7). Spleen cells were fused with SP2/0 myeloma cells, cloned, and the hybridomas screened by cell surface staining of mPD-L2 transfected 300.19 and COS cells and for lack of reactivity with untransfected cells and mPD-L1 transfected cells. Monoclonal antibodies were produced that specifically recognize mPD-L2.

EXAMPLE 6: EXPRESSION OF PD-L2

To analyze the expression PD-L2, mRNA expression was analyzed by Northern blot hybridization in a variety of tissues and in activated antigen presenting cells. Human and murine PD-L2 mRNAs are expressed highly in normal placenta, and are expressed at low levels in normal spleen, lymph nodes, and thymus. PD-L2 is also expressed in human heart, but is expressed at a low level in mouse heart. Other tissues in which PD-L2 is expressed are human pancreas, lung and liver. PD-L2 mRNA was not detected in unstimulated human monocytes but was upregulated by IFN- γ stimulation. The induction of PD-L2 was slightly delayed in kinetics as compared to the upregulation of PD-L1 (Freeman, G.J. *et al.* (2000) *J. Exp. Med.* 192:1027-1034). Northern blot analysis of murine tumor cell lines revealed PD-L2 mRNA expression in lines of lymphoid origin such as PU5-1.8 (myeloid lymphoma), RAW 264.7

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(macrophage lymphoma), R1.1 (T lymphoma), L1210 (lymphocytic leukemia), P388D1 (monocyte/macrophage lymphoma), and P815 (mastocytoma). PD-L2 was expressed poorly or not at all in fibroblast cell lines (M-MSV Balb/3T3, K-Balb, LM) and the P19 teratocarcinoma cell line. PD-L2 is also expressed in hepatoma (Hepa 1-6) and neuroblastoma (NB41A3) cell lines.

EXAMPLE 7:PD-L2-PD-1 INTERACTION INHIBITS TCR MEDIATED RESPONSES

To investigate the role of the PD-L2-PD-1 pathway in T cell activation, a Dynal bead-based T cell activation system was used. Purified T cells from Balb/c lymph nodes were activated with beads coated with anti-CD3 plus control.Ig or mPD-L2.Ig. Proliferation was measured by ³H-thymidine incorporation. T cells activated with anti-CD3 plus mPD-L2.Ig coated beads showed a marked decrease in proliferation relative to anti-CD3 plus control.Ig activated cells (Figure 8). Thus, engagement of PD-1 on T cells by PD-L2 leads to inhibition of T cell proliferation.

In order to study antigen specific signals, CHO cells co-expressing PD-L2 and I-A^d and CHO cells expressing similar levels of I-A^d alone (Figure 7) were compared in their ability to activate DO11.10 CD4⁺ T cells. Because PD-1 is upregulated after activation, pre-activated DO11.10 T cells were used to enable maximal PD-L2 interaction. Pre-activated CD4⁺ T cells were incubated with a range of concentrations of OVA (323-339) peptide and mitomycin-C treated CHO transfectants. As seen in Figure 9, pre-activated DO11.10 CD4⁺ T cells gave a moderate proliferative response to OVA peptide presented by CHO.I-A^d. In contrast, this response was significantly reduced in the presence of PD-L2. Interleukin (IL)-4, IFN- γ and IL-10 productions were also markedly reduced (Fig. 9). IL-2 was not detected under these conditions of activation. These data show that PD-1-PD-L2 interactions can inhibit TCR mediated proliferation and cytokine production. The inhibitory effects of the PD-1-PD-L2 pathway and the expression of PD-L2 in non-lymphoid organs points to a role in controlling autoimmune reactions.

EXAMPLE 8:PD-L2 CAN INHIBIT TCR-CD28 SIGNALS

Since optimal T cell clonal expansion requires both TCR and CD28 signals, the interplay between the TCR plus CD28 and PD-L2-PD-1 signals was examined. To address this issue, the following CHO transfectants were used: CHO.I-A^d.B7-2 and

CHO.I-A^d.B7-2.PD-L2. The ability of these CHO transfectants to stimulate previously activated DO11.10 CD4⁺ T cells with OVA peptide was compared. The expression of I-A^d and B7-2 was similarly high on these CHO transfectants (Fig. 7). The level of PD-L2 expression, as measured by PD-1.Ig binding, was high. As expected, introduction of B7-2 led to an increase in proliferative responses by T cells at all antigen concentrations, with the most marked stimulation at low antigen concentrations (Figure 10). Co-expression of PD-L2 on CHO transfectants inhibited TCR and B7-2 mediated proliferative responses at low peptide concentrations (0.01 µg/ml and 0.001 µg/ml) (Figure 10). At 0.01 µg/ml peptide concentration, PD-L2 significantly inhibited TCR-B7-2 mediated cytokine production, consistent with the inhibition of proliferation (Figure 11). At 0.1 µg/ml peptide concentration, where there was only a weak inhibition of proliferation, cytokine production was inhibited when DO11.10 CD4⁺ T cells were cultured with peptide and CHO.I-A^d.B7-2.PD-L2 transfectants (Figure 12). Therefore, PD-1 engagement by PD-L2 can downregulate TCR-CD28 mediated stimulation of cytokine production.

To determine whether the diminished cytokine production was due to reduced mRNA levels, cytokine mRNA levels were measured by RNAase protection assay. Interleukin-4, IL-10, IL-13, IL-2, IL-6 and IFN-γ mRNAs were readily detected in previously activated DO11.10 CD4⁺ T cells after stimulation with 0.01 µg/ml OVA peptide presented by CHO.I-A^d.B7-2. However, the introduction of PD-L2 into the CHO.I-A^d.B7-2 transfectants significantly reduced mRNA levels for both T_H1 and T_H2 cytokines. There was minimal expression of cytokine mRNAs when previously activated T cells were incubated alone or with peptide presented by CHO.I-A^d. These results further demonstrate the capacity of the PD-L2-PD-1 pathways to antagonize a B7-CD28 signal when antigenic stimulation is weak or limiting.

To assess whether the ability of the PD-L2-PD-1 pathway to inhibit at low antigen concentrations was related to variations in levels of CD28 and PD-1 expression at different antigen concentrations, surface expression of PD-1 and CD28 was examined. Table 1 shows the mean fluorescence intensity of PD-1, CD28 and CD25 on DO11.10 CD4⁺ T cells following different activation conditions.

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Table 1: MFI of CD28, PD-1, and CD25 on freshly isolated and previously activated DO11.10 CD4⁺ T cells and during restimulation with CHO.I-A^d.B7-2

Antibody		MFI							
Freshly isolated CD4 ⁺ DO11.10 T cells	CD28	33							
	PD-1	14							
	CD25	27							
	Isotype	16							
Previously activated CD4 ⁺ DO11.10 T cells	Expt. 1				Expt. 2				
	CD28	50				117			
	PD-1	51				51			
	CD25	373				2705			
Restimulated CD4 ⁺ DO11.10 T cells	Isotype	12				17			
	Peptide Conc. (μ g/ml)	0.001	0.01	0.1	1.0	0.001	0.01	0.1	1.0
	Expt. 1				Expt. 2				
	CD28	77	106	123	165	77	121	338	516
	PD-1	78	56	41	39	117	92	84	55
	CD25	334	417	560	1154	2507	2602	2971	7021
	Isotype	11	14						

CD4⁺ T cells were incubated with biotinylated anti-PD-1, biotinylated anti-CD28, biotinylated anti-CD25, or biotinylated isotype control and developed with streptavidin-PE.

- Freshly isolated DO11.10 CD4⁺ T cells expressed CD28, but neither PD-1 nor
- 5 CD25. T cells stimulated with 1 μ g/ml of peptide presented by splenic APCs upregulated CD28 modestly and strongly upregulated PD-1 and CD25. After restimulation of previously activated DO11.10 CD4⁺ T cells with various concentrations of peptide presented by CHO.I-A^d.B7.2, CD28 and CD25 expression increased on CD4⁺ T cells with higher peptide concentration. In contrast, PD-1 expression was highest on
- 10 T cells activated at low peptide concentration and decreased at higher peptide concentrations. The higher expression of PD-1 at lower antigen doses further suggests a mechanism whereby the PD-L2-PD-1 pathway can attenuate weak antigen responses.

EXAMPLE 9: MECHANISM OF ACTION OF THE PD-1-PD-L2 PATHWAY

- 15 Cross-linking of CTLA-4 has been shown to inhibit cell cycle progression in naive T cells (Krummel, M.F. and Allison, J.P. (1996) *J. Exp. Med.* 183:2533-2540;

Walunas, T.L. *et al.* (1996) *J. Exp. Med.* 183:2541-2550). As PD-1 was isolated from murine cell lines undergoing apoptosis, a possible mechanism of action of the PD-1/PD-L2 pathway might be to increase programmed cell death (*e.g.*, activation-induced cell death or AICD). To address this issue, DO11.10 CD4⁺ T cells were

5 restimulated with 0.01 µg/ml peptide and various CHO transfectants, and cell cycle progression was analyzed. After 48 hours, cells were recovered, stained with CD4-FITC, permeabilized, and incubated with propidium iodide to analyze the G₀/G₁, S/G₂ and sub-diploid populations. CD4⁺ T cells restimulated with peptide presented by CHO-IA^d or CHO-IA^d/PD-L2 both have a large proportion of cells in the sub-diploid

10 population, indicative of apoptosis (Figure 13). These results were confirmed by annexin staining. In cultures where CD4⁺ T cells were stimulated by peptide presented by CHO-IA^d (1 µg/ml), there were increased number of cells in the S/G₂ phase, indicating that the cells were in cycle. The introduction of PD-L2 into IA^d transfectants led to an increased number of cells in the G₀/G₁ phase, suggesting cell cycle arrest.

15 Cell cycle progression in the presence of B7-2 and PD-L2 signals was then compared. CD4⁺ T cells stimulated by 0.01 µg/ml peptide presented by CHO.IA^d.B7-2 showed an increased number of cells in the S/G₂ phase and a decreased number in the sub-diploid population indicating cells were in cycle and rescued from apoptosis by B7-CD28 costimulation (Figure 14). As seen with the CHO.IA^d transfectants at higher

20 peptide concentrations, introduction of PD-L2 into B7-2 transfectants led to an increased number of cells in the G₀/G₁ phase and a corresponding decrease in those in S phase. There was no difference in the proportion of apoptotic cells between B7-2 and B7-2.PD-L2 stimulated T cells, indicating that PD-1 crosslinking did not lead to an increase in cell death.

25

EXAMPLE 10: STIMULATION OF T CELL ACTIVATION BY INHIBITION OF PD-1:PD-LIGAND INTERACTION

As shown above, signaling via PD-1 is dominated by strong TCR/CD28 costimulatory signals; the PD-1 signaling pathway inhibits moderate TCR/CD28

30 costimulatory signals, with cytokine production being reduced first without a decrease in T cell proliferation. As the TCR/CD28 costimulatory signals weaken, the PD-1 pathway dominates, with a great reduction in cytokine production accompanied by a reduction in proliferation. Accordingly, in order to determine whether inhibition of the PD-1 pathway via inhibition of the interaction with PD-L1 or PD-L2 would enhance T cell

activation, mixed lymphocyte reactions (MLRs) were performed using weakly functioning antigen presenting cells (APCs) (*i.e.*, antigen presenting cells with weak TCR/CD28 costimulation).

5 Mature dendritic cells are potent APCs. However, treatment with IL-10 reduces their potency. Previous reports indicate that IL-10 greatly reduces dendritic cell APC potency, and this has been attributed to a reduction in the expression of MHC, B7-1, and B7-2. However, experiments herein indicate that the reduction is modest; moreover, IL-10 treated dendritic cells express PD-L1 and PD-L2.

10 Immature myeloid dendritic cells were isolated by culturing human peripheral blood monocytes in IL-4 and GM-CSF. Exposure of immature dendritic cells to an inflammatory cocktail of IL-1 β , TNF- α , IL-6, and PGE₂ elicits the development of mature dendritic cells that function as APCs. However, the addition of IL-10 to the inflammatory cytokines given during the maturation phase results in APCs that function only 1/6 to 1/3 as well.

15 T cell activation assays (MLRs) were performed as described generally above, using IL-10 treated dendritic cells as APCs, in the presence of antibodies to PD-L1 and/or PD-L2, or control antibodies. The addition of anti-PD-L1 or PD-L2 mAb to cultures of IL-10 treated dendritic cells plus allogeneic T cells resulted in a 3-fold increase in T cell proliferation, as compared to control IgG treated cultures (Figure 15).

20 A combination of anti-PD-L1 and anti-PD-L2 antibodies resulted in an increase in stimulation greater than that seen with either antibody alone (Figure 16). This stimulation is consistent with the result expected from blockade of the immunoinhibitory signal mediated by PD-L1 and/or PD-L2 binding to PD-1.

25 Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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What is claimed:

1. A method of identifying a compound which modulates T cell activation at a first and second antigen concentration comprising:
 - 5 a) contacting a T cell expressing a PD-L2 target molecule with a test compound at a first antigen concentration;
 - b) determining the ability of the test compound to modulate T cell proliferation or cytokine production at the first antigen concentration;
 - c) contacting a T cell expressing a PD-L2 target molecule with the
 - 10 test compound at a second antigen concentration; and
 - d) determining the ability of the test compound to modulate T cell proliferation or cytokine production at the second antigen concentration,thereby identifying a compound which modulates T cell activation at a first and second antigen concentration.
- 15 2. The assay of claim 1, wherein the PD-L2 target molecule is PD-1.

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FIG. 1

Human PD-L2 nucleotide sequence

1 GCAAACCTTAAGCTGAATGAACAACTTTTCTCTCTTGAATATATCTTAACGCCAA
ATTTTGAGTGCTTTTGTGTTACCCATCCTCATATGTCCAGCTGGAAAGAAATCCTG
GGTTGGAGCTACTGCATGTTGATTGTTTGTCTTTCCCTTTGGCTGTTTATTTGG
TGGCTACTATAAGGAAATCTAACACAAACAGCAACTGTTTTGTTGTTTACTTTT
GCATCTTACTTGTGGAGCTGTGGCAAGTCCTCATATCAAATACAGAACATGATCT
TCCTCCTGCTAATGTTGAGCCTGGAATTGCAGCTTCACCAGATAGCAGCTTATTC
ACAGTGACAGTCCCTAAGGAAGTACATAATAGAGCATGGCAGCAATGTGACCCCT
GGAATGCAACTTTGACACTGGAAGTCATGTGAACCTTGGAGCAATAACAGCCAGTT
TGCAAAGGTGGAAATGATACATCCCAACCCGTGAAAGAGCCACTTTGCTGGAG
GAGCAGCTGCCCTAGGGAAGGCTCGTTCCACATACCTCAAGTCCAAGTGAGGGA
CGAAGGACAGTACCAATGCATAATCATCTATGGGGTCGCCCTGGGACTACAAGTACC
TGACTCTGAAAGTCAAGCTTCCTACAGGAAATAAACACTCACATCCTAAAGGTT
CCAGAAACAGATGAGGTAGAGCTCACCTGCCAGGCTACAGGTTATCCTCTGGCAGA
AGTATCCTGGCCAAACGTACAGCTTCTGCGCAACACCAAGCCACTCCAGGACCCCTG
AAGGCCCTTACCAGGTACACAGTGTCTGCGCCTAAAGCCACCCCTGGCAGAAAC
TTCAGCTCTGTGTTCTGGAATCTCACGTGAGGGAAGTTACTTTGGCCAGCATTGA
CCTTCAAAGTCAGATGGAACCCAGGACCCATCCAACTTGGCTGCTTCACATTTTCA
TCCCTCCTGCATCATGCTTTTCTTTTATAGCCACAGTGATAGCCCTAAGAAAA
CAACTCTGTCAAAGCTGTATTCTTCAAAGACACAAAGAAAGACCTGTCAACAC
AACAAAGAGGGAAGTGAACAGTGTCTATCTGAACCTGTGGTCTTGGGAGCCAGGGTG
ACCTGATATGACATCTAAAGAGCTTCTGACTCTGAACAGAAATTCGGTGGCCTG
CAGAGCTTGCATTTGCACATTTTCAAATGCCCTTTGGATGACCCAGCA 1223

Human PD-L2 amino acid sequence

1 MIFLLMLSLQLQLHQLAALFTVTVPRELYIIHGSNVTLECNFDTGSHVNLGAIT
ASLQKVENDTSPHRERATLLEEQLPLKASFHIPQVQVRDEGQYQCIIYGVANDY
KYLTLKVKASYRKINTHILKVPETDEVELTQATGYPLAEVSWPNVSVFANTSHSR
TPEGLYQVTSVLRLLKPPPRNFSCVFWNTHVRELTLASIDLQSQMEPRTHPTWLLH
IFIPSCIIAFIFATVIALRKQLCQKLYSSKDTTKRPVTTTKREVNSAI 273

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FIG. 2

Mouse PD-L2 nucleotide sequence

1 GAATTGGGCAGCAGGCTCAAAATGTGGCATATCTTTTGTGTCTCCTTCTGTCTCCCAA
CTAGAGAGAACACACACTTACGGCTCTCTGTCCGGGACAGGTTTGGCTTGTTCGTGTGTT
TGGCTTCCAGGGAACCTGATACAAGGAGACACTGTGTGCTTGCCTTTTCTGTGTCT
TCTGTAGAGAGCTGTCTGGGTGGTCTGATATTGACACAGACCATCTGCTCTCTGTCTG
CCGATACTGAACCTGAGCTTACACTTCACTCTCTAGACAGCTTTATTACACCTGAC
AGCCCCATAAGAGTGTACACCTGTAGACAGCTCGGCAGCAGTGTGAGSCTGGAGTGGC
ATTTTGACCAGCAGAGATGCATCGAAGTGAAGGGAGTTCAGGACAGTTTTCGAGAAG
GTAGAAAATGATACGTTCTCTCAAGTGAAGAGGCCAATCGCTGTGGAGAGAGCGCT
GCCCTTGGGAAGAGCTTTGTCCACATCTGCTAGTGTCCAAGTGTGAGAGATTTCCGGG
AGTACGTTGGCTGTGATCTGCGGGGCGCTGGGACATCAAGTACTCTGAGCGTGTG
AAAGTCAAAGCTTCTTACATGAGCATAGACATAGGATCTGGAGGTTCCAGGTAC
AGGGGAGGTGCGACCTTACTTCCGAGGCTAGAGGTTATCCCTAGCAGAAGTGTCTCT
GGCAAAATGTCAGTGTCTCTCGCAACACCGACCATCAGAACCCCCGAGGCGCT
TACCAGGTCACCAAGTGTCTCGCCTCAAGCTCAGCCTAGCAGAAATCTCAGCTGT
CATGTTCTGGAATGCTACATGAAGAGCTGACTTACGCCATCTGAGCCTCTGA
CTCGGATGGAACCCAAAGTCCCGAAGACGTGGCCACTCATGTTTTCATCCCGGCC
TGCACTATCGCTTTGATCTTCTGCGCATAGTGATATCCAGAGAAGAGGATCTA
GGGGAAGCTGTATTACGGAAGAAGTGGTCTCTCTCCGACATCTGGACCTGCGGT
CTTGGGAGTTGGGAAGGATCTGATGGGAACCCCTCAAGACACTCTCGACATCAAAGT
GAGAATCTTCGAGAGCTGCCATTTGCATCTTTGAACCTTTGGACGGTGGCCAG
GGCTCCGAAGAGGAGCTGTGAAGACTGACAACTCTTCCCTCTGTCTCAAGACTCTCT
GAACGACAAGACCCCAATGGCACTTTAGACTTACCCTGGGATCTGGGCCCAAGT
GAGGSCCTAAGGCTCCTAATGACTTTCCAGGTGAGAACAAAAGGAATTTGCTCTCG
CCCCACCCCACTCTCTGTTTCCGAGGGGACATCGAAATTTCCAGTTACATAAA
ATAGATTGTCAATGAGCTTATTATAGAGCTCATTTTCTCGGGGGCTTGGAGGCT
TCAGACAGGGTTTTCATAACCAAGAGCTATAACTGTGTTTTCACAGACTCTTGA
AATCTCGGCGGCTCTGAAGTTCTAATTAACTCGGAAGCATTTAAGCAACACGTCAA
GTGCCCCCTGTGTGGTATTGTTTCTACTTTTCTGTTTTAAAGTGTGAGTCCAA
GGTAATTTGTTGTGAGCTGTGATATCACTGTTTCTGTCTCTTCTTCACTACA
TCTTTTAAAAACAAAAAATAAAAAA

Mouse PD-L2 amino acid sequence

1 MLLLLPILNLSLQHPVAALFTVTPAPKEVYTVDVGSSSVLECDFFDRRECTELEGIR
ASLQKVENDTSLQSERATLLEEQLPLGKALPHIPSVQVRDSGQYRCLVICGAAWDY
KYLTVKVKASMYMRIDTRILEVPFGTEGVQLTCQARGYPLAEYSWQNVSVFANTSHIR
TPEGLQVTSVYLRKLQPSRNFSCMFNAHMKELTSALIDPLSRMEFPKVPRTWPLH
VFIPACTIALIFLAVIUIQKRRI

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FIG. 3

PD-L2

Domains Order: signal, IgV, IgC, transmembrane,
cytoplasmic

Murine 247 aa

Signal MLLLLPILNLSLQHPVAA
Peptide

IgV LFTVTAPKEVYTVDVGSSVSLECDFDRECTELEGRASLQKVENDTSLQS
ERATLLEEQLPLGKALFHIPSVQVRDSGQYRCLVICGAADYKYLTVKVK

IgC ASYMRIDTRILEVPGTGEVQLTCQARGYPLAEVSWQNVSPANTSHIRTPE
GLYQVTSVLRLLKPQPSRNFSCMFVNAHMKELTSIIIDPLSRMEPKVPR

Trans- TWPLHVFIPACTIALIFLAIVII
membrane

Cyto- QRKRI
plasmic

Extracellular

Human 273 aa

Signal MIFLLMLSLQLHQIAA
peptide

IgV LFTVTVPKELYIIIEHGSNVTLECNFDTGSHVNLGAITASLQKVENDTSPHR
ERATLLEEQLPLGKASFHIPQVQVRDEGQYQCIIYGVAWDYKYLTLKVK

IgC ASYRKINTHILKVPETDEVELTCQATGYPLAEVSWPNVSPANTSHSRTP
GLYQVTSVLRLLKPPPGRNFSVFWNTHVRELTASIDLQSQMEPRTHP

Trans- TWLLHIFIPSCIIAFIFIATVIAL
membrane

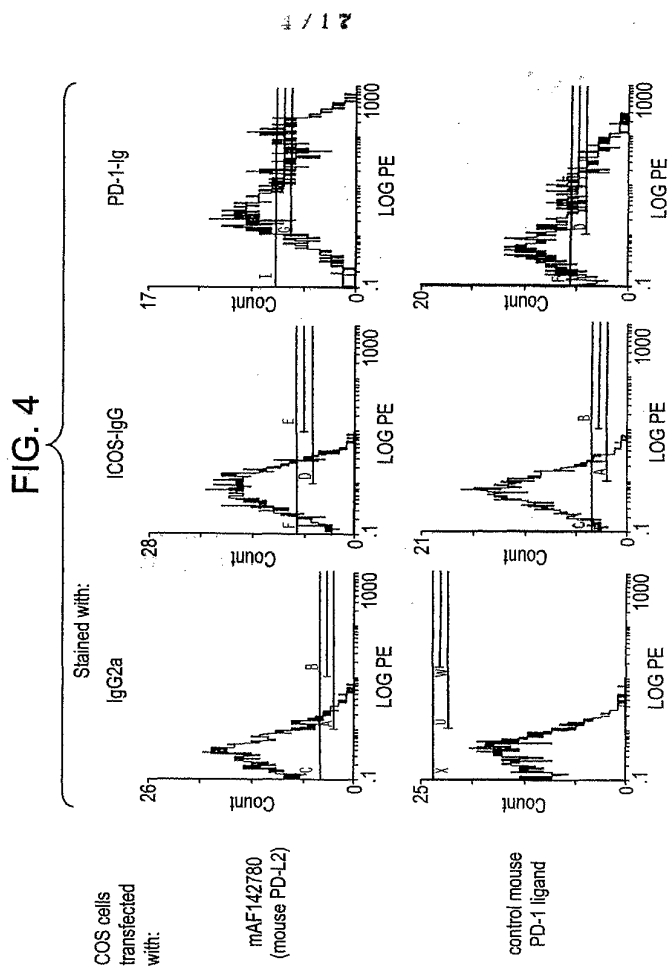
Cyto- RKQLCQKLYSSKDTTKRPVTTTKREVNSAI
plasmic

Extracellular

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FIG. 5

murine PD-L2 vs human PD-L2

70% identity

mPD-L2: 15 HPVAALFTVTAPKEVYTVDVGSVSLECDFFRRECTELEGIRASLQKVENDTSLQSERAT 74
H +AALFTVT PRE+Y ++ GS+VLEC+FD L I ASLQKVENDTSLQSERAT 74
hPD-L2: 15 HQIAALFTVTVPKELYIIEHGSNVTLECNFTGSHVNLGAIATASLQKVENDTSPHRERAT 74

mPD-L2: 75 LLEEQLPLGKALFHIPSQVVRDSQYRCIVICGAAMDYKYLTVKVKASIMRIDTRILEVP 134
LLEEQLPLGKA FHIP VQVRD GQY+C+I I G AWDYKYLTVKVKASY +I+T IL+VP
hPD-L2: 75 LLEEQLPLGKASFHIPSQVVRDEGQYQYCIILYGVAMDYKYLTVKVKASYRKNTHILKVP 134

mPD-L2: 135 GTGEVQLTQARGYPLAEVSWQNVSVFANTSHIRTEGELYQVTSVLRKPQPSRNFSCMF 194
T EV+LTCOA GYPLAEVSW NVSVFANTSH RTEGELYQVTSVLRKP P RNFSCT+P
hPD-L2: 135 ETDEVELTQATGYPLAEVSWFNVSVFANTSHSRTEGELYQVTSVLRKPPEGRNFSCVF 194

mPD-L2: 195 WNAHMKELTSALIDPLSRMEKPVRTWPLHVFIPACTIALIFIAIVIQKRRI 247
WN H++ELT A ID S+MEP+ TW LH+FIIP+C IA IF+A VI RK++
mPD-L2: 195 WNTHVRELTASIDLQSQMEPRTHPTWLLHIFIPSCIIAIFITATVIALRKQL 247

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FIG. 6

```
mPD-L2 MLLLLPILNLSQLEPVAAFTVTAKEVTTVDVSSVSLECDPDRRECTELEGI....
hPD-L2 MIFLLIMSLLELOHQIAAFTVTVPRRLYTHHSGSNVTLECNPDGSHVNGAI....
mPD-L1 ~MRITAGITFTACCHLLRAFTITAEKELYVVEYGSNVTDCREFEVERELDLALVYWE
hPD-L1 ~MRITAVVFTFMTYWHLLNAFTVTVPRKELYVVEYGSNMTDECKEPVEKCLDIADLVYWE
1.....10.....20.....30.....40.....50.....

mPD-L2 ...RASLQKV...ENDTSLQ...SERATLLEBOLPLCKALPHIPSVQVRDSCQVRCLVIC
hPD-L2 ...TASLQKV...ENDTSEH...REKATLLEBOLPLCKASFHIPQVQVRDSCQVCCITHV
mPD-L1 KEDQVIOFVAGEEULKPOHSNFRGASPKDOLKGNALQATDVKLODAGVYCCITISV
hPD-L1 MEKKNIIICVHSEEDLKVDHSSVQEARLKPDLSLGNALQNTVYKLODAGVYFCMESV
61.....70.....80.....90.....100.....110.....

mPD-L2 QAWDYKYLTVKKNASVMRDTRTLEVPGTCEVQLTQAEQYPLAEVSMON.....VSV
hPD-L2 QAWDYKYLTVKKNASVMKINTHLEKVPGTCEVQLTQAEQYPLAEVSMEN.....VSV
mPD-L1 GGAIDYKRIIVKKNASVMKINRI.SVDPATSEHELTQAEQYPLAEVMTNSDRQVLSG
hPD-L1 GGAIDYKRIIVKKNASVMKINRI.VVDQVTSHELTQAEQYPLAEVMTNSDRQVLSG
121.....130.....140.....150.....160.....170.....

mPD-L2 PANTSIRTPEGLYQVTSVLRLKQESRNFSQFNNAMKELTSA...ITDPLSRMCKVVP
hPD-L2 PANTSIRTPEGLYQVTSVLRLKQESRNFSQFNNHVRRLTSA...SIDLQSCMEPRTH
mPD-L1 KRSVTSRTEGMLNVTSLHVNANADVCTFWRSCQGNHTAELTPELPATHEPSON
hPD-L1 KTYTNSKREKIFNVTSPLINTNTNEDICTERRLDEPNHTAELVPELPATHEPNE
181.....190.....200.....210.....220.....230.....

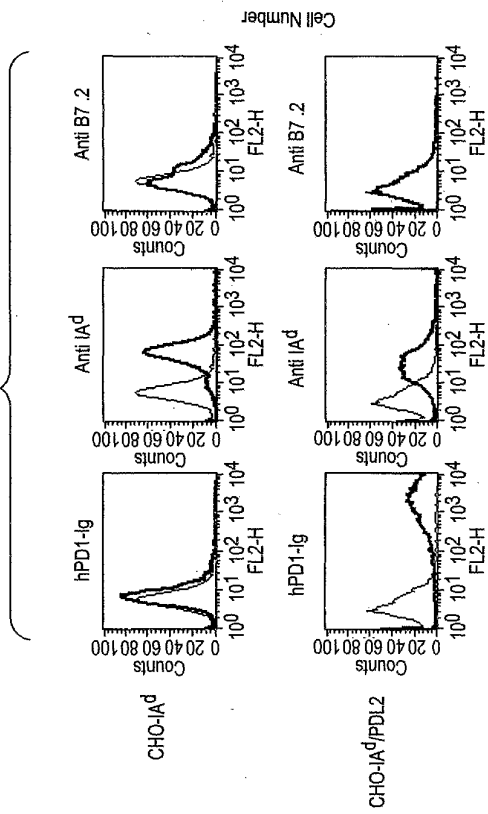
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hPD-L2 RTWPLEVFTPSCIALIPLAIVTALRKQLCQKLYSSDITTKRPVTTTKREVNSAI--
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hPD-L1 RH...ELYLDALLCLGVLTFTFRDRNG.RWMDVKKCGIQDTNSKKOSDTHLEET
241.....250.....260.....270.....280.....290.....
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FIG. 7A

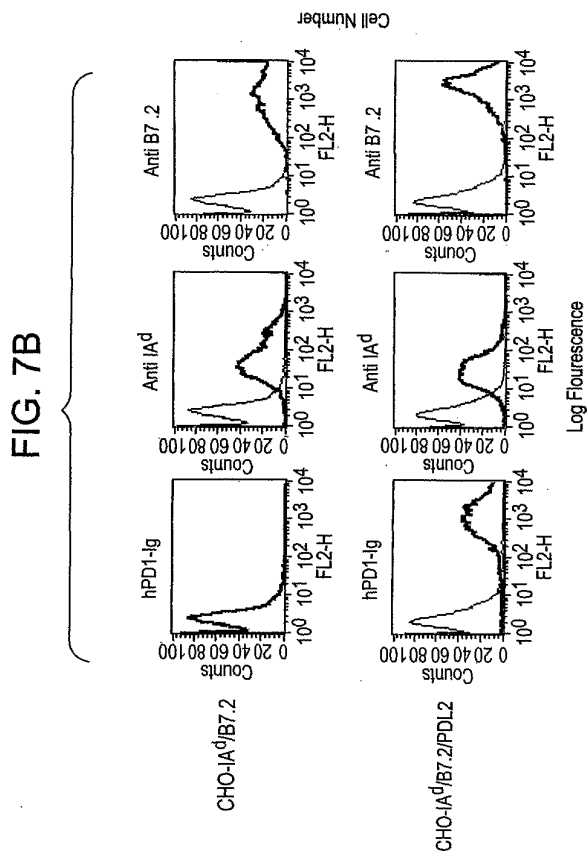


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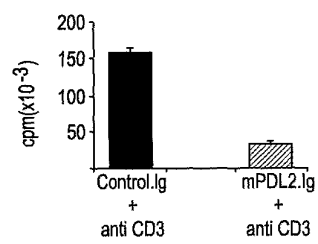


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FIG. 8



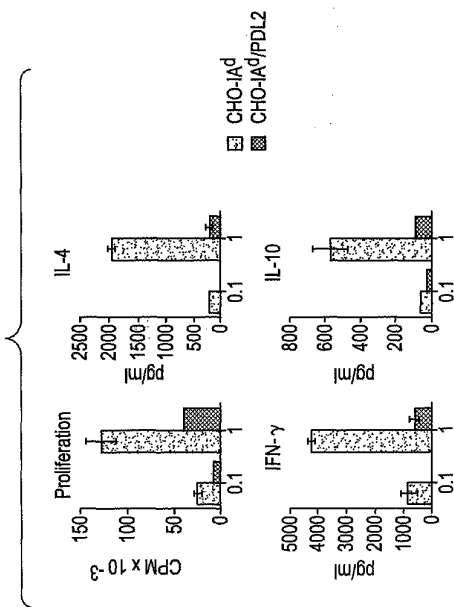
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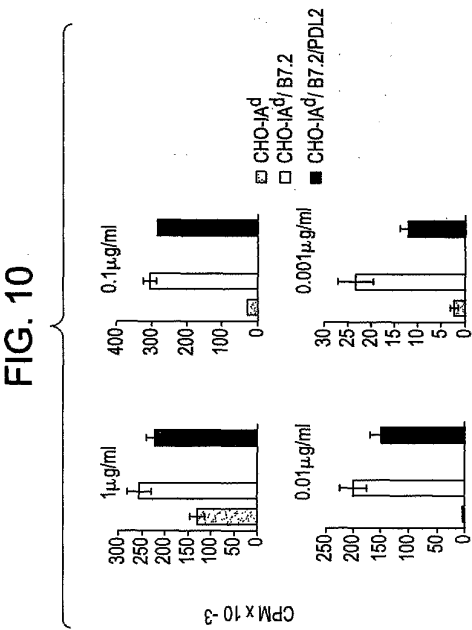
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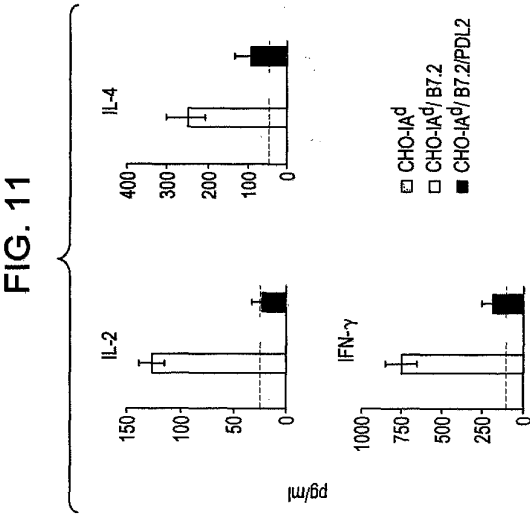
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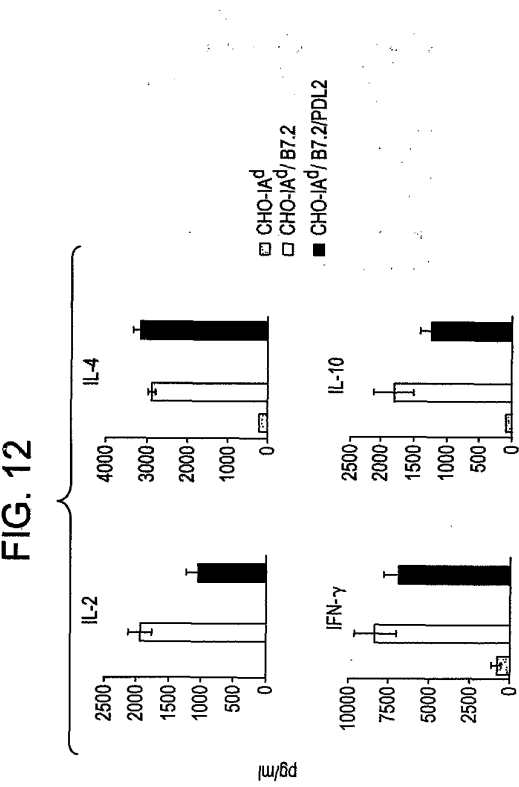
FIG. 9



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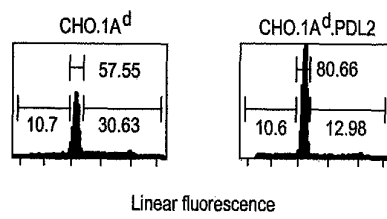


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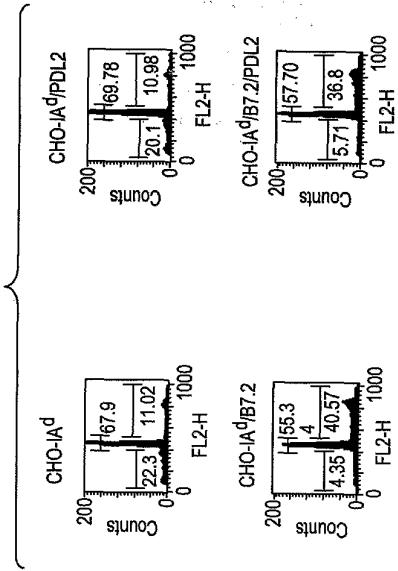
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FIG. 13



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FIG. 14



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FIG. 15

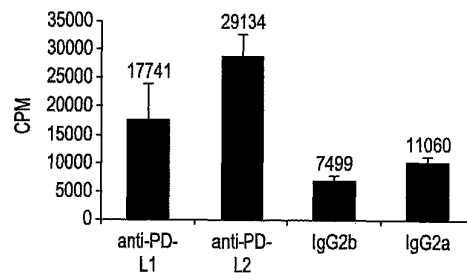
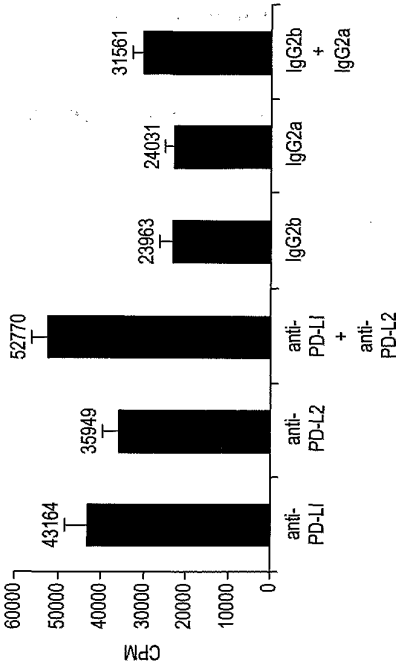


FIG. 16



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(54) Title: PD-L2 MOLECULES; PD-1 LIGANDS AND USES THEREFOR

(57) Abstract: The invention provides isolated nucleic acid molecules, designated PD-L2 nucleic acid molecules, which encode novel B7-related molecules which are ligands for PD-1. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing PD-L2 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a PD-L2 gene has been introduced or disrupted. The invention further provides isolated PD-L2 polypeptides, fusion proteins, antigenic peptides and anti-PD-L2 antibodies. The invention still further provides methods for promoting or inhibiting the interaction between PD-L2 and PD-1. Diagnostic and treatment methods utilizing compositions of the invention are also provided.

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(54) Title: PD-L2 MOLECULES: PD-1 LIGANDS AND USES THEREFOR

(57) Abstract: The invention provides isolated nucleic acid molecules, designated PD-L2 nucleic acid molecules, which encode novel B7-related molecules which are ligands for PD-1. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing PD-L2 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a PD-L2 gene has been introduced or disrupted. The invention further provides isolated PD-L2 polypeptides, fusion proteins, antigenic peptides and anti-PD-L2 antibodies. The invention still further provides methods for promoting or inhibiting the interaction between PD-L2 and PD-1. Diagnostic and treatment methods utilizing compositions of the invention are also provided.

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PD-L2 MOLECULES: NOVEL PD-1 LIGANDS AND USES THEREFOR

Related Applications

5 This application claims priority to U.S. Provisional Application Serial No. 60/214,563, filed June 28, 2000; U.S. Provisional Application Serial No. 60/270,822, filed February 23, 2001; and U.S. Provisional Application Serial No. 60/271,114, filed February 23, 2001. The entire contents of each of these applications are incorporated herein by this reference.

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Background of the Invention

15 In order for T cells to respond to foreign polypeptides, two signals must be provided by antigen-presenting cells (APCs) to resting T lymphocytes (Jenkins, M. and Schwartz, R. (1987) *J. Exp. Med.* 165:302-319; Mueller, D.L. *et al.* (1990) *J. Immunol.* 144:3701-3709). The first signal, which confers specificity to the immune response, is transduced via the T cell receptor (TCR) following recognition of foreign antigenic peptide presented in the context of the major histocompatibility complex (MHC). The second signal, termed costimulation, induces T cells to proliferate and become functional (Lenschow *et al.* (1996) *Annu. Rev. Immunol.* 14:233). Costimulation is
20 neither antigen-specific, nor MHC-restricted, and is thought to be provided by one or more distinct cell surface molecules expressed by APCs (Jenkins, M.K. *et al.* (1988) *J. Immunol.* 140:3324-3330; Linsley, P.S. *et al.* (1991) *J. Exp. Med.* 173:721-730; Gimmi, C.D. *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:6575-6579; Young, J.W. *et al.* (1992) *J. Clin. Invest.* 90:229-237; Koulova, L. *et al.* (1991) *J. Exp. Med.* 173:759-762; Reiser, H. *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:271-275; van-Seventer, G.A. *et al.* (1990) *J. Immunol.* 144:4579-4586; LaSalle, J.M. *et al.* (1991) *J. Immunol.* 147:774-80; Dustin, M.I. *et al.* (1989) *J. Exp. Med.* 169:503; Armitage, R.J. *et al.* (1992) *Nature* 357:80-82; Liu, Y. *et al.* (1992) *J. Exp. Med.* 175:437-445).

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The CD80 (B7-1) and CD86 (B7-2) proteins, expressed on APCs, are critical costimulatory molecules (Freeman *et al.* (1991) *J. Exp. Med.* 174:625; Freeman *et al.* (1989) *J. Immunol.* 143:2714; Azuma *et al.* (1993) *Nature* 366:76; Freeman *et al.* (1993) *Science* 262:909). B7-2 appears to play a predominant role during primary immune responses, while B7-1, which is upregulated later in the course of an immune response, may be important in prolonging primary T cell responses or costimulating secondary T cell responses (Bluestone (1995) *Immunity* 2:555).

One ligand to which B7-1 and B7-2 bind, CD28, is constitutively expressed on resting T cells and increases in expression after activation. After signaling through the T cell receptor, ligation of CD28 and transduction of a costimulatory signal induces T cells to proliferate and secrete IL-2 (Linsley, P.S. *et al.* (1991) *J. Exp. Med.* 173:721-730; Gimmi, C.D. *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:6575-6579; June, C.H. *et al.* (1990) *Immunol. Today* 11:211-6; Harding, F.A. *et al.* (1992) *Nature* 356:607-609). A second ligand, termed CTLA4 (CD152) is homologous to CD28 but is not expressed on resting T cells and appears following T cell activation (Brunet, J.F. *et al.* (1987) *Nature* 328:267-270). CTLA4 appears to be critical in negative regulation of T cell responses (Waterhouse *et al.* (1995) *Science* 270:985). Blockade of CTLA4 has been found to remove inhibitory signals, while aggregation of CTLA4 has been found to provide inhibitory signals that downregulate T cell responses (Allison and Krummel (1995) *Science* 270:932). The B7 molecules have a higher affinity for CTLA4 than for CD28 (Linsley, P.S. *et al.* (1991) *J. Exp. Med.* 174:561-569) and B7-1 and B7-2 have been found to bind to distinct regions of the CTLA4 molecule and have different kinetics of binding to CTLA4 (Linsley *et al.* (1994) *Immunity* 1:793). A new molecule related to CD28 and CTLA4, ICOS, has been identified (Hutloff *et al.* (1999) *Nature* 397:263; WO 98/38216), as has its ligand, which is a new B7 family member (Aicher A. *et al.* (2000) *J. Immunol.* 164:4689-96; Mages H.W. *et al.* (2000) *Eur. J. Immunol.* 30:1040-7; Brodie D. *et al.* (2000) *Curr. Biol.* 10:333-6; Ling V. *et al.* (2000) *J. Immunol.* 164:1653-7; Yoshinaga S.K. *et al.* (1999) *Nature* 402:827-32). If T cells are only stimulated through the T cell receptor, without receiving an additional costimulatory signal, they become nonresponsive, anergic, or die, resulting in downmodulation of the immune response.

Immune cells have receptors that transmit activating signals. For example, T cells have T cell receptors and the CD3 complex, B cells have B cell receptors, and myeloid cells have Fc receptors. In addition, immune cells bear receptors that transmit

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signals that provide costimulatory signals or receptors that transmit signals that inhibit receptor-mediated signaling. For example, CD28 transmits a costimulatory signal to T cells. After ligation of the T cell receptor, ligation of CD28 results in a costimulatory signal characterized by, *e.g.*, upregulation of IL-2 α , IL-2 β , and IL-2 γ receptor, increased transcription of IL-2 messenger RNA, and increased expression of cytokine genes (including IL-2, IFN- γ , GM-CSF, and TNF- α). Transmission of a costimulatory signal allows the cell to progress through the cell cycle and, thus, increases T cell proliferation (Greenfield *et al.* (1998) *Crit. Rev. Immunol.* 18:389). Binding of a receptor on a T cell which transmits a costimulatory signal to the cell (*e.g.*, ligation of a costimulatory receptor that leads to cytokine secretion and/or proliferation of the T cell) by a costimulatory ligand results in costimulation. Thus, inhibition of an interaction between a costimulatory ligand and a receptor that transmits a costimulatory signal on immune cells results in a downmodulation of the immune response and/or specific unresponsiveness, termed immune cell anergy. Inhibition of this interaction can be accomplished using, *e.g.*, anti-CD28 Fab fragments, antibodies to B7 family molecules, or by using a soluble form of a receptor to which a B7 family member molecule can bind as a competitive inhibitor (*e.g.*, CTLA4Ig).

Inhibitory receptors that bind to costimulatory molecules have also been identified on immune cells. Activation of CTLA4, for example, transmits a negative signal to a T cell. Engagement of CTLA4 inhibits IL-2 production and can induce cell cycle arrest (Krummel and Allison (1996) *J. Exp. Med.* 183:2533). In addition, mice that lack CTLA4 develop lymphoproliferative disease (Tivol *et al.* (1995) *Immunity* 3:541; Waterhouse *et al.* (1995) *Science* 270:985). The blockade of CTLA4 with antibodies may remove an inhibitory signal, whereas aggregation of CTLA4 with antibody transmits an inhibitory signal. Therefore, depending upon the receptor to which a costimulatory molecule binds (*i.e.*, a costimulatory receptor such as CD28 or an inhibitory receptor such as CTLA4), B7 molecules including B7-4 can promote T cell costimulation or inhibition.

PD-1 is a member of the immunoglobulin family of molecules (Ishida *et al.* (1992) *EMBO J.* 11:3887; Shinohara *et al.* (1994) *Genomics* 23:704). PD-1 was previously identified using a subtraction cloning based approach designed to identify modulators of programmed cell death (Ishida *et al.* (1992) *EMBO J.* 11:3887-95; Woronicz *et al.* (1995) *Curr. Top. Microbiol. Immunol.* 200:137). PD-1 is believed to play a role in regulating lymphocyte survival, *e.g.*, during clonal selection (Honjo (1992)

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Science 258:591; Agata *et al.* (1996) *Int. Immunology* 8:765; Nishimura *et al.* (1996) *Int. Immunology* 8:773). PD-1 has an extracellular region containing an immunoglobulin superfamily domain, a transmembrane domain, and an intracellular region which includes an immunoreceptor tyrosine kinase-based inhibitory motif (ITIM) (Ishida *et al.* (1992) *supra*; Shinohara *et al.* (1994) *supra*; US Patent 5,698,520). This features also define a larger family of molecules, called the immunoinhibitory receptors, which also includes gp49B, PIR-B, and the killer inhibitory receptors (KIRs) (Vivier and Daeron (1997) *Immunology Today* 18:286). It is often assumed that the tyrosyl phosphorylated ITIM motif of these receptors interacts with the SH2-domain-containing phosphatases, which leads to inhibitory signals. A subset of these immunoinhibitory receptors binds to MHC molecules, for example the KIRs, and CTLA4 binds to B7-1 and B7-2. It has been proposed that there is a phylogenetic relationship between the MHC and B7 genes (Henry *et al.* (1999) *Immunology Today* 20:285-288).

PD-1 was also implicated as a regulator of B cell responses (Nishimura (1998) *Int. Immunology* 10:1563). Unlike CTLA4, which is found only on T cells, PD-1 is also found on B cells (in response anti-IgM) and on a subset of thymocytes and myeloid cells (Agata *et al.* (1996) *supra*; Nishimura *et al.* (1996) *Int. Immunology* 8:773).

The importance of the B7:CD28/CTLA4 costimulatory pathway has been demonstrated *in vitro* and in several *in vivo* model systems. Blockade of this costimulatory pathway results in the development of antigen-specific tolerance in murine and human systems (Harding, F.A. *et al.* (1992) *Nature* 356:607-609; Lenschow, D.J. *et al.* (1992) *Science* 257:789-792; Turka, L.A. *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:11102-11105; Gimmi, C.D. *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6586-6590; Boussiotis, V. *et al.* (1993) *J. Exp. Med.* 178:1753-1763). Conversely, expression of B7 by B7-negative murine tumor cells induces T-cell mediated specific immunity accompanied by tumor rejection and long lasting protection to tumor challenge (Chen, L. *et al.* (1992) *Cell* 71:1093-1102; Townsend, S.E. and Allison, J.P. (1993) *Science* 259:368-370; Baskar, S. *et al.* (1993) *Proc. Natl. Acad. Sci* 90:5687-5690.). Therefore, manipulation of the costimulatory pathways offers great potential to stimulate or suppress immune responses in humans.

Summary of the Invention

The present invention is based, at least in part, on the discovery of novel nucleic acid molecules and polypeptides encoded by such nucleic acid molecules, referred to

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herein as PD-L2 nucleic acid and polypeptide molecules, which are members of the B7 family and are ligands for PD-1. Interaction of PD-L2 with PD-1 transmits a negative signal to immune cells, downregulating immune responses. Preferred PD-L2 molecules are expressed on the surface of professional antigen presenting cells (*e.g.*, B lymphocytes, monocytes, dendritic cells, and Langerhans cells) and other antigen presenting cells (*e.g.*, keratinocytes, endothelial cells, astrocytes, fibroblasts, and oligodendrocytes), down-regulate lymphocyte activation, and/or are bound by antibodies which recognize PD-L2 molecules. The PD-L2 nucleic acid and polypeptide molecules of the present invention are useful, *e.g.*, in modulating the immune response.

Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding PD-L2 polypeptides, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of PD-L2-encoding nucleic acids.

In one embodiment, a PD-L2 nucleic acid molecule of the invention is at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the nucleotide sequence (*e.g.*, to the entire length of the nucleotide sequence) shown in SEQ ID NO:1 or 3, or a complement thereof.

In a preferred embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown in SEQ ID NO:1 or 3, or a complement thereof. In another embodiment, the nucleic acid molecule includes the nucleic acid sequence shown in SEQ ID NO:3 and nucleotides 1-273 of SEQ ID NO:1. In a further embodiment, the nucleic acid molecule includes the nucleic acid sequence shown in SEQ ID NO:3 and nucleotides 1096-1223 of SEQ ID NO:1. In another preferred embodiment, the nucleic acid molecule consists of the nucleotide sequence shown in SEQ ID NO:1 or 3.

In another embodiment, a PD-L2 nucleic acid molecule includes a nucleotide sequence encoding a polypeptide having an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2. In a preferred embodiment, a PD-L2 nucleic acid molecule includes a nucleotide sequence encoding a polypeptide having an amino acid sequence at least about 71%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the entire length of the amino acid sequence of SEQ ID NO:2.

In another preferred embodiment, an isolated nucleic acid molecule encodes the amino acid sequence of human PD-L2. In yet another preferred embodiment, the nucleic acid molecule includes a nucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO:2. In yet another preferred embodiment, the nucleic acid molecule is at least about 50, 100, 150, 200, 250, 300, 350, 400, 450, 500,

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550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more nucleotides in length. In a further preferred embodiment, the nucleic acid molecule is at least about 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more nucleotides in length and encodes a polypeptide having a PD-L2 activity (as described herein).

Another embodiment of the invention features nucleic acid molecules, preferably PD-L2 nucleic acid molecules, which specifically detect PD-L2 nucleic acid molecules relative to nucleic acid molecules encoding non-PD-L2 polypeptides. For example, in one embodiment, such a nucleic acid molecule is at least about 880, 900, 950, 1000, 1050, 1100, 1150 or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, or a complement thereof. In another embodiment, such a nucleic acid molecule is at least 20, 30, 40, 50, 100, 150, 200, 250, 300 or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising nucleotides 1-358 of SEQ ID NO:1, or a complement thereof. In a further embodiment, such a nucleic acid molecule is at least 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more nucleotides in length, includes at least 15 (*i.e.*, 15 contiguous) nucleotides of the sequence comprising nucleotides 1-358 of SEQ ID NO:1, or a complement thereof, and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, or a complement thereof.

In preferred embodiments, the nucleic acid molecules are at least about 880 nucleotides in length and hybridize under stringent conditions to the nucleotide molecule set forth in SEQ ID NO:1 (*i.e.*, to 880 contiguous nucleotides of SEQ ID NO:1), or a complement thereof. In other preferred embodiments, the nucleic acid molecules are at least about 15 nucleotides in length and hybridize under stringent conditions to nucleotides 1-358 of the nucleotide molecule set forth in SEQ ID NO:1 (*i.e.*, to 15 contiguous nucleotides of nucleotides 1-358 of SEQ ID NO:1), or a complement thereof. In further preferred embodiments, the nucleic acid molecules are at least 15 nucleotides in length, include at least 15 (*i.e.*, 15 contiguous) nucleotides of the sequence comprising nucleotides 1-358 of SEQ ID NO:1, or a complement thereof, and hybridize under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, or a complement thereof.

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In still other preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the nucleic acid molecule hybridizes to a complement of a nucleic acid molecule comprising SEQ ID NO:1 or 3, or a complement thereof, under stringent conditions.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to a PD-L2 nucleic acid molecule, *e.g.*, is antisense to the coding strand of a PD-L2 nucleic acid molecule as shown in SEQ ID NO:1 or 3.

Another aspect of the invention provides a vector comprising a PD-L2 nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. In yet another embodiment, the invention provides a host cell containing a nucleic acid molecule of the invention. The invention also provides a method for producing a polypeptide, preferably a PD-L2 polypeptide, by culturing in a suitable medium, a host cell, *e.g.*, a mammalian host cell such as a non-human mammalian cell, of the invention containing a recombinant expression vector, such that the polypeptide is produced.

Another aspect of this invention features isolated or recombinant PD-L2 polypeptides (*e.g.*, proteins, polypeptides, peptides, or fragments or portions thereof). In one embodiment, an isolated PD-L2 polypeptide includes at least one or more of the following domains: a signal peptide domain, an IgV domain, an IgC domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain.

In a preferred embodiment, a PD-L2 polypeptide includes at least one or more of the following domains: a signal peptide domain, an IgV domain, an IgC domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain, and has an amino acid sequence at least about 71%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO:2. In another preferred embodiment, a PD-L2 polypeptide includes at least one or more of the following domains: a signal peptide domain, an IgV domain, an IgC domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain, and has a PD-L2 activity (as described herein).

In yet another preferred embodiment, a PD-L2 polypeptide includes at least one or more of the following domains: a signal peptide domain, an IgV domain, an IgC domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain,

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and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3.

In another embodiment, the invention features fragments or portions of the polypeptide having the amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 15 amino acids (*i.e.*, contiguous amino acids) of the amino acid sequence of SEQ ID NO:2. In another embodiment, a PD-L2 polypeptide comprises or consists of the amino acid sequence of SEQ ID NO:2.

In another embodiment, the invention features a PD-L2 polypeptide which is encoded by a nucleic acid molecule consisting of a nucleotide sequence at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a nucleotide sequence of SEQ ID NO:1 or 3, or a complement thereof. This invention further features a PD-L2 polypeptide which is encoded by a nucleic acid molecule consisting of a nucleotide sequence which hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3.

The polypeptides of the present invention or portions thereof, *e.g.*, biologically active portions thereof, can be operatively linked to a non-PD-L2 polypeptide (*e.g.*, heterologous amino acid sequences) to form fusion polypeptides. The invention further features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind polypeptides of the invention, preferably PD-L2 polypeptides. In addition, the PD-L2 polypeptides (or biologically active portions thereof) or modulators of the PD-L2 molecules can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of a PD-L2 nucleic acid molecule, protein, or polypeptide in a biological sample by contacting the biological sample with an agent capable of detecting a PD-L2 nucleic acid molecule, protein, or polypeptide, such that the presence of a PD-L2 nucleic acid molecule, protein or polypeptide is detected in the biological sample.

In another aspect, the present invention provides a method for detecting the presence of PD-L2 activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of PD-L2 activity, such that the presence of PD-L2 activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating PD-L2 activity, comprising contacting a cell capable of expressing PD-L2 with an agent that modulates PD-L2 activity, such that PD-L2 activity in the cell is modulated. In one embodiment, the agent inhibits PD-L2 activity. In another embodiment, the agent stimulates PD-L2 activity. In a further embodiment, the agent interferes with or enhances the interaction between a PD-L2 polypeptide and its natural binding partner(s), *e.g.*, PD-1. In a preferred embodiment, the binding partner is PD-1. In one embodiment, the agent is an antibody that specifically binds to a PD-L2 polypeptide. In a further embodiment, the agent is a combination of an antibody that specifically binds to a PD-L2 polypeptide and an antibody that specifically binds to a PD-L1 polypeptide. In another embodiment, the agent is a peptide, peptidomimetic, or other small molecule that binds to a PD-L2 polypeptide. In yet another embodiment, the agent is another PD-L1 ligand which can modulate the interaction between PD-L2 and PD-1. In still another embodiment, the agent modulates expression of PD-L2 by modulating transcription of a PD-L2 gene, translation of a PD-L2 mRNA, or post-translational modification of a PD-L2 polypeptide. In another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of a PD-L2 mRNA or a PD-L2 gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder or condition characterized by aberrant, insufficient, or unwanted PD-L2 polypeptide or nucleic acid expression or activity by administering an agent which is a PD-L2 modulator to the subject. In one embodiment, the PD-L2 modulator is a PD-L2 polypeptide. In another embodiment the PD-L2 modulator is a PD-L2 nucleic acid molecule. In a further embodiment, the PD-L2 modulator is an antibody that specifically binds to a PD-L2 polypeptide. In another embodiment, the PD-L2 modulator is a combination of an antibody that specifically binds to a PD-L2 polypeptide and an antibody that specifically binds to a PD-L1 polypeptide. In yet another embodiment, the PD-L2 modulator is a peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder or condition characterized by aberrant, insufficient, or unwanted PD-L2 polypeptide or nucleic acid expression or activity is an immune response disorder or condition that would benefit from modulation of PD-L2 activity. In another embodiment, the invention further provides treating the subject with an additional agent that modulates an immune response.

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In still another embodiment, the invention provides a vaccine comprising an antigen and an agent that reduces or inhibits PD-L2 activity. In a preferred embodiment, the vaccine inhibits the interaction between PD-L2 and its natural binding partner(s). In a more preferred embodiment, the binding partner is PD-1.

5 The present invention also provides diagnostic assays for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a PD-L2 polypeptide; (ii) mis-regulation of the gene; and (iii) aberrant post-translational modification of a PD-L2 polypeptide, wherein a wild-type form of the gene encodes a polypeptide with a PD-L2 activity.

10 In another aspect the invention provides methods for identifying a compound that binds to or modulates the activity of a PD-L2 polypeptide, by providing an indicator composition comprising a PD-L2 polypeptide having PD-L2 activity, contacting the indicator composition with a test compound, and determining the effect of the test compound on PD-L2 activity in the indicator composition to identify a compound that
15 modulates the activity of a PD-L2 polypeptide.

In another aspect, this invention provides a method for modulating an immune response by modulating the interaction between PD-1 and PD-L2.

In one aspect, the invention features a method for modulating the interaction of PD-L2 with its natural binding partner(s) on an immune cell comprising contacting an
20 antigen presenting cell which expresses PD-L2 with an agent selected from the group consisting of: a form of PD-L2, a form of PD-1, or an agent that modulates the interaction of PD-L2 and its natural binding partner(s) such that the interaction of PD-L2 with its natural binding partner(s) on an immune cell is modulated. In a preferred embodiment, an agent that modulates the interaction of PD-L2 and its natural binding
25 partner(s) (*e.g.*, PD-1) is an antibody that specifically binds to PD-L2. In another preferred embodiment, the agent is a combination of an antibody that specifically binds to PD-L2 and an antibody that specifically binds to PD-L1.

In one embodiment, the interaction of PD-L2 with its natural binding partner(s) is upregulated. In another embodiment, the interaction of PD-L2 with its natural binding
30 partner(s) is downregulated.

In one embodiment, the method further comprises contacting the immune cell or the antigen presenting cell with an additional agent that modulates an immune response.

In one embodiment, the step of contacting is performed *in vitro*. In another embodiment, the step of contacting is performed *in vivo*.

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In one embodiment, the immune cell is selected from the group consisting of: a T cell, a B cell, and a myeloid cell.

In one embodiment, the PD-L2 binding partner is PD-1.

5 In another aspect, the invention pertains to a method for inhibiting activation in an immune cell via a non-apoptotic mechanism comprising increasing the activity or expression of PD-L2 in a cell such that immune cell activation is inhibited.

In yet another aspect, the invention pertains to a vaccine comprising an antigen and an agent that inhibits the interaction between PD-L2 and its natural binding partner(s).

10 In still another aspect, the invention pertains to a vaccine comprising an antigen and an agent that promotes the interaction between PD-L2 and its natural binding partner(s).

In one embodiment, the PD-L2 binding partner is PD-1.

15 In another aspect, the invention pertains to a method for treating a subject having a condition that would benefit from upregulation of an immune response comprising administering an agent that inhibits the interaction between PD-L2 and its natural binding partner(s) on cells of the subject such that a condition that would benefit from upregulation of an immune response is treated.

20 In one embodiment, the agent comprises a blocking antibody or a small molecule that binds to PD-L2 and inhibits the interaction between PD-L2 and its natural binding partner(s). In another embodiment, the agent comprises a combination of an antibody that specifically binds to PD-L2 and an antibody that specifically binds to PD-L1.

In another embodiment, the method further comprises administering a second agent that upregulates an immune response to the subject.

25 In one embodiment, the condition is selected from the group consisting of: a tumor, a pathogenic infection, or an immunosuppressive disease.

In another embodiment, the PD-L2 binding partner is PD-1.

30 In one aspect, the invention pertains to a method for treating a subject having a condition that would benefit from downregulation of an immune response comprising administering an agent that stimulates the interaction between PD-L2 and its natural binding partner(s) on cells of the subject such that a condition that would benefit from downregulation of an immune response is treated.

In one embodiment agent comprises an antibody or a small molecule that stimulates the interaction between PD-L2 and its natural binding partner(s).

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In another embodiment, the method further comprises administering a second agent that downregulates an immune response to the subject.

In another embodiment, the condition is selected from the group consisting of: a transplant, an allergy, and an autoimmune disorder.

5 In one embodiment, the PD-L2 binding partner is PD-1.

In another aspect, the invention pertains to a cell-based assay for screening for compounds which modulate the activity of PD-L2 comprising contacting a cell expressing a PD-L2 target molecule with a test compound and determining the ability of the test compound to modulate the activity of the PD-L2 target molecule

10 In still another aspect, the invention pertains to a cell-free assay for screening for compounds which modulate the binding of PD-L2 to a target molecule comprising contacting a PD-L2 polypeptide or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the PD-L2 polypeptide or biologically active portion thereof.

15 In another embodiment, the invention pertains to a method of identifying a compound which modulates T cell activation at a first and second antigen concentration comprising contacting a T cell expressing a PD-L2 target molecule with a test compound at a first antigen concentration, determining the ability of the test compound to modulate T cell proliferation or cytokine production at the first antigen concentration, contacting a T cell expressing a PD-L2 target molecule with the test compound at a
20 second antigen concentration, and determining the ability of the test compound to modulate T cell proliferation or cytokine production at the second antigen concentration, thereby identifying a compound which modulates T cell activation at a first and second antigen concentration.

25 In one embodiment, the PD-L2 target molecule is PD-1.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

30 *Figure 1* depicts the cDNA sequence and predicted amino acid sequence of human PD-L2. The nucleotide sequence corresponds to nucleic acids 1-1223 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1-273 of SEQ ID NO:2. The coding region without the 5' or 3' untranslated regions of the human PD-L2 gene is shown in SEQ ID NO:3.

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Figure 2 depicts the cDNA sequence and amino acid sequence of mouse PD-L2. The nucleotide sequence corresponds to nucleic acids 1-1655 of SEQ ID NO:4. The amino acid sequence corresponds to amino acids 1-247 of SEQ ID NO:5. The coding region without the 5' or 3' untranslated region of the mouse PD-L2 gene is shown in

5 SEQ ID NO:6.

Figure 3 depicts the amino acid sequences of the human and mouse PD-L2 polypeptides (SEQ ID NO:2 and SEQ ID NO:5, respectively) and illustrates the signal peptide, IgV, IgC, extracellular, transmembrane, and cytoplasmic domains.

Figure 4 illustrates the results of FACS analysis of the binding of IgG2a (control Ig), ICOS-IgG, and control PD-1-Ig to COS cells transfected with mouse PD-L2 or with a control mouse PD-1 ligand.

Figure 5 depicts an alignment of the amino acid sequences of the mouse and human PD-L2 polypeptides (SEQ ID NO:5 and SEQ ID NO:2, respectively). Identical amino acids are illustrated between the two sequences.

15 *Figure 6* depicts an alignment of the amino acid sequences of the mouse PD-L2 (SEQ ID NO:5), human PD-L2 (SEQ ID NO:2), mouse PD-L1 (SEQ ID NO:11), and human PD-L1 (SEQ ID NO:12).

Figure 7 depicts the binding of PD-1 to PD-L2. CHO cells expressing I-A^d alone or I-A^d and B7-2 were either not transfected or stably transfected with mouse PD-L2. Cells were stained with hPD-1-Ig and stained with PE-goat anti-mouse IgG2a (thick line). CHO cells were stained separately using PE-anti-I-A^d or PE-anti-B7-2 (thick line). The thin lines indicate staining with isotype control monoclonal antibody. Ten-thousand events were analyzed.

Figure 8 depicts inhibition of TCR-mediated responses by PD-L2-PD-1 interaction. Purified T cells from BALB/c lymph nodes were stimulated at a 2:1 bead:cell ratio with tosyl beads coated with anti-CD3 + control Ig or anti-CD3 + mPD-L2-Ig. Proliferation was measured after 72 hours. These data are representative of more than eight independent experiments.

25 *Figure 9* depicts inhibition of TCR-mediated responses by PD-L2-PD-1 interaction. Splenocytes from DO11.10 transgenic mice were activated with OVA peptide (1 µg/ml). CD4⁺ T cells were isolated and rested overnight. Previously activated T cells (10⁵) were restimulated with peptide (1 or 0.1 µg/ml) presented by CHO-I-A^d or CHO-I-A^d-PD-L2 for 48 hours. [³H]thymidine incorporation was measured in triplicate. Aliquots of supernatants were collected at 36 hours after

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initiation of cultures and cytokines measured by ELISA. These data are representative of four independent experiments.

Figure 10 depicts inhibition of TCR and CD28 mediated responses by PD-L2-PD-1 interaction. Splenocytes from DO11.10 transgenic mice were activated with OVA peptide (1 µg/ml) for 4 days. CD4⁺ T cells were isolated and rested overnight. Previously activated T cells (10⁵) were restimulated, with varying peptide concentrations, by the indicated CHO transfectants for 48 hours. [³H]thymidine incorporation was measured in triplicate. These data are representative of six independent experiments.

Figure 11 depicts inhibition of TCR and CD28 mediated responses by PD-L2-PD-1 interaction. Previously activated T cells (10⁵) were cultured with 0.01 µg/ml OVA peptide presented by the indicated CHO transfectants. Aliquots of supernatants were collected at 36 hours after initiation of cultures and cytokines measured by ELISA. The broken line indicates the sensitivity of the ELISA.

Figure 12 depicts inhibition of TCR and CD28 mediated responses by PD-L2-PD-1 interaction. Previously activated T cells (10⁵) were cultured with 0.1 µg/ml OVA peptide presented by the indicated CHO transfectants. Aliquots of supernatants were collected at 36 hours after initiation of cultures and cytokines measured by ELISA. The broken line indicates the sensitivity of the ELISA.

Figure 13 depicts cell cycle arrest and apoptosis as a result of the engagement of the PD-L2-PD-1 pathway. Previously activated T cells were restimulated with OVA peptide (1 µg/ml) and the indicated CHO transfectants. Cells were collected after 36 hours of culture, stained with anti-CD4 and fixed in 70% ethanol. Cells were resuspended in propidium iodide solution. FACS profiles are propidium iodide staining of the CD4⁺ population. Subdiploid, diploid, and supradiploid populations are indicated. Ten-thousand events were collected and analyzed at a constant flow rate. These data are representative of three independent experiments.

Figure 14 depicts cell cycle arrest and apoptosis as a result of the engagement of the PD-L2-PD-1 pathway. Previously activated T cells were restimulated with OVA peptide (0.01 µg/ml) and the indicated CHO transfectants. Cells were collected after 36 hours of culture, stained with anti-CD4 and fixed in 70% ethanol. Cells were resuspended in propidium iodide solution. FACS profiles are propidium iodide staining of the CD4⁺ population. Subdiploid, diploid, and supradiploid populations are

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indicated. Ten-thousand events were collected and analyzed at a constant flow rate. These data are representative of three independent experiments.

Figure 15 depicts the enhancement of T cell proliferation in the presence of anti-PD-L1 or anti PD-L2 antibodies. Allogeneic CD4+ T cells were stimulated in a mixed lymphocyte reaction by IL-10 treated dendritic cells.

Figure 16 depicts the enhancement of T cell proliferation in the presence of anti-PD-L1, anti PD-L2 antibodies, or a combination of anti-PD-L1 and anti-PD-L2 antibodies. Allogeneic CD4+ T cells were stimulated in a mixed lymphocyte reaction by IL-10 treated dendritic cells.

10

Detailed Description of the Invention

In addition to the previously characterized B lymphocyte activation antigens, *e.g.*, B7-1 and B7-2, there are other antigens on the surface of antigen-presenting cells (*e.g.*, B cells, monocytes, dendritic cell, Langerhans cells, keratinocytes, endothelial cells, astrocytes, fibroblasts, and oligodendrocytes) which modulate the activation of B cells, T cells, and other immune cells. The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as PD-L2 polypeptides, which bind to the PD-1 receptor and down-regulate the activation of these immune cells and/or to downregulate immune responses. These novel molecules play a role in the modulating the immune response.

The instant discovery that PD-L2 binds to PD-1 places PD-L2 in a family of inhibitory ligands, and sequence analysis places PD-L2 in the B7 family. While engagement of a costimulatory receptor results in a costimulatory signal in an immune cell, engagement of an inhibitory receptor, *e.g.*, CTLA4 or PD-1 (for example by crosslinking or by aggregation), leads to the transmission of an inhibitory signal in an immune cell resulting in downmodulation of immune cell responses and/or in immune cell anergy. While transmission of an inhibitory signal leads to downmodulation in immune cell responses (and a resulting downmodulation in the overall immune response), the prevention of an inhibitory signal (*e.g.*, by using a non-activating antibody against PD-1) in immune cells leads to upmodulation of immune cell responses (and a resulting upmodulation of an immune response).

PD-L2 has homology to PD-L1, a previously described ligand for PD-1 (see the co-pending U.S. Application Serial No. 09/644,934; International Publication WO 01/14557; Dong, H. *et al.* (1999) *Nat. Med.* 5:1365-1369; and Freeman, G.J. *et al.*

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(2000) *J. Exp. Med.* 192:1027-1034 the contents of each of which are incorporated herein by reference).

The instant invention makes available agents useful for modulating the activity and/or expression of PD-L2; agents for modulating the interaction between PD-L2 and its natural binding partner(s) (*e.g.*, PD-1), and agents for modulating the immune response via modulation of the interaction between PD-L2 and its natural binding partner(s) (*e.g.*, PD-1). Exemplary modulatory agents for use in these methods are described further as follows.

10 Definitions

As used herein, the term "immune cell" includes cells that are of hematopoietic origin and that play a role in the immune response. Immune cells include lymphocytes, such as B cells and T cells; natural killer cells; and myeloid cells, such as monocytes, macrophages, eosinophils, mast cells, basophils, and granulocytes.

15 As used herein, the term "T cell" includes CD4+ T cells and CD8+ T cells. The term T cell also includes both T helper 1 type T cells and T helper 2 type T cells. The term "antigen presenting cell" includes professional antigen presenting cells (*e.g.*, B lymphocytes, monocytes, dendritic cells, and Langerhans cells) as well as other antigen presenting cells (*e.g.*, keratinocytes, endothelial cells, astrocytes, fibroblasts, and
20 oligodendrocytes).

As used herein, the term "immune response" includes T cell-mediated and/or B cell-mediated immune responses that are influenced by modulation of T cell costimulation. Exemplary immune responses include B cell responses (*e.g.*, antibody production) T cell responses (*e.g.*, cytokine production, and cellular cytotoxicity) and
25 activation of cytokine responsive cells, *e.g.*, macrophages. As used herein, the term "downmodulation" with reference to the immune response includes a diminution in any one or more immune responses, while the term "upmodulation" with reference to the immune response includes an increase in any one or more immune responses. It will be understood that upmodulation of one type of immune response may lead to a
30 corresponding downmodulation in another type of immune response. For example, upmodulation of the production of certain cytokines (*e.g.*, IL-10) can lead to downmodulation of cellular immune responses.

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As used herein, the term "costimulatory receptor" includes receptors which transmit a costimulatory signal to an immune cell, *e.g.*, CD28 or ICOS. As used herein, the term "inhibitory receptors" includes receptors which transmit a negative signal to an immune cell (*e.g.*, CTLA4 or PD-1).

5 As used herein, the term "costimulate", with reference to activated immune cells, includes the ability of a costimulatory molecule to provide a second, non-activating, receptor-mediated signal (a "costimulatory signal") that induces proliferation or effector function. For example, a costimulatory signal can result in cytokine secretion, *e.g.*, in a T cell that has received a T cell-receptor-mediated signal. Immune cells that have
10 received a cell receptor-mediated signal, *e.g.*, via an activating receptor, are referred to herein as "activated immune cells."

An inhibitory signal as transduced by an inhibitory receptor can occur even if a costimulatory receptor (such as CD28 or ICOS) is not present on the immune cell and, thus, is not simply a function of competition between inhibitory receptors and
15 costimulatory receptors for binding of costimulatory molecules (Fallarino *et al.* (1998) *J. Exp. Med.* 188:205). Transmission of an inhibitory signal to an immune cell can result in unresponsiveness, anergy or programmed cell death in the immune cell. Preferably, transmission of an inhibitory signal operates through a mechanism that does not involve apoptosis. As used herein the term "apoptosis" includes programmed cell
20 death which can be characterized using techniques which are known in the art. Apoptotic cell death can be characterized, *e.g.*, by cell shrinkage, membrane blebbing, and chromatin condensation culminating in cell fragmentation. Cells undergoing apoptosis also display a characteristic pattern of internucleosomal DNA cleavage.

Depending upon the form of the PD-L2 molecule that binds to a receptor, a
25 signal can be either transmitted (*e.g.*, by a multivalent form of a PD-L2 molecule that results in crosslinking of the receptor or by a soluble form of PD-L2 that binds to Fc receptors on antigen presenting cells) or inhibited (*e.g.*, by a soluble, monovalent form of a PD-L2 molecule or a soluble form of PD-L2 that is altered using methods known in the art such that it does not bind to Fc receptors on antigen presenting cells), *e.g.*, by
30 competing with activating forms of PD-L2 molecules for binding to the receptor. However, there are instances in which a soluble molecule can be stimulatory. The effects of the various modulatory agents can be easily demonstrated using routine screening assays as described herein.

As used herein, the term "activating receptor" includes immune cell receptors that bind antigen, complexed antigen (*e.g.*, in the context of MHC molecules), or antibodies. Such activating receptors include T cell receptors (TCRs), B cell receptors (BCRs), cytokine receptors, LPS receptors, complement receptors, and Fc receptors.

For example, T cell receptors are present on T cells and are associated with CD3 molecules. T cell receptors are stimulated by antigen in the context of MHC molecules (as well as by polyclonal T cell activating reagents). T cell activation via the TCR results in numerous changes, *e.g.*, protein phosphorylation, membrane lipid changes, ion fluxes, cyclic nucleotide alterations, RNA transcription changes, protein synthesis changes, and cell volume changes.

The term "B cell receptor" (BCR) as used herein includes the complex between membrane Ig (mIg) and other transmembrane polypeptides (*e.g.*, Ig α and Ig β) found on B cells. The signal transduction function of mIg is triggered by crosslinking of receptor molecules by oligomeric or multimeric antigens. B cells can also be activated by anti-immunoglobulin antibodies. Upon BCR activation, numerous changes occur in B cells, including tyrosine phosphorylation.

The term "Fc receptor" (FcRs) include cell surface receptors for the Fc portion of immunoglobulin molecules (Igs). Fc receptors are found on many cells which participate in immune responses. Among the human FcRs that have been identified so far are those which recognize IgG (designated Fc γ R), IgE (Fc ϵ R1), IgA (Fc α R), and polymerized IgM/A (Fc $\mu\alpha$ R). FcRs are found in the following cell types: Fc ϵ R I (mast cells), Fc ϵ R II (many leukocytes), Fc α R (neutrophils), and Fc $\mu\alpha$ R (glandular epithelium, hepatocytes) (Hogg, N. (1988) *Immunol. Today* 9:185-86). The widely studied Fc γ Rs are central in cellular immune defenses, and are responsible for stimulating the release of mediators of inflammation and hydrolytic enzymes involved in the pathogenesis of autoimmune disease (Unkeless, J.C. (1988) *Annu. Rev. Immunol.* 6:251-87). The Fc γ Rs provide a crucial link between effector cells and the lymphocytes that secrete Ig, since the macrophage/monocyte, polymorphonuclear leukocyte, and natural killer (NK) cell Fc γ Rs confer an element of specific recognition mediated by IgG. Human leukocytes have at least three different receptors for IgG: hFc γ RI (found on monocytes/macrophages), hFc γ RII (on monocytes, neutrophils, eosinophils, platelets, possibly B cells, and the K562 cell line), and Fc γ III (on NK cells, neutrophils, eosinophils, and macrophages).

With respect to T cells, transmission of a costimulatory signal to a T cell involves a signaling pathway that is not inhibited by cyclosporin A. In addition, a costimulatory signal can induce cytokine secretion (*e.g.*, IL-2 and/or IL-10) in a T cell and/or can prevent the induction of unresponsiveness to antigen, the induction of anergy, or the induction of cell death in the T cell.

As used herein, the term "inhibitory signal" refers to a signal transmitted via an inhibitory receptor (*e.g.*, CTLA4 or PD-1) molecule on an immune cell. Such a signal antagonizes a signal via an activating receptor (*e.g.*, via a TCR, CD3, BCR, or Fc molecule) and can result, *e.g.*, in inhibition of: second messenger generation; proliferation; or effector function in the immune cell, *e.g.*, reduced phagocytosis, antibody production, or cellular cytotoxicity, or the failure of the immune cell to produce mediators (such as cytokines (*e.g.*, IL-2) and/or mediators of allergic responses); or the development of anergy.

As used herein, the term "unresponsiveness" includes refractivity of immune cells to stimulation, *e.g.*, stimulation via an activating receptor or a cytokine. Unresponsiveness can occur, *e.g.*, because of exposure to immunosuppressants or high doses of antigen. As used herein, the term "anergy" or "tolerance" includes refractivity to activating receptor-mediated stimulation. Such refractivity is generally antigen-specific and persists after exposure to the tolerizing antigen has ceased. For example, anergy in T cells (as opposed to unresponsiveness) is characterized by lack of cytokine production, *e.g.*, IL-2. T cell anergy occurs when T cells are exposed to antigen and receive a first signal (a T cell receptor or CD-3 mediated signal) in the absence of a second signal (a costimulatory signal). Under these conditions, reexposure of the cells to the same antigen (even if reexposure occurs in the presence of a costimulatory molecule) results in failure to produce cytokines and, thus, failure to proliferate. Anergic T cells can, however, mount responses to unrelated antigens and can proliferate if cultured with cytokines (*e.g.*, IL-2). For example, T cell anergy can also be observed by the lack of IL-2 production by T lymphocytes as measured by ELISA or by a proliferation assay using an indicator cell line. Alternatively, a reporter gene construct can be used. For example, anergic T cells fail to initiate IL-2 gene transcription induced by a heterologous promoter under the control of the 5' IL-2 gene enhancer or by a multimer of the AP1 sequence that can be found within the enhancer (Kang *et al.* (1992) *Science* 257:1134).

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Modulation of a costimulatory signal results in modulation of effector function of an immune cell. Thus, the term "PD-L2 activity" includes the ability of a PD-L2 polypeptide to bind its natural binding partner(s), *e.g.*, PD-1, the ability to modulate immune cell costimulatory or inhibitory signals, and the ability to modulate the immune response.

5 With respect to PD-1, the term "activity" includes the ability of a PD-1 polypeptide to modulate an inhibitory signal in an activated immune cell, *e.g.*, by engaging a natural ligand on an antigen presenting cell. PD-1 transmits an inhibitory signal to an immune cell in a manner similar to CTLA4. Modulation of an inhibitory
10 signal in an immune cell results in modulation of proliferation of and/or cytokine secretion by an immune cell. PD-1 can also modulate a costimulatory signal by competing with a costimulatory receptor for binding of its natural ligand(s). Thus, the term "PD-1 activity" includes the ability of a PD-1 polypeptide to bind its natural ligand(s), the ability to modulate immune cell costimulatory or inhibitory signals, and
15 the ability to modulate the immune response.

As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

As used herein, an "antisense" nucleic acid molecule comprises a nucleotide
20 sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule, complementary to an mRNA sequence or complementary to the coding strand of a gene. Accordingly, an antisense nucleic acid molecule can hydrogen bond to a sense nucleic acid molecule.

25 As used herein, the term "coding region" refers to regions of a nucleotide sequence comprising codons which are translated into amino acid residues, whereas the term "noncoding region" refers to regions of a nucleotide sequence that are not translated into amino acids (*e.g.*, 5' and 3' untranslated regions).

As used herein, the term "vector" refers to a nucleic acid molecule capable of
30 transporting another nucleic acid molecule to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are

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introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of
5 directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “recombinant expression vectors” or simply “expression vectors”. In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, “plasmid” and “vector” may be used interchangeably as the plasmid is the most commonly used form of vector. However,
10 the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

As used herein, the term “host cell” is intended to refer to a cell into which a nucleic acid molecule of the invention, such as a recombinant expression vector of the
15 invention, has been introduced. The terms “host cell” and “recombinant host cell” are used interchangeably herein. It should be understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the
20 parent cell, but are still included within the scope of the term as used herein.

As used herein, a “transgenic animal” refers to a non-human animal, preferably a mammal, more preferably a mouse, in which one or more of the cells of the animal includes a “transgene”. The term “transgene” refers to exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which
25 remains in the genome of the mature animal, for example directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal.

As used herein, a “homologous recombinant animal” refers to a type of transgenic non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous gene has been altered by homologous recombination between the
30 endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

As used herein, an "isolated protein" refers to a protein that is substantially free of other proteins, cellular material and culture medium when isolated from cells or produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized.

5 An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the PD-L2 protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of PD-L2 protein in which
10 the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of PD-L2 protein having less than about 30% (by dry weight) of non- PD-L2 protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non- PD-L2 protein, still more
15 preferably less than about 10% of non- PD-L2 protein, and most preferably less than about 5% non- PD-L2 protein. When the PD-L2 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein
20 preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of PD-L2 protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one
25 embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of PD-L2 protein having less than about 30% (by dry weight) of chemical precursors or non- PD-L2 chemicals, more preferably less than about 20% chemical precursors or non- PD-L2 chemicals, still more preferably less than about 10% chemical precursors or non- PD-L2 chemicals, and most preferably less than about 5% chemical precursors or non- PD-L2 chemicals.

30 The term "antibody", as used herein, includes an "antigen-binding portion" of an antibody (or simply "antibody portion"), as well as whole antibody molecules. The term "antigen-binding portion", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, PD-L2). It has been shown that the antigen-binding function of an antibody can be performed by

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fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; (v) a dAb fragment (Ward *et al.* (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Osbourn *et al.* 1998 *Nat. Biotechnol.* 16:778). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Any VH and VL sequences of specific scFv can be linked to human immunoglobulin constant region cDNA or genomic sequences, in order to generate expression vectors encoding complete IgG molecules or other isotypes. VH and VL can also be used in the generation of Fab, Fv, or other fragments of immunoglobulins using either protein chemistry or recombinant DNA technology. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P. *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J. *et al.* (1994) *Structure* 2:1121-1123).

Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecules, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S.M. *et al.* (1995) *Hum. Antibodies Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S.M. *et al.* (1994) *Mol. Immunol.* 31:1047-1058). Antibody portions, such as Fab and F(ab')₂ fragments,

can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

5 Antibodies may be polyclonal or monoclonal; xenogeneic, allogeneic, or syngeneic; or modified forms thereof, *e.g.*, humanized, chimeric, etc. Preferably, antibodies of the invention bind specifically or substantially specifically to PD-L2 molecules. The terms "monoclonal antibodies" and "monoclonal antibody composition", as used herein, refer to a population of antibody molecules that contain
10 only one species of an antigen binding site capable of immunoreacting with a particular epitope of an antigen, whereas the term "polyclonal antibodies" and "polyclonal antibody composition" refer to a population of antibody molecules that contain multiple species of antigen binding sites capable of interacting with a particular antigen. A monoclonal antibody composition, typically displays a single binding affinity for a
15 particular antigen with which it immunoreacts.

 The term "humanized antibody", as used herein, is intended to include antibodies made by a non-human cell having variable and constant regions which have been altered to more closely resemble antibodies that would be made by a human cell. For example, by altering the non-human antibody amino acid sequence to incorporate amino acids
20 found in human germline immunoglobulin sequences. The humanized antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs. The term "humanized antibody", as used herein, also includes antibodies in which CDR
25 sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

 An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds PD-L2 is substantially free of antibodies that
30 specifically bind antigens other than PD-L2). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

PD-L2 Nucleic Acid and Polypeptide Molecules

The term "family" when referring to the polypeptide and nucleic acid molecules of the invention is intended to mean two or more polypeptide or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first polypeptide of human origin, as well as other, distinct polypeptides of human origin or alternatively, can contain homologues of non-human origin, *e.g.*, monkey polypeptides. Members of a family may also have common functional characteristics.

For example, the family of PD-L2 polypeptides of the present invention preferably comprises least one "signal peptide domain". As used herein, a "signal sequence" or "signal peptide" includes a peptide containing about 15 or more amino acids which occurs at the N-terminus of secretory and membrane bound polypeptides and which contains a large number of hydrophobic amino acid residues. For example, a signal sequence contains at least about 10-30 amino acid residues, preferably about 15-25 amino acid residues, more preferably about 18-20 amino acid residues, and even more preferably about 19 amino acid residues, and has at least about 35-65%, preferably about 38-50%, and more preferably about 40-45% hydrophobic amino acid residues (*e.g.*, Valine, Leucine, Isoleucine or Phenylalanine). Such a "signal sequence", also referred to in the art as a "signal peptide", serves to direct a polypeptide containing such a sequence to a lipid bilayer, and is cleaved in secreted and membrane bound polypeptides. A signal sequence was identified in the amino acid sequence of native human PD-L2 at about amino acids 1-19 of SEQ ID NO:2 (Figure 3). A signal sequence was also identified in the amino acid sequence of native mouse PD-L2 at about amino acids 1-19 of SEQ ID NO:5 (Figure 3).

In another embodiment of the invention, a PD-L2 polypeptide of the present invention is identified based on the presence of a "transmembrane domain". As used herein, the term "transmembrane domain" includes an amino acid sequence of about 15 amino acid residues in length which spans the plasma membrane. More preferably, a transmembrane domain includes about at least 20, 25, 30, 35, 40, or 45 amino acid residues and spans the plasma membrane. Transmembrane domains are rich in hydrophobic residues, and typically have an alpha-helical structure. In a preferred embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a

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transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, tyrosines, or tryptophans. Transmembrane domains are described in, for example, Zagotta, W.N. *et al.* (1996) *Annu. Rev. Neurosci.* 19:235-263, the contents of which are incorporated herein by reference. Amino acid residues 220-243 of the native human PD-L2 polypeptide, and amino acid residues 201-243 of the predicted mature polypeptide, are predicted to comprise transmembrane domains (see Figure 3). Amino acid residues 220-242 of the native mouse PD-L2 polypeptide, and amino acid residues 201-223 of the predicted mature polypeptide, are predicted to comprise transmembrane domains (see Figure 3). Accordingly, PD-L2 polypeptides having at least 71-80%, or more preferably about 80-90% homology with a transmembrane domain of human PD-L2 are within the scope of the invention.

In another embodiment, a PD-L2 molecule of the present invention is identified based on the presence of an "IgC domain" or an "IgV domain" in the polypeptide or corresponding nucleic acid molecule. As used herein, IgV and IgC domains are recognized in the art as Ig superfamily member domains. These domains correspond to structural units that have distinct folding patterns called Ig folds. Ig folds are comprised of a sandwich of two β sheets, each consisting of antiparallel β strands of 5-10 amino acids with a conserved disulfide bond between the two sheets in most, but not all, domains. IgC domains of Ig, TCR, and MHC molecules share the same types of sequence patterns and are called the C1 set within the Ig superfamily. Other IgC domains fall within other sets. IgV domains also share sequence patterns and are called V set domains. IgV domains are longer than C-domains and form an additional pair of β strands. Amino acid residues 20-120 of the native human PD-L2 polypeptide, and amino acid residues 1-101 of the predicted mature polypeptide, are predicted to comprise IgV domains (see Figure 3). Amino acid residues 20-120 of the native mouse PD-L2 polypeptide, and amino acid residues 1-101 of the predicted mature polypeptide, are also predicted to comprise IgV domains (see Figure 3). Amino acid residues 121-219 of the native human PD-L2 polypeptide, and amino acid residues 102-200 of the predicted mature polypeptide, are predicted to comprise IgC domains (see Figure 3). Amino acid residues 121-219 of the native mouse PD-L2 polypeptide, and amino acid residues 102-200 of the predicted mature polypeptide, are also predicted to comprise IgC domains (see Figure 3). In a preferred embodiment, the presence of an IgV domain is required for binding of PD-L2 to its natural binding partner, *e.g.*, PD-1.

In another embodiment, a PD-L2 molecule of the present invention is identified based on the presence of a "extracellular domain" in the polypeptide or corresponding nucleic acid molecule. As used herein, the term "extracellular domain" represents the N-terminal amino acids which extend as a tail from the surface of a cell. An
 5 extracellular domain of the present invention includes an IgV domain and an IgC domain, and may include a signal peptide domain. Amino acid residues 1-219 of the native human PD-L2 polypeptide, and amino acid residues 1-200 of the predicted mature polypeptide, are predicted to comprise extracellular domains (see Figure 3).
 10 Amino acid residues 1-219 of the native mouse PD-L2 polypeptide, and amino acid residues 1-200 of the predicted mature polypeptide, are also predicted to comprise extracellular domains (see Figure 3).

In still another embodiment, a PD-L2 molecule of the present invention is identified based on the presence of a "cytoplasmic domain" in the polypeptide or corresponding nucleic acid molecule. As used herein, the term "cytoplasmic domain"
 15 represents the C-terminal amino acids which extend as a tail into the cytoplasm of a cell. Amino acid residues 244-273 of the native human PD-L2 polypeptide, and amino acid residues 225-273 of the predicted mature polypeptide, are predicted to comprise cytoplasmic domains (see Figure 3). Amino acid residues 243-247 of the native mouse PD-L2 polypeptide, and amino acid residues 224-228 of the predicted mature
 20 polypeptide, are also predicted to comprise cytoplasmic domains.

In a preferred embodiment, the PD-L2 molecules of the invention include at least one or more of the following domains: a signal peptide domain, an IgV domain, an IgC domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain.

Isolated polypeptides of the present invention, preferably PD-L2 polypeptides,
 25 have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2, or are encoded by a nucleotide sequence sufficiently identical to SEQ ID NO:1 or 3. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid
 30 residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30%, 40%, or 50% homology, preferably 60% homology, more preferably 70%-80%, and even more

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preferably 90-95% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently identical. Furthermore, amino acid or nucleotide sequences which share at least 30%, 40%, or 50%, preferably 60%, more preferably 70-80%, or 90-95%

5 homology and share a common functional activity are defined herein as sufficiently identical.

As used interchangeably herein, "PD-L2 activity", "biological activity of PD-L2" or "functional activity of PD-L2", refers to an activity exerted by a PD-L2 protein, polypeptide or nucleic acid molecule on a PD-L2-responsive cell or tissue, or on a PD-L2 polypeptide binding partner, as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, a PD-L2 activity is a direct activity, such as an association with a PD-L2 binding partner. As used herein, a "target molecule" or "binding partner" is a molecule with which a PD-L2 polypeptide binds or interacts in nature, such that PD-L2-mediated function is achieved. In an exemplary embodiment, a PD-L2 target molecule is the receptor PD-1. Alternatively, a PD-L2 activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the PD-L2 polypeptide with its natural binding partner, *e.g.*, PD-1. The biological activities of PD-L2 are described herein. For example, the PD-L2 polypeptides of the present invention can have one or more of the following activities: 1) bind to and/or modulate the activity of the receptor PD-1 or other PD-L2 natural binding partners, 2) modulate intra- or intercellular signaling, 3) modulate activation of immune cells, *e.g.*, T lymphocytes, and 4) modulate the immune response of an organism, *e.g.*, a mouse or human organism.

Accordingly, another embodiment of the invention features isolated PD-L2 proteins and polypeptides having a PD-L2 activity. Other preferred polypeptides are PD-L2 polypeptides having one or more of the following domains: a signal peptide domain, an IgV domain, an IgC domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain, and, preferably, a PD-L2 activity.

Additional preferred PD-L2 polypeptides have at least one extracellular domain, and one or more of a signal peptide domain, an IgV domain, an IgC domain, an transmembrane domain, and a cytoplasmic domain, and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising a complement of the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6.

The nucleotide sequence of the isolated human PD-L2 cDNA and the predicted amino acid sequence of the human PD-L2 polypeptide are shown in Figure 1 and in SEQ ID NO:1 and 2, respectively. The nucleotide sequence of the isolated mouse PD-L2 cDNA and the amino acid sequence of the mouse PD-L2 polypeptide are shown in Figure 2 and in SEQ ID NO:4 and 5, respectively.

The human PD-L2 gene, which is approximately 1223 nucleotides in length, encodes a polypeptide having a molecular weight of approximately 30.0 kD and which is approximately 273 amino acid residues in length. The mouse PD-L2 gene, which is approximately 1655 nucleotides in length, encodes a polypeptide having a molecular weight of approximately 27.2 kD and which is approximately 247 amino acid residues in length.

Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode PD-L2 polypeptides or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify PD-L2-encoding nucleic acid molecules (*e.g.*, PD-L2 mRNA) and fragments for use as PCR primers for the amplification or mutation of PD-L2 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid molecule is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid molecule) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated PD-L2 nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb,

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2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid molecule is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium, when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1,3,4, or 6, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of SEQ ID NO:1, 3, 4, or 6 as a hybridization probe, PD-L2 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J. *et al. Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989*).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, 3, 4, or 6 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, 3, 4, or 6.

A nucleic acid molecule of the invention can be amplified using cDNA, mRNA or, alternatively, genomic DNA as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid molecule so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to PD-L2 nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6. This cDNA may comprise sequences encoding the human PD-L2 polypeptide (*i.e.*, "the coding region", from nucleotides 274-1092), as well as 5' untranslated sequences (nucleotides 1-273) and 3' untranslated sequences (nucleotides 1093-1223) of SEQ ID NO:1. Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1 (*i.e.*, nucleotides 274-1092, corresponding to SEQ ID NO:3). Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention comprises SEQ ID NO:3 and nucleotides 1-274 of SEQ ID NO:1. In yet another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:3 and nucleotides 1093-1223 of SEQ ID

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NO:1. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:1 or SEQ ID NO:3. In still another embodiment, the nucleic acid molecule can comprise the coding region of SEQ ID NO:1 (*e.g.*, nucleotides 274-1092, corresponding to SEQ ID NO:3), as well as a stop codon (*e.g.*, nucleotides 1093-1095 of SEQ ID NO:1).

This cDNA may also comprise sequences encoding the mouse PD-L2 polypeptide (*i.e.*, the coding region, from nucleotides 210-950), as well as 5' untranslated sequences (nucleotides 1-209) and 3' untranslated sequences (nucleotides 951-1655) of SEQ ID NO:4. Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:4 (*i.e.*, nucleotides 210-950, corresponding to SEQ ID NO:6). Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention comprises SEQ ID NO:6 and nucleotides 1-210 of SEQ ID NO:4. In yet another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:6 and nucleotides 951-1655 of SEQ ID NO:4. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:4 or SEQ ID NO:6. In still another embodiment, the nucleic acid molecule can comprise the coding region of SEQ ID NO:4 (*e.g.*, nucleotides 210-950, corresponding to SEQ ID NO:4), as well as a stop codon (*e.g.*, nucleotides 951-953 of SEQ ID NO:4).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6 such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, respectively, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the entire length of the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, or a portion of any of these nucleotide sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, 3, 4, or 6, for example, a fragment which can be used as a probe or primer or a fragment which encodes a portion

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of a PD-L2 polypeptide, *e.g.*, a biologically active portion of a PD-L2 polypeptide. The nucleotide sequences determined from the cloning of the human PD-L2 gene allow for the generation of probes and primers designed for use in identifying and/or cloning other PD-L2 family members, as well as PD-L2 homologues from other species. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, 3, 4, or 6; of an anti-sense sequence of SEQ ID NO:1, 3, 4, or 6; or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, 3, 4, or 6.

In one embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is greater than about 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1150 or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, 3, 4, or 6, or the complement thereof. In a further embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is greater than about 880-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1150 or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1 or 3, or the complement thereof. In yet another embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is greater than 50-100, 100-150, 150-200, 200-250, 250-300 or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising nucleotides 1-358 of SEQ ID NO:1, or a complement thereof. In yet a further embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is greater than about 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 850-900, 900-950, 950-1000-1050-1100, 1100-1150 or more nucleotides in length, includes at least about 15 (*i.e.*, 15 contiguous) nucleotides of the sequence comprising nucleotides 1-358 of SEQ ID NO:1, or a complement thereof, and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, or a complement thereof.

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Probes based on the PD-L2 nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous polypeptides. In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a PD-L2 polypeptide, such as by measuring a level of a PD-L2-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting PD-L2 mRNA levels or determining whether a genomic PD-L2 gene has been mutated or deleted.

10 A nucleic acid fragment encoding a "biologically active portion of a PD-L2 polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6 which encodes a polypeptide having a PD-L2 biological activity (*e.g.*, the ability to bind to its natural binding partner(s) (*e.g.*, PD-1), and/or modulate immune cell activity), expressing the encoded portion of the PD-L2 polypeptide (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the PD-L2 polypeptide.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6 due to degeneracy of the genetic code and thus encode the same PD-L2 polypeptides as those encoded by the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a polypeptide having an amino acid sequence shown in SEQ ID NO:2 or 5.

In addition to the PD-L2 nucleotide sequences shown in SEQ ID NO:1, 3, 4, or 6, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the PD-L2 polypeptides may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the PD-L2 genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a PD-L2 polypeptide, preferably a mammalian PD-L2 polypeptide, and can further include non-coding regulatory sequences, and introns.

Allelic variants of human or mouse PD-L2 include both functional and non-functional PD-L2 polypeptides. Functional allelic variants are naturally occurring amino acid sequence variants of the human or mouse PD-L2 polypeptide that maintain

the ability to bind natural PD-L2 binding partner(s), *e.g.*, PD-1, and/or modulate lymphocyte activation. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2 or 5, or substitution, deletion or insertion of non-critical residues in non-critical regions of the polypeptide.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human or mouse PD-L2 polypeptide that do not have the ability to either bind natural PD-L2 binding partners, *e.g.*, PD-1, and/or modulate any of the PD-L2 activities described herein. Non-functional allelic variants will typically contain a non-conservative substitution, deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2 or 5, or a substitution, insertion or deletion in critical residues or critical regions of the polypeptide, *e.g.*, in an IgV domain.

The present invention further provides non-human, non-mouse orthologues of the human or mouse PD-L2 polypeptide. Orthologues of the human or mouse PD-L2 polypeptide are polypeptides that are isolated from non-human, non-mouse organisms and possess the same PD-1-binding activity and/or lymphocyte activation-modulating activity of the PD-L2 polypeptide. Orthologues of the human or mouse PD-L2 polypeptide can readily be identified as comprising an amino acid sequence that is substantially identical to SEQ ID NO:2 or 5.

Moreover, nucleic acid molecules encoding other PD-L2 family members and, thus, which have a nucleotide sequence which differs from the PD-L2 sequences of SEQ ID NO:1, 3, 4, or 6 are intended to be within the scope of the invention. For example, another PD-L2 cDNA can be identified based on the nucleotide sequence of mouse or human PD-L2. Moreover, nucleic acid molecules encoding PD-L2 polypeptides from different species, and which, thus, have a nucleotide sequence which differs from the PD-L2 sequences of SEQ ID NO:1, 3, 4, or 6 are intended to be within the scope of the invention. For example, a monkey PD-L2 cDNA can be identified based on the nucleotide sequence of the mouse or human PD-L2.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the PD-L2 cDNAs of the invention can be isolated based on their homology to the PD-L2 nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Nucleic acid molecules corresponding to natural

allelic variants and homologues of the PD-L2 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the PD-L2 gene.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under
5 stringent conditions to the nucleic acid molecule comprising nucleotides 1-358 of the nucleotide sequence of SEQ ID NO:1, or nucleotides 1-85 of SEQ ID NO:3. In other embodiment, the nucleic acid is at least 880-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1150 or more nucleotides in length.

As used herein, the term "hybridizes under stringent conditions" is intended to
10 describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to
15 those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions
20 includes hybridization in 4X or 6X sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70°C. A further preferred, non-limiting example of stringent hybridization conditions includes hybridization at 6X SSC at 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. A preferred, non-
25 limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X or 6X SSC, at about 50-60°C (or alternatively hybridization in 6X
30 SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is 0.15M NaCl, 10mM NaH₂PO₄, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the

hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, $T_m(^{\circ}\text{C}) = 2(\# \text{ of A + T bases}) + 4(\# \text{ of G + C bases})$. For hybrids between 18 and 49 base pairs in length, $T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log_{10}[\text{Na}^+]) + 0.41(\% \text{G+C}) - (600/\text{N})$, where N is the number of bases in the hybrid, and $[\text{Na}^+]$ is the concentration of sodium ions in the hybridization buffer ($[\text{Na}^+]$ for 1xSSC = 0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (e.g., BSA or salmon or herring sperm carrier DNA), detergents (e.g., SDS), chelating agents (e.g., EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M NaH_2PO_4 , 7% SDS at about 65°C, followed by one or more washes at 0.02M NaH_2PO_4 , 1% SDS at 65°C, see e.g., Church and Gilbert (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995 (or alternatively 0.2X SSC, 1% SDS).

Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, 3, 4, or 6 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (i.e., encodes a natural polypeptide).

In addition to naturally-occurring allelic variants of the PD-L2 sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, 3, 4, or 6, thereby leading to changes in the amino acid sequence of the encoded PD-L2 polypeptides, without altering the functional ability of the PD-L2 polypeptides. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, 3, 4, or 6. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of PD-L2 (e.g., the sequence of SEQ ID NO:2 or 5) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the PD-L2 polypeptides of the present invention,

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e.g., those present in an extracellular domain, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the PD-L2 polypeptides of the present invention and other members of the PD-L2 family are not likely to be amenable to alteration.

5 Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding PD-L2 polypeptides that contain changes in amino acid residues that are not essential for activity. Such PD-L2 polypeptides differ in amino acid sequence from SEQ ID NO:2 or 5, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a polypeptide, wherein the
10 polypeptide comprises an amino acid sequence at least about 71%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2 or 5.

An isolated nucleic acid molecule encoding a PD-L2 polypeptide identical to the polypeptide of SEQ ID NO:2 or 5 can be created by introducing one or more nucleotide
15 substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded polypeptide. Mutations can be introduced into SEQ ID NO:1, 3, 4, or 6 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more
20 predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar
25 side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a PD-L2 polypeptide is preferably replaced
30 with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a PD-L2 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for PD-L2 biological activity to identify mutants that retain activity. Following

mutagenesis of SEQ ID NO:1, 3, 4, or 6, the encoded polypeptide can be expressed recombinantly and the activity of the polypeptide can be determined.

In a preferred embodiment, a mutant PD-L2 polypeptide can be assayed for the ability to bind to and/or modulate the activity of a natural PD-L2 binding partner, *e.g.*,
5 PD-1, modulate intra- or intercellular signaling, modulate activation of T lymphocytes, and/or modulate the immune response of an organism.

Yet another aspect of the invention pertains to isolated nucleic acid molecules encoding a PD-L2 fusion proteins. Such nucleic acid molecules, comprising at least a first nucleotide sequence encoding a PD-L2 protein, polypeptide or peptide operatively
10 linked to a second nucleotide sequence encoding a non- a PD-L2 protein, polypeptide or peptide, can be prepared by standard recombinant DNA techniques.

In addition to the nucleic acid molecules encoding PD-L2 polypeptides described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence
15 which is complementary to a "sense" nucleic acid encoding a polypeptide, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire PD-L2 coding strand, or to only a portion thereof. In one embodiment, an
20 antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding a PD-L2. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the coding region of human PD-L2 corresponds to SEQ ID NO:3). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of
25 the coding strand of a nucleotide sequence encoding PD-L2. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding human or mouse PD-L2 disclosed herein (*e.g.*, SEQ ID NO:3 or 6, respectively), antisense nucleic acids of the invention
30 can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of PD-L2 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of PD-L2 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site

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of PD-L2 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid molecule of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid molecule

5 (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides

10 which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-

15 methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-

20 (3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target

25 nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a PD-L2 polypeptide to thereby inhibit expression of the polypeptide, e.g., by inhibiting transcription and/or translation. The hybridization can

30 be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified

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to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gautier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-*o*-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haseloff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave PD-L2 mRNA transcripts to thereby inhibit translation of PD-L2 mRNA. A ribozyme having specificity for a PD-L2-encoding nucleic acid can be designed based upon the nucleotide sequence of a PD-L2 cDNA disclosed herein (*i.e.*, SEQ ID NO:1, 3, 4, or 6). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a PD-L2-encoding mRNA. See, *e.g.*, Cech *et al.*, US Patent No. 4,987,071 and Cech *et al.*, U.S. Patent No. 5,116,742. Alternatively, PD-L2 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, PD-L2 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the PD-L2 (*e.g.*, the PD-L2 promoter and/or enhancers; *e.g.*, nucleotides 1-273 of SEQ ID NO:1 or nucleotides 1-209 of SEQ ID NO:4) to form triple helical structures that prevent transcription of the

PD-L2 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioessays* 14(12):807-15.

In yet another embodiment, the PD-L2 nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup, B. and Nielsen, P. E. (1996) *Bioorg. Med. Chem.* 4(1):5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup and Nielsen (1996) *supra* and Perry-O'Keefe *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93:14670-675.

PNAs of PD-L2 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of PD-L2 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes (*e.g.*, S1 nucleases (Hyrup and Nielsen (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup and Nielsen (1996) *supra*; Perry-O'Keefe *et al.* (1996) *supra*).

In another embodiment, PNAs of PD-L2 can be modified (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of PD-L2 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes (*e.g.*, RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds

between the nucleobases, and orientation (Hyrup and Nielsen (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup and Nielsen (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17):3357-63. For example, a DNA chain can be synthesized on a solid support using standard
 5 phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a bridge between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acids Res.* 17:5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.*
 10 (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5:1119-1124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating
 15 transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. WO 88/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO 89/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Biotechniques*
 20 6:958-976) or intercalating agents (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

Alternatively, the expression characteristics of an endogenous PD-L2 gene
 25 within a cell line or microorganism may be modified by inserting a heterologous DNA regulatory element into the genome of a stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous PD-L2 gene. For example, an endogenous PD-L2 gene which is normally "transcriptionally silent", *i.e.*, a PD-L2 gene which is normally not expressed, or is expressed only at very
 30 low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, a transcriptionally silent, endogenous PD-L2 gene may be activated by insertion of a promiscuous regulatory element that works across cell types.

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A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with an endogenous PD-L2 gene, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described, *e.g.*, in Chappel, U.S. Patent No.

5 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991.

II. Isolated PD-L2 Polypeptides and Anti-PD-L2 Antibodies

One aspect of the invention pertains to isolated PD-L2 polypeptides, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-PD-L2 antibodies. In one embodiment, native PD-L2 polypeptides can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, PD-L2 polypeptides are produced by recombinant DNA techniques. Alternative to recombinant expression, a PD-L2 protein or polypeptide can be synthesized chemically using

10 standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the PD-L2 polypeptide is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language

20 "substantially free of cellular material" includes preparations of PD-L2 polypeptide in which the polypeptide is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of PD-L2 polypeptide having less than about 30% (by dry weight) of non-PD-L2 protein (also referred to herein as a

25 "contaminating protein"), more preferably less than about 20% of non-PD-L2 protein, still more preferably less than about 10% of non-PD-L2 protein, and most preferably less than about 5% non-PD-L2 protein. When the PD-L2 polypeptide or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably

30 less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of PD-L2 polypeptide in which the polypeptide is separated from chemical precursors or other chemicals which are involved in the synthesis of the

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polypeptide. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of PD-L2 polypeptide having less than about 30% (by dry weight) of chemical precursors or non-PD-L2 chemicals, more preferably less than about 20% chemical precursors or non-PD-L2 chemicals, still more preferably less than about 10% chemical precursors or non-PD-L2 chemicals, and most preferably less than about 5% chemical precursors or non-PD-L2 chemicals.

As used herein, a "biologically active portion" of a PD-L2 polypeptide includes a fragment of a PD-L2 polypeptide which participates in an interaction between a PD-L2 molecule and a non-PD-L2 molecule, *e.g.*, a natural ligand of PD-L2, *e.g.*, PD-1.

Biologically active portions of a PD-L2 polypeptide include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the PD-L2 polypeptide, *e.g.*, the amino acid sequence shown in SEQ ID NO:2 or 5, which include fewer amino acids than the full length PD-L2 polypeptides, and exhibit at least one activity of a PD-L2 polypeptide. Typically, biologically active portions comprise a domain or motif with at least one activity of the PD-L2 polypeptide, *e.g.*, modulating PD-1 activity. A biologically active portion of a PD-L2 polypeptide can be a polypeptide which is, for example, 25, 50, 75, 100, 125, 150, 175, 200, 225 or more amino acids in length. Biologically active portions of a PD-L2 polypeptide can be used as targets for developing agents which modulate a PD-L2-mediated activity, *e.g.*, immune cell activation.

In one embodiment, a biologically active portion of a PD-L2 polypeptide comprises at least a portion of an extracellular domain. It is to be understood that a preferred biologically active portion of a PD-L2 polypeptide of the present invention may contain at least a portion of an extracellular domain (*e.g.*, comprising an IgV and/or an IgC domain), and one or more of the following domains: a signal peptide domain, a transmembrane domain, and a cytoplasmic domain. Moreover, other biologically active portions, in which other regions of the polypeptide are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native PD-L2 polypeptide.

In a preferred embodiment, the PD-L2 polypeptide has an amino acid sequence shown in SEQ ID NO:2 or 5. In other embodiments, the PD-L2 polypeptide is substantially identical to SEQ ID NO:2 or 5, and retains the functional activity of the polypeptide of SEQ ID NO:2 or 5, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above.

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Accordingly, in another embodiment, the PD-L2 polypeptide is a polypeptide which comprises an amino acid sequence at least about 71%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2 or 5.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (e.g., when aligning a second sequence to the human PD-L2 amino acid sequence of SEQ ID NO:2 having 273 amino acid residues, at least 82, preferably at least 109, more preferably at least 137, even more preferably at least 164, and even more preferably at least 191, 218, 246 or more amino acid residues are aligned; when aligning a second amino acid sequence to the mouse PD-L2 amino acid sequence of SEQ ID NO:5 having 247 amino acid residues, at least 74, preferably at least 99, more preferably at least 124, even more preferably at least 148, and even more preferably at least 173, 198, 222 or more amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available online through the Genetics Computer Group), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1,

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2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available online through the Genetics Computer Group), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of Meyers, E. and Miller, W. (*Comput. Appl. Biosci.* 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0 or 2.0U), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

10 The nucleic acid and polypeptide sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to PD-L2 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 100, wordlength = 3 to obtain amino acid sequences homologous to PD-L2 polypeptide molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See the internet website for the National Center for Biotechnology Information.

25 The invention also provides PD-L2 chimeric or fusion proteins. As used herein, a PD-L2 "chimeric protein" or "fusion protein" comprises a PD-L2 polypeptide operatively linked to a non-PD-L2 polypeptide. A "PD-L2 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a PD-L2 molecule, whereas a "non-PD-L2 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a polypeptide which is not substantially homologous to the PD-L2 polypeptide, *e.g.*, a polypeptide which is different from the PD-L2 polypeptide and which is derived from the same or a different organism. Within a PD-L2 fusion protein, the PD-L2 polypeptide can correspond to all or a portion of a PD-L2 polypeptide. In a preferred embodiment, a PD-L2 fusion protein comprises at least one biologically active portion of a PD-L2 polypeptide. In another preferred embodiment, a

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PD-L2 fusion protein comprises at least two domains of a PD-L2 polypeptide. Within the fusion protein, the term "operatively linked" is intended to indicate that the PD-L2 polypeptide and the non-PD-L2 polypeptide are fused in-frame to each other. The non-PD-L2 polypeptide can be fused to the N-terminus or C-terminus of the PD-L2 polypeptide and corresponds to a moiety that alters the solubility, binding affinity, stability, or valency of the PD-L2 polypeptide.

For example, in one embodiment, the fusion protein is a GST-PD-L2 fusion protein in which the PD-L2 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant PD-L2.

In another embodiment, the fusion protein is a PD-L2 polypeptide containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of PD-L2 can be increased through use of a heterologous signal sequence.

In a preferred embodiment, the fusion protein is an Ig-PD-L2 fusion protein in which the PD-L2 sequences are fused to a portion of an Ig molecule. The Ig portion of the fusion protein can include an immunoglobulin constant region, *e.g.*, a human C γ 1 domain or a C γ 4 domain (*e.g.*, the hinge, CH2, and CH3 regions of human IgC γ 1 or human IgC γ 4 (see, *e.g.*, Capon *et al.*, U.S. Patent No. 5,116,964; 5,580,756; 5,844,095, and the like, incorporated herein by reference). A resulting fusion protein may have altered PD-L2 solubility, binding affinity, stability and/or valency (*i.e.*, the number of binding sites per molecule) and may increase the efficiency of protein purification.

Particularly preferred PD-L2 Ig fusion proteins include an extracellular domain portion of PD-L2 coupled to an immunoglobulin constant region (*e.g.*, the Fc region). The immunoglobulin constant region may contain genetic modifications which reduce or eliminate effector activity inherent in the immunoglobulin structure. For example, DNA encoding an extracellular portion of a PD-L2 polypeptide can be joined to DNA encoding the hinge, CH2, and CH3 regions of human IgG γ 1 and/or IgG γ 4 modified by site-directed mutagenesis, *e.g.*, as taught in WO 97/28267.

The PD-L2 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The PD-L2 fusion proteins can be used to affect the bioavailability of a PD-L2 binding partner, *e.g.*, PD-1. Use of PD-L2 fusion proteins may be useful therapeutically for the treatment of conditions or disorders that would benefit from modulation of the immune response.

Moreover, the PD-L2-fusion proteins of the invention can be used as immunogens to produce anti-PD-L2 antibodies in a subject, to purify PD-L2-binding proteins, and in screening assays to identify molecules which inhibit the interaction of PD-L2 with its natural binding partner, *e.g.*, PD-1.

5 Preferably, a PD-L2 chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini,
10 filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene
15 fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A PD-L2-encoding nucleic acid can be cloned into such an expression vector such that
20 the fusion moiety is linked in-frame to the PD-L2 polypeptide.

The present invention also pertains to variants of the PD-L2 polypeptides which function as either PD-L2 agonists (mimetics) or as PD-L2 antagonists. Variants of the PD-L2 polypeptides can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of a PD-L2 polypeptide. An agonist of the PD-L2 polypeptides can retain
25 substantially the same, or a subset, of the biological activities of the naturally occurring form of a PD-L2 polypeptide. An antagonist of a PD-L2 polypeptide can inhibit one or more of the activities of the naturally occurring form of the PD-L2 polypeptide by, for example, competitively modulating a PD-L2-mediated activity of a PD-L2 polypeptide. Thus, specific biological effects can be elicited by treatment with a variant of limited
30 function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the polypeptide has fewer side effects in a subject relative to treatment with the naturally occurring form of the PD-L2 polypeptide.

In one embodiment, variants of a PD-L2 polypeptide which function as either PD-L2 agonists (mimetics) or as PD-L2 antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a PD-L2 polypeptide for PD-L2 polypeptide agonist or antagonist activity. In one embodiment, a variegated library of PD-L2 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of PD-L2 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential PD-L2 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of PD-L2 sequences therein. There are a variety of methods which can be used to produce libraries of potential PD-L2 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential PD-L2 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acids Res.* 11:477).

In addition, libraries of fragments of a PD-L2 polypeptide coding sequence can be used to generate a variegated population of PD-L2 fragments for screening and subsequent selection of variants of a PD-L2 polypeptide. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a PD-L2 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the PD-L2 polypeptide.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of PD-

L2 polypeptides. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify PD-L2 variants (Arkin and Youvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delagrave *et al.* (1993) *Protein Eng.* 6(3):327-331).

In one embodiment, cell-based assays can be exploited to analyze a variegated PD-L2 library. For example, a library of expression vectors can be transfected into a cell line. The transfected cells are then contacted with PD-1-expressing cells and the effect of expression of the mutant on interaction of wild-type PD-L2 with its natural ligand(s), *e.g.*, PD-1, can be detected. Plasmid DNA can then be recovered from the cells which score for inhibition of signaling via PD-1 (leading to T cell upregulation), or alternatively, potentiation of signaling by PD-1 (leading to T cell downregulation), and the individual clones further characterized.

In addition to PD-L2 polypeptides consisting only of naturally-occurring amino acids, PD-L2 peptidomimetics are also provided. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compounds are termed "peptide mimetics" or "peptidomimetics" (Fauchere, J. (1986) *Adv. Drug Res.* 15:29; Veber and Freidinger (1985) *TINS* p.392; and Evans *et al.* (1987) *J. Med. Chem* 30:1229, which are incorporated herein by reference) and are usually developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides can be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (*i.e.*, a polypeptide that has a biological or pharmacological activity), such as human or mouse PD-L2, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: -CH₂NH-, -CH₂S-, -CH₂-CH₂-, -CH=CH- (cis and trans), -COCH₂-, -CH(OH)CH₂-, and -CH₂SO-, by methods known in the art and further described in the following references: Spatola, A.F. in *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins* Weinstein, B., ed., Marcel

Dekker, New York, p. 267 (1983); Spatola, A.F., Vega Data (March 1983), Vol. 1, Issue 3, "Peptide Backbone Modifications"; Morley, J.S. (1980) *Trends. Pharm. Sci.* pp.463-468; Hudson, D. *et al.* (1979) *Int. J. Pept. Prot. Res.* 14:177-185 (-CH₂NH-, CH₂CH₂-); Spatola, A.F. *et al.* (1986) *Life. Sci.* 38:1243-1249 (-CH₂-S); Hann, M.M. (1982) *J. Chem. Soc. Perkin. Trans. I* 307-314 (-CH-CH-, cis and trans); Almquist, R.G. *et al.* (1980) *J. Med. Chem.* 23:1392-1398 (-COCH₂-); Jennings-White, C. *et al.* (1982) *Tetrahedron Lett.* 23:2533 (-COCH₂-); Szelke, M. *et al.*, European Patent Application No. EP 45665 (1982) CA: 97:39405 (-CH(OH)CH₂-); Holladay, M.W. *et al.* (1983) *Tetrahedron. Lett.* 24:4401-4404 (-C(OH)CH₂-); and Hruby, V.J. (1982) *Life Sci.* 31:189-199 (-CH₂-S-); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is -CH₂NH-. Such peptide mimetics may have significant advantages over polypeptide embodiments, including, for example: more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (*e.g.*, a broad-spectrum of biological activities), reduced antigenicity, and others. Labeling of peptidomimetics usually involves covalent attachment of one or more labels, directly or through a spacer (*e.g.*, an amide group), to non-interfering position(s) on the peptidomimetic that are predicted by quantitative structure-activity data and/or molecular modeling. Such non-interfering positions generally are positions that do not form direct contacts with the macromolecules(s) to which the peptidomimetic binds to produce the therapeutic effect. Derivatization (*e.g.*, labeling) of peptidomimetics should not substantially interfere with the desired biological or pharmacological activity of the peptidomimetic.

Systematic substitution of one or more amino acids of a PD-L2 amino acid sequence with a D-amino acid of the same type (*e.g.*, D-lysine in place of L-lysine) can be used to generate more stable peptides. In addition, constrained peptides comprising a PD-L2 amino acid sequence or a substantially identical sequence variation can be generated by methods known in the art (Rizo and Gierasch (1992) *Annu. Rev. Biochem.* 61:387, incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

The amino acid sequences of the PD-L2 polypeptides identified herein will enable those of skill in the art to produce polypeptides corresponding to PD-L2 peptide sequences and sequence variants thereof. Such polypeptides can be produced in prokaryotic or eukaryotic host cells by expression of polynucleotides encoding a PD-L2

peptide sequence, frequently as part of a larger polypeptide. Alternatively, such peptides can be synthesized by chemical methods. Methods for expression of heterologous polypeptides in recombinant hosts, chemical synthesis of polypeptides, and *in vitro* translation are well known in the art and are described further in Maniatis *et al.*,
 5 *Molecular Cloning: A Laboratory Manual* (1989), 2nd Ed., Cold Spring Harbor, N.Y.; Berger and Kimmel, *Methods in Enzymology*, Volume 152, Guide to Molecular Cloning Techniques (1987), Academic Press, Inc., San Diego, Calif.; Merrifield, J. (1969) *J. Am. Chem. Soc.* 91:501; Chaiken I.M. (1981) *CRC Crit. Rev. Biochem.* 11:255; Kaiser *et al.* (1989) *Science* 243:187; Merrifield, B. (1986) *Science* 232:342; Kent, S.B.H. (1988)
 10 *Annu. Rev. Biochem.* 57:957; and Offord, R.E. (1980) *Semisynthetic Proteins*, Wiley Publishing, which are incorporated herein by reference).

Peptides can be produced, typically by direct chemical synthesis, and used *e.g.*, as agonists or antagonists of a PD-L2/PD-1 interaction. Peptides can be produced as modified peptides, with nonpeptide moieties attached by covalent linkage to the N-terminus and/or C-terminus. In certain preferred embodiments, either the carboxy-terminus or the amino-terminus, or both, are chemically modified. The most common
 15 modifications of the terminal amino and carboxyl groups are acetylation and amidation, respectively. Amino-terminal modifications such as acylation (*e.g.*, acetylation) or alkylation (*e.g.*, methylation) and carboxy-terminal modifications such as amidation, as
 20 well as other terminal modifications, including cyclization, can be incorporated into various embodiments of the invention. Certain amino-terminal and/or carboxy-terminal modifications and/or peptide extensions to the core sequence can provide advantageous physical, chemical, biochemical, and pharmacological properties, such as: enhanced stability, increased potency and/or efficacy, resistance to serum proteases, desirable
 25 pharmacokinetic properties, and others. Peptides can be used therapeutically to treat disease, *e.g.*, by altering costimulation in a patient.

An isolated PD-L2 polypeptide, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind PD-L2 using standard techniques for polyclonal and monoclonal antibody preparation. A full-length PD-L2 polypeptide can
 30 be used or, alternatively, the invention provides antigenic peptide fragments of PD-L2 for use as immunogens. In one embodiment, an antigenic peptide of PD-L2 comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 or 5 and encompasses an epitope of PD-L2 such that an antibody raised against the peptide forms a specific immune complex with the PD-L2 polypeptide. Preferably, the

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antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of PD-L2 that are located in the extracellular domain of the polypeptide, *e.g.*, hydrophilic regions, as well as regions with high antigenicity.

A PD-L2 immunogen typically is used to prepare antibodies by immunizing a suitable subject (*e.g.*, rabbit, goat, mouse, or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed PD-L2 polypeptide or a chemically synthesized PD-L2 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic PD-L2 preparation induces a polyclonal anti-PD-L2 antibody response.

Accordingly, another aspect of the invention pertains to anti-PD-L2 antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as a PD-L2. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind PD-L2 molecules. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of PD-L2. A monoclonal antibody composition thus typically displays a single binding affinity for a particular PD-L2 polypeptide with which it immunoreacts.

Polyclonal anti-PD-L2 antibodies can be prepared as described above by immunizing a suitable subject with a PD-L2 immunogen. The anti-PD-L2 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized PD-L2. If desired, the antibody molecules directed against PD-L2 can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the anti-PD-L2 antibody titers are highest, antibody-producing

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cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497 (see also Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol. Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally Kenneth, R.H. in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); Lerner, E.A. (1981) *Yale J. Biol. Med.* 54:387-402; Gefter, M.L. *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a PD-L2 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds PD-L2.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-PD-L2 monoclonal antibody (see, *e.g.*, Galfre, G. *et al.* (1977) *Nature* 266:55052; Gefter *et al.* (1977) *supra*; Lerner (1981) *supra*; and Kenneth (1980) *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a

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monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind PD-L2, e.g., using a standard ELISA assay.

Further methods for producing antibodies that bind PD-L2 are described in Examples 4 and 5.

- 5 Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-PD-L2 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with PD-L2 to thereby isolate immunoglobulin library members that bind PD-L2. Kits for generating and screening phage display libraries are commercially available
- 10 (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene SurfZAP™ Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.*, U.S. Patent No. 5,223,409; Kang *et al.*, PCT International Publication No. WO 92/18619;
- 15 Dower *et al.*, PCT International Publication No. WO 91/17271; Winter *et al.*, PCT International Publication WO 92/20791; Markland *et al.*, PCT International Publication No. WO 92/15679; Breitling *et al.*, PCT International Publication WO 93/01288; McCafferty *et al.*, PCT International Publication No. WO 92/01047; Garrard *et al.*, PCT International Publication No. WO 92/09690; Ladner *et al.*, PCT International
- 20 Publication No. WO 90/02809; Fuchs *et al.* (1991) *Biotechnology (NY)* 9:1369-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J.* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrard *et al.* (1991) *Biotechnology (NY)*
- 25 9:1373-1377; Hoogenboom *et al.* (1991) *Nucleic Acids Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* (1990) *Nature* 348:552-554.

- Additionally, recombinant anti-PD-L2 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions,
- 30 which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.*, International Application No. PCT/US86/02269; Akira *et al.*, European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496;

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- Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, PCT International Publication No. WO 86/01533; Cabilly *et al.*, U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Cancer Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S.L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *Biotechniques* 4:214; Winter, U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeven *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

- An anti-PD-L2 antibody (*e.g.*, monoclonal antibody) can be used to isolate PD-L2 by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-PD-L2 antibody can facilitate the purification of natural PD-L2 from cells and of recombinantly produced PD-L2 expressed in host cells. Moreover, an anti-PD-L2 antibody can be used to detect PD-L2 polypeptide (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the PD-L2 polypeptide. Anti-PD-L2 antibodies can be used diagnostically to monitor polypeptide levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid molecule encoding a PD-L2 polypeptide (or a portion

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thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein
5 additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated
10 along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most
15 commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of
20 the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of
25 interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for
30 example, in Goeddel (1990) *Methods Enzymol.* 185:3-7. Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as

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the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., PD-L2 polypeptides, mutant forms of PD-L2 polypeptides, fusion proteins, and the like).

5 The recombinant expression vectors of the invention can be designed for expression of PD-L2 polypeptides in prokaryotic or eukaryotic cells. For example, PD-L2 polypeptides can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors), yeast cells, or mammalian cells. Suitable host cells are discussed further in Goeddel (1990) *supra*. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of polypeptides in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino terminus of the recombinant polypeptide. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant polypeptide; 2) to increase the solubility of the recombinant polypeptide; and 3) to aid in the purification of the recombinant polypeptide by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant polypeptide to enable separation of the recombinant polypeptide from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant polypeptide.

Purified fusion proteins can be utilized in PD-L2 activity assays (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for PD-L2 polypeptides, for example. In a preferred embodiment, a PD-L2 fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated

recipients. The pathology of the subject recipient is then examined after sufficient time has passed (*e.g.*, six weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.* (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.* (1990) *Methods Enzymol.* 185:60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV5 promoter.

One strategy to maximize recombinant polypeptide expression in *E. coli* is to express the polypeptide in a host bacteria with an impaired capacity to proteolytically cleave the recombinant polypeptide (Gottesman, S. (1990) *Methods Enzymol.* 185:119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.* (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the PD-L2 expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari *et al.* (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.* (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (Invitrogen Corp, San Diego, CA).

Alternatively, PD-L2 polypeptides can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of polypeptides in cultured insect cells (*e.g.*, Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements.

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For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J. *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example by the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campe and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to PD-L2 mRNA. Regulatory sequences operatively linked to a nucleic acid molecule cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific, or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the

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regulation of gene expression using antisense genes, see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a PD-L2 nucleic acid molecule of the invention is introduced, *e.g.*, a PD-L2 nucleic acid molecule within a recombinant expression vector or a PD-L2 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a PD-L2 polypeptide can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a PD-L2 polypeptide or

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can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a PD-L2 polypeptide. Accordingly, the invention further provides methods for producing a PD-L2 polypeptide using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention (into which a recombinant expression vector encoding a PD-L2 polypeptide has been introduced) in a suitable medium such that a PD-L2 polypeptide is produced. In another embodiment, the method further comprises isolating a PD-L2 polypeptide from the medium or the host cell.

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which PD-L2-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous PD-L2 sequences have been introduced into their genome or homologous recombinant animals in which endogenous PD-L2 sequences have been altered. Such animals are useful for studying the function and/or activity of a PD-L2 and for identifying and/or evaluating modulators of PD-L2 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous PD-L2 gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing a PD-L2-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a

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pseudopregnant female foster animal. The PD-L2 cDNA sequence of SEQ ID NO:1 or 4 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human PD-L2 gene, such as a monkey or rat PD-L2 gene, can be used as a transgene. Alternatively, a PD-L2 gene homologue, such as another PD-L2 family member, can be isolated based on hybridization to the PD-L2 cDNA sequences of SEQ ID NO:1, 3, 4, or 6 (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a PD-L2 transgene to direct expression of a PD-L2 polypeptide to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a PD-L2 transgene in its genome and/or expression of PD-L2 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a PD-L2 polypeptide can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a PD-L2 gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the PD-L2 gene. The PD-L2 gene can be a human gene (*e.g.*, the cDNA of SEQ ID NO:1 or 3), but more preferably, is a non-human homologue of a human PD-L2 gene (*e.g.*, a cDNA isolated by stringent hybridization with the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6). For example, a mouse PD-L2 gene (*e.g.*, the cDNA of SEQ ID NO:3 or 6) can be used to construct a homologous recombination nucleic acid molecule, *e.g.*, a vector, suitable for altering an endogenous PD-L2 gene in the mouse genome. In a preferred embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous recombination, the endogenous PD-L2 gene is functionally disrupted (*i.e.*, no longer encodes a functional polypeptide; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed

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such that, upon homologous recombination, the endogenous PD-L2 gene is mutated or otherwise altered but still encodes functional polypeptide (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous PD-L2 polypeptide). In the homologous recombination nucleic acid molecule, the altered portion of the PD-L2 gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the PD-L2 gene to allow for homologous recombination to occur between the exogenous PD-L2 gene carried by the homologous recombination nucleic acid molecule and an endogenous PD-L2 gene in a cell, *e.g.*, an embryonic stem cell. The additional flanking PD-L2 nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, *e.g.*, Thomas, K.R. and Capecchi, M.R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The homologous recombination nucleic acid molecule is introduced into a cell, *e.g.*, an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced PD-L2 gene has homologously recombined with the endogenous PD-L2 gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells can then be injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, E.J., ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, *e.g.*, vectors, or homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos. WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the

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FLP recombinase system of *S. cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected polypeptide are required. Such animals can be provided through the construction of

5 "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected polypeptide and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature*

10 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte

15 is then cultured such that it develops to the morula or blastocyte stage and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

20 IV. Pharmaceutical Compositions

The PD-L2 molecules, *e.g.*, the PD-L2 nucleic acid molecules, fragments of PD-L2 polypeptides, and anti-PD-L2 antibodies (also referred to herein as "active compounds" or "modulating agents") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically

25 comprise the nucleic acid molecule, polypeptide, or antibody and a carrier, *e.g.*, a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and

30 agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS).

In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., modulating agents such as a PD-L2 nucleic acid molecule, a fragment of a PD-L2 polypeptide, an anti-PD-L2 antibody, or a combination of an anti-PD-L2 antibody and an anti-PD-L1 antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active

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compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention
5 enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid.
10 Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically
15 acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in US Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject
20 to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art
25 of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and
30 therapeutic effects is the therapeutic index and can be expressed as the ratio LD₅₀/ED₅₀. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity of PD-L2. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs,

organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the scope of knowledge of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram). It is

furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include

taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs
 5 or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II)
 10 (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological
 15 response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such polypeptides may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet
 20 derived growth factor, tissue plasminogen activator; or biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well
 25 known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer
 30 Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985); and Thorpe *et al.* "The Preparation And Cytotoxic

Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).
Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in US Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used
5 as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for
example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by
stereotactic injection (see, *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-
3057). The pharmaceutical preparation of the gene therapy vector can include the gene
therapy vector in an acceptable diluent, or can comprise a slow release matrix in which
10 the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery
vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the
pharmaceutical preparation can include one or more cells which produce the gene
delivery system.

The pharmaceutical compositions can be included in a container, pack, or
15 dispenser together with instructions for administration.

V. Uses and Methods of the Invention

The PD-L2 molecules, *e.g.*, the PD-L2 nucleic acid molecules, polypeptides,
polypeptide homologues, and antibodies described herein can be used in one or more of
20 the following methods: a) screening assays; b) predictive medicine (*e.g.*, diagnostic
assays, prognostic assays, and monitoring clinical trials); and c) methods of treatment
(*e.g.*, therapeutic and prophylactic, *e.g.*, by up- or down-modulating the immune
response). As described herein, a PD-L2 polypeptide of the invention has one or more
of the following activities: 1) binds to and/or modulates the activity of its natural
25 binding partner(s), *e.g.*, PD-1, 2) modulates intra- or intercellular signaling, 3)
modulates activation of T lymphocytes, 4) modulates the immune response of an
organism, *e.g.*, a mammalian organism, such as a mouse or human.

The isolated nucleic acid molecules of the invention can be used, for example, to
express PD-L2 polypeptide (*e.g.*, via a recombinant expression vector in a host cell in
30 gene therapy applications), to detect PD-L2 mRNA (*e.g.*, in a biological sample) or a
genetic alteration in a PD-L2 gene, and to modulate PD-L2 activity, as described further
below. The PD-L2 polypeptides can be used to treat conditions or disorders
characterized by insufficient or excessive production of a PD-L2 polypeptide or
production of PD-L2 inhibitors. In addition, the PD-L2 polypeptides can be used to

screen for naturally occurring PD-L2 binding partner(s) (in addition to PD-1), to screen for drugs or compounds which modulate PD-L2 activity, as well as to treat conditions or disorders characterized by insufficient or excessive production of PD-L2 polypeptide or production of PD-L2 polypeptide forms which have decreased, aberrant or unwanted activity compared to PD-L2 wild-type polypeptide (*e.g.*, immune system disorders such as severe combined immunodeficiency, multiple sclerosis, systemic lupus erythematosus, type I diabetes mellitus, lymphoproliferative syndrome, inflammatory bowel disease, allergies, asthma, graft-versus-host disease, and transplant rejection; immune responses to infectious pathogens such as bacteria and viruses; and immune system cancers such as lymphomas and leukemias). Moreover, the anti-PD-L2 antibodies of the invention can be used to detect and isolate PD-L2 polypeptides, regulate the bioavailability of PD-L2 polypeptides, and modulate PD-L2 activity, *e.g.*, by modulating the interaction between PD-L2 and its natural binding partner(s) (*e.g.*, PD-1).

15

A. Screening Assays:

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to PD-L2 polypeptides, have a stimulatory or inhibitory effect on, for example, PD-L2 expression or PD-L2 activity, or have a stimulatory or inhibitory effect on the interaction between PD-L2 and its natural binding partner(s), *e.g.*, PD-1.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to the PD-L2 protein or polypeptide or biologically active portion thereof, *e.g.*, modulate the ability of the PD-L2 polypeptide to interact with its natural binding partner(s). In a preferred embodiment, the binding partner is PD-1. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a PD-L2 protein or polypeptide or biologically active portion thereof (*e.g.*, cofactor or coenzyme analogs, or inhibitory molecules).

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In a preferred embodiment, the invention provides assays for screening candidate or test compounds which have a stimulatory or inhibitory effect on the interaction between PD-L2 and its natural binding partner(s). In an exemplary embodiment, the binding partner is PD-1. The test compounds of the present invention can be obtained

using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994) *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:1865-1869) or phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:6378-6382; Felici (1991) *J. Mol. Biol.* 222:301-310; Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a PD-L2 polypeptide or biologically active portion thereof is contacted with a test compound, and the ability of the test compound to modulate PD-L2 activity is determined. Determining the ability of the test compound to modulate PD-L2 activity can be accomplished by monitoring, for example, the ability of PD-L2 to bind to its natural binding partner(s), *e.g.*, PD-1, and modulate immune cell activity. The immune cell can be, *e.g.*, a T cell, a B cell, or a myeloid cell. Determining the ability of the test compound to modulate PD-L2 binding to PD-1 can be accomplished, for example, by coupling PD-1 with a radioisotope or enzymatic label such that binding of the PD-1 to PD-L2 can be determined by detecting the labeled PD-1 in a complex. Alternatively, PD-L2 could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate PD-L2 binding to PD-1 in a complex. Determining the ability of the test compound to bind PD-L2 can be accomplished, for example, by coupling the compound with a radioisotope or enzymatic label such that binding of the

compound to PD-L2 can be determined by detecting the labeled PD-L2 compound in a complex. For example, compounds (*e.g.*, PD-1) can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (*e.g.*, PD-1) to interact with PD-L2 without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with PD-L2 without the labeling of either the compound or the PD-L2 (McConnell, H.M. *et al.* (1992) *Science* 257:1906-1912). As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and PD-L2.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a PD-L2 binding partner with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the PD-L2 binding partner. In a preferred embodiment, the binding partner is PD-1. Determining the ability of the test compound to modulate the activity of a PD-L2 binding partner can be accomplished, for example, by determining the ability of the PD-L2 polypeptide to bind to or interact with the PD-L2 binding partner.

Determining the ability of the PD-L2 polypeptide, or a biologically active fragment thereof, to bind to or interact with a PD-L2 binding partner, *e.g.*, PD-1, can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the PD-L2 polypeptide to bind to or interact with a PD-L2 binding partner can be accomplished by determining the activity of the binding partner. For example, the activity of the binding partner can be determined by detecting induction of a cellular second messenger (*e.g.*, tyrosine kinase or phosphatase activity), detecting catalytic/enzymatic activity of an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a target-regulated cellular response. For example,

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determining the ability of the PD-L2 polypeptide to bind to or interact with a natural PD-L2 binding partner, *e.g.*, PD-1, can be accomplished by measuring the ability of a compound to modulate immune cell costimulation or inhibition in a proliferation assay, or by interfering with the ability of a PD-L2 polypeptide to bind to antibodies that
5 recognize a portion of the PD-L2 polypeptide. In one embodiment, compounds that modulate T cell activation can be identified by determining the ability of a compound to modulate T cell proliferation or cytokine production. In a preferred embodiment, compounds that modulate T cell activation can be identified by determining the ability of a compound to modulate T cell proliferation or cytokine production at more than one
10 antigen concentration.

In yet another embodiment, an assay of the present invention is a cell-free assay in which a PD-L2 polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the PD-L2 polypeptide or biologically active portion thereof is determined. Preferred biologically active portions
15 of the PD-L2 polypeptides to be used in assays of the present invention include fragments which participate in interactions with non-PD-L2 molecules, *e.g.*, at least a portion of an extracellular domain which binds to a PD-L2 binding partner. In a further preferred embodiment, the binding partner is PD-1. Binding of the test compound to the PD-L2 polypeptide can be determined either directly or indirectly as described above.
20 In a preferred embodiment, the assay includes contacting the PD-L2 polypeptide or biologically active portion thereof with a known compound which binds PD-L2 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a PD-L2 polypeptide, wherein determining the ability of the test compound to interact with a PD-L2 polypeptide
25 comprises determining the ability of the test compound to preferentially bind to PD-L2 or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which a PD-L2 polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the
30 PD-L2 polypeptide or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a PD-L2 polypeptide can be accomplished, for example, by determining the ability of the PD-L2 polypeptide to bind to a PD-L2 binding partner by one of the methods described above for determining direct binding. In a preferred embodiment, the binding partner is PD-1. Determining

the ability of the PD-L2 polypeptide to bind to a PD-L2 binding partner can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA) (Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345; Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705). As used herein, "BIA" is a technology for
 5 studying biospecific interactions in real time, without labeling any of the interactants (e.g., BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a PD-L2 polypeptide can be accomplished by determining the
 10 ability of the PD-L2 polypeptide to further modulate the activity of a downstream effector of a PD-L2 binding partner (e.g., a downstream effector of PD-1). For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

The cell-free assays of the present invention are amenable to use of both soluble and/or membrane-bound forms of polypeptides (e.g., PD-L2 polypeptides or biologically active portions thereof, or binding partners to which PD-L2 binds, e.g., PD-1). In the case of cell-free assays in which a membrane-bound form a polypeptide is
 15 used (e.g., a cell-surface PD-L2), it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the polypeptide is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecylpoly(ethylene glycol ether)_n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate
 20 (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either PD-L2 or its binding partner to facilitate separation of complexed from uncomplexed forms of one or both of the
 25 polypeptides, as well as to accommodate automation of the assay. In a preferred embodiment, the binding partner is PD-1. Binding of a test compound to a PD-L2 polypeptide, or interaction of a PD-L2 polypeptide with its binding partner in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates,

test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the polypeptides to be bound to a matrix. For example, glutathione-S-transferase/PD-L2 fusion proteins or glutathione-S-transferase/binding partner fusion proteins can be adsorbed onto
5 glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed binding partner polypeptide or PD-L2 polypeptide, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtitre
10 plate wells are washed to remove any unbound components, the matrix is immobilized in the case of beads, and complex formation is determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of PD-L2 binding or activity determined using standard techniques.

15 Other techniques for immobilizing polypeptides on matrices can also be used in the screening assays of the invention. For example, either a PD-L2 polypeptide or a PD-L2 binding partner can be immobilized utilizing conjugation of biotin and streptavidin. In a preferred embodiment, the binding partner is PD-1. Biotinylated PD-L2 polypeptide or binding partners can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals,
20 Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which are reactive with PD-L2 polypeptide or binding partners but which do not interfere with binding of the PD-L2 polypeptide to its binding partner can be derivatized to the wells of the plate, and unbound binding partner
25 or PD-L2 polypeptide is trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the PD-L2 polypeptide or binding partner, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the PD-L2 polypeptide or
30 binding partner.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a PD-L2 polypeptide can be accomplished by determining the ability of the test compound to modulate the activity of a molecule that functions downstream of PD-L2, *e.g.*, by interacting with the cytoplasmic domain of a PD-L2

binding partner, *e.g.*, PD-1. For example, levels of second messengers, the activity of the interacting molecule on an appropriate target, or the binding of the interactor to an appropriate target can be determined as previously described.

In another embodiment, modulators of PD-L2 expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of PD-L2 mRNA or polypeptide in the cell is determined. The level of expression of PD-L2 mRNA or polypeptide in the presence of the candidate compound is compared to the level of expression of PD-L2 mRNA or polypeptide in the absence of the candidate compound. The candidate compound can then be identified as a modulator of PD-L2 expression based on this comparison. For example, when expression of PD-L2 mRNA or polypeptide is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of PD-L2 mRNA or polypeptide expression. Alternatively, when expression of PD-L2 mRNA or polypeptide is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of PD-L2 mRNA or polypeptide expression. The level of PD-L2 mRNA or polypeptide expression in the cells can be determined by methods described herein for detecting PD-L2 mRNA or polypeptide.

In yet another aspect of the invention, the PD-L2 polypeptides can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other polypeptides which bind to or interact with PD-L2 ("PD-L2-binding proteins", "PD-L2 binding partners", or "PD-L2-bp") and are involved in PD-L2 activity. An example of such a binding protein is PD-1. Such PD-L2-binding proteins are also likely to be involved in the propagation of signals by the PD-L2 polypeptides or PD-L2 targets as, for example, downstream elements of a PD-L2-mediated signaling pathway. Alternatively, such PD-L2-binding polypeptides may be PD-L2 inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a PD-L2 polypeptide is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a

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library of DNA sequences, that encodes an unidentified polypeptide ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" polypeptides are able to interact, *in vivo*, forming a PD-L2-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the polypeptide which interacts with the PD-L2 polypeptide.

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell-free assay, and the ability of the agent to modulate the activity of a PD-L2 polypeptide can be confirmed *in vivo*, *e.g.*, in an animal such as an animal model for cellular transformation and/or tumorigenesis.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a PD-L2 modulating agent, an antisense PD-L2 nucleic acid molecule, a PD-L2-specific antibody, or a PD-L2 binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the PD-L2 nucleotide sequences, described herein, can be used to map the location of the PD-L2 genes on a chromosome. The mapping of the PD-L2 sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, PD-L2 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the PD-L2 nucleotide sequences. Computer analysis of the PD-L2 sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the PD-L2 sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes (D'Eustachio, P. *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the PD-L2 nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a PD-L2 sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:6223-27), pre-

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screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results in a reasonable amount of time. For a review of this technique, see Verma *et al.*, *Human Chromosomes: A Manual of Basic Techniques* (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data (such data are found, for example, in McKusick, V., Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the PD-L2 gene can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are

visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

5 2. Tissue Typing

 The PD-L2 sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is
10 digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in US Patent 5,272,057).

15 Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the PD-L2 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and
20 subsequently sequence it.

 Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals
25 and from tissue. The PD-L2 nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some
30 degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 or 4 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding

amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 or 6 are used, a more appropriate number of primers for positive individual identification would be 500-2000.

If a panel of reagents from PD-L2 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

10 3. Use of PD-L2 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.*, another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 or 4 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the PD-L2 nucleotide sequences or portions thereof, *e.g.*, fragments derived from the noncoding regions of SEQ ID NO:1 or 4 having a length of at least 20 bases, preferably at least 30 bases.

The PD-L2 nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, lymphocytes. This can be very useful in cases where a forensic pathologist is presented

with a tissue of unknown origin. Panels of such PD-L2 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, PD-L2 primers or probes can be used to screen tissue culture for contamination (*i.e.*, screen for the presence of a mixture of
5 different types of cells in a culture).

C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for
10 prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining PD-L2 polypeptide and/or nucleic acid expression as well as PD-L2 activity, in the context of a biological sample (*e.g.*, blood, serum, cells, or tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at
15 risk of developing a disorder, associated with aberrant or unwanted PD-L2 expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with PD-L2 polypeptide, nucleic acid expression or activity. For example, mutations in a PD-L2 gene can be assayed in a biological sample. Such assays can be used for
20 prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with PD-L2 polypeptide, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of PD-L2 in clinical trials.

25 These and other agents are described in further detail in the following sections.

1. Diagnostic Assays

An exemplary method for detecting the presence or absence of PD-L2 polypeptide or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting PD-L2 polypeptide or nucleic acid (e.g., mRNA or genomic DNA) that encodes PD-L2 polypeptide such that the presence of PD-L2 polypeptide or nucleic acid is detected in the biological sample. A preferred agent for detecting PD-L2 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to PD-L2 mRNA or genomic DNA. The nucleic acid probe can be, for example, the PD-L2 nucleic acid set forth in SEQ ID NO:1, 3, 4, or 6, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to PD-L2 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting PD-L2 polypeptide is an antibody capable of binding to PD-L2 polypeptide, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

The term "biological sample" is intended to include tissues, cells, and biological fluids isolated from a subject, as well as tissues, cells, and fluids present within a subject. That is, the detection method of the invention can be used to detect PD-L2 mRNA, polypeptide, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of PD-L2 mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of PD-L2 polypeptide include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of PD-L2 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of PD-L2 polypeptide include introducing into a subject a labeled anti-PD-

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L2 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains polypeptide molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from
5 the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting PD-L2 polypeptide, mRNA, or genomic DNA,
10 such that the presence of PD-L2 polypeptide, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of PD-L2 polypeptide, mRNA or genomic DNA in the control sample with the presence of PD-L2 polypeptide, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of PD-L2 in a
15 biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting PD-L2 polypeptide or mRNA in a biological sample; means for determining the amount of PD-L2 in the sample; and means for comparing the amount of PD-L2 in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect
20 PD-L2 polypeptide or nucleic acid.

2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant or
25 unwanted PD-L2 expression or activity. As used herein, the term "aberrant" includes a PD-L2 expression or activity which deviates from the wild type PD-L2 expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For
30 example, aberrant PD-L2 expression or activity is intended to include the cases in which a mutation in the PD-L2 gene causes the PD-L2 gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional PD-L2 polypeptide or a polypeptide which does not function in a wild-type fashion, *e.g.*, a polypeptide which does not interact with a PD-L2 binding partner (*e.g.*, PD-1), or one

which interacts with a non-PD-L2 binding partner. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as immune cell activation. For example, the term unwanted includes a PD-L2 expression or activity which is undesirable in a subject.

5 The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in PD-L2 polypeptide activity or nucleic acid expression, such as an autoimmune disorder, an immunodeficiency disorder, an immune system cancer, or a tendency to have spontaneous abortions. Alternatively, the
10 prognostic assays can be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation of PD-L2 polypeptide activity or nucleic acid expression, such as an autoimmune disorder, and immunodeficiency disorder, an immune system cancer, or a tendency to have spontaneous abortions. Thus, the present invention provides a method for identifying a disease or disorder associated with
15 aberrant or unwanted PD-L2 expression or activity in which a test sample is obtained from a subject and PD-L2 polypeptide or nucleic acid (*e.g.*, mRNA or genomic DNA) is detected, wherein the presence of PD-L2 polypeptide or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted PD-L2 expression or activity. As used herein, a "test sample" refers to a
20 biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, cerebrospinal fluid or serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, polypeptide, peptide, nucleic acid, small molecule, or other drug
25 candidate) to treat a disease or disorder associated with aberrant or unwanted PD-L2 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for an autoimmune disorder, immunodeficiency disorder, immune system cancer, or tendency to have spontaneous abortions. Thus, the present invention provides methods for determining whether a
30 subject can be effectively treated with an agent for a disorder associated with aberrant or unwanted PD-L2 expression or activity in which a test sample is obtained and PD-L2 polypeptide or nucleic acid expression or activity is detected (*e.g.*, wherein the abundance of PD-L2 polypeptide or nucleic acid expression or activity is diagnostic for

a subject that can be administered the agent to treat a disorder associated with aberrant or unwanted PD-L2 expression or activity).

The methods of the invention can also be used to detect genetic alterations in a PD-L2 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in PD-L2 polypeptide activity or nucleic acid expression, such as an autoimmune disorder, an immunodeficiency disorder, an immune system cancer, or a tendency to have spontaneous abortions. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one alteration affecting the integrity of a gene encoding a PD-L2 polypeptide, or the mis-expression of the PD-L2 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a PD-L2 gene, 2) an addition of one or more nucleotides to a PD-L2 gene, 3) a substitution of one or more nucleotides of a PD-L2 gene, 4) a chromosomal rearrangement of a PD-L2 gene, 5) an alteration in the level of a messenger RNA transcript of a PD-L2 gene, 6) aberrant modification of a PD-L2 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a PD-L2 gene, 8) a non-wild type level of a PD-L2 polypeptide, 9) allelic loss of a PD-L2 gene, and 10) inappropriate post-translational modification of a PD-L2 polypeptide. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in a PD-L2 gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., US Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in a PD-L2 gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a PD-L2 gene under conditions such that hybridization and amplification of the PD-L2 gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the

amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a PD-L2 gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, US Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in PD-L2 can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotide probes (Cronin, M.T. *et al.* (1996) *Hum. Mutat.* 7:244-255; Kozal, M.J. *et al.* (1996) *Nat. Med.* 2:753-759). For example, genetic mutations in PD-L2 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin *et al.* (1996) *supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential, overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the PD-L2 gene and detect mutations by comparing the sequence of the sample PD-L2 with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert (1977) *Proc. Natl. Acad. Sci. USA* 74:560 or Sanger (1977) *Proc. Natl. Acad. Sci. USA* 74:5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (Naeve, C.W. (1995) *Biotechniques* 19:448-53), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the PD-L2 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes formed by hybridizing (labeled) RNA or DNA containing the wild-type PD-L2 sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digest the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:4397 and Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in PD-L2 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis*

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15:1657-1662). According to an exemplary embodiment, a probe based on a PD-L2 sequence, *e.g.*, a wild-type PD-L2 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the
5 like. See, for example, US Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in PD-L2 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci*
10 *USA* 86:2766; see also Cotton (1993) *Mutat. Res.* 285:125-144 and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control PD-L2 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change.
15 The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends*
20 *Genet.* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to ensure that it does
25 not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys. Chem.* 265:12753).

Examples of other techniques for detecting point mutations include, but are not
30 limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163; Saiki *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6230). Such allele specific

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oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

- Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

- The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a PD-L2 gene.

- Furthermore, any cell type or tissue in which PD-L2 is expressed may be utilized in the prognostic assays described herein.

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3. Monitoring of Effects During Clinical Trials

- Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of a PD-L2 polypeptide (*e.g.*, the modulation of cell proliferation and/or migration) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase PD-L2 gene expression, polypeptide levels, or upregulate PD-L2 activity, can be monitored in clinical trials of subjects exhibiting decreased PD-L2 gene expression, polypeptide levels, or downregulated PD-L2 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease PD-L2 gene expression,

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polypeptide levels, or downregulate PD-L2 activity, can be monitored in clinical trials of subjects exhibiting increased PD-L2 gene expression, polypeptide levels, or PD-L2 activity. In such clinical trials, the expression or activity of a PD-L2 gene, and preferably, other genes that have been implicated in, for example, a PD-L2-associated disorder can be used as a "read out" or marker of the phenotype of a particular cell.

For example, and not by way of limitation, genes, including PD-L2, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates PD-L2 activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on PD-L2-associated disorders (*e.g.*, disorders characterized by dysregulated PD-1 activity), for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of PD-L2 and other genes implicated in the PD-L2-associated disorder, respectively. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of polypeptide produced, by one of the methods as described herein, or by measuring the levels of activity of PD-L2 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, polypeptide, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a PD-L2 polypeptide, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the PD-L2 polypeptide, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the PD-L2 polypeptide, mRNA, or genomic DNA in the pre-administration sample with the PD-L2 polypeptide, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of PD-L2 to higher levels than detected, *i.e.*, to increase the effectiveness of the agent.

Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of PD-L2 to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent. According to such an embodiment, PD-L2 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence
5 of an observable phenotypic response.

D. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder characterized by insufficient or excessive production of PD-L2 protein or production of PD-L2 protein forms which
10 have decreased or aberrant activity compared to PD-L2 wild type protein. Moreover, the anti- PD-L2 antibodies of the invention can be used to detect and isolate PD-L2 proteins, regulate the bioavailability of PD-L2 proteins, and modulate PD-L2 activity *e.g.*, by modulating the interaction of PD-L2 with PD-1.

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1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted PD-L2 expression or activity, by administering to the subject a PD-L2 polypeptide or an agent which
20 modulates PD-L2 expression or at least one PD-L2 activity. Subjects at risk for a disease or disorder which is caused or contributed to by aberrant or unwanted PD-L2 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the PD-L2 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its
25 progression. Depending on the type of PD-L2 aberrancy, for example, a PD-L2 polypeptide, PD-L2 agonist or PD-L2 antagonist (*e.g.*, an anti-PD-L2 antibody or a combination of anti-PD-L2 and anti-PD-L1 antibodies) agent can be used for treating the subject. The appropriate agent can be determined based on screening assays
30 described herein.

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating PD-L2 expression or activity or interaction with its natural binding partners, *e.g.*, PD-1, for

therapeutic purposes. PD-L2 has been demonstrated to inhibit the costimulation and proliferation of activated immune cells and to transmit an inhibitory signal to immune cells via PD-1. Accordingly, the activity and/or expression of PD-L2, as well as the interaction between PD-L2 and PD-1 can be modulated in order to modulate the immune response. Because PD-L2 binds to inhibitory receptors, upregulation of PD-L2 activity results in downregulation of immune responses, whereas downregulation of PD-L2 activity results in upregulation of immune responses. In a preferred embodiment, PD-L2 binds to inhibitory receptors. In a particularly preferred embodiment, PD-L2 binds to PD-1.

Modulatory methods of the invention involve contacting a cell with a PD-L2 polypeptide or agent that modulates one or more of the activities of PD-L2 polypeptide activity associated with the cell, *e.g.*, an agent that modulates expression or activity of PD-L2 and/or modulates the interaction of PD-L2 and its natural binding partner(s). In a preferred embodiment, the binding partner is PD-1. An agent that modulates PD-L2 polypeptide activity can be an agent as described herein, such as a nucleic acid or a polypeptide, a naturally-occurring binding partner of a PD-L2 polypeptide (*e.g.*, PD-1), a PD-L2 antibody, a combination of PD-L2 and PD-L1 antibodies, a PD-L2 agonist or antagonist, a peptidomimetic of a PD-L2 agonist or antagonist, a PD-L2 peptidomimetic, or other small molecule. Soluble forms of PD-L2 may also be used to interfere with the binding of PD-1 to any of its natural binding partner(s) or ligands.

An agent that modulates the expression of PD-L2 is, *e.g.*, an antisense nucleic acid molecule, triplex oligonucleotide, ribozyme, or recombinant vector for expression of a PD-L2 polypeptide. For example, an oligonucleotide complementary to the area around a PD-L2 polypeptide translation initiation site can be synthesized. One or more antisense oligonucleotides can be added to cell media, typically at 200 µg/ml, or administered to a patient to prevent the synthesis of a PD-L2 polypeptide. The antisense oligonucleotide is taken up by cells and hybridizes to a PD-L2 mRNA to prevent translation. Alternatively, an oligonucleotide which binds double-stranded DNA to form a triplex construct to prevent DNA unwinding and transcription can be used. As a result of either, synthesis of PD-L2 polypeptide is blocked. When PD-L2 expression is modulated, preferably, such modulation occurs by a means other than by knocking out the PD-L2 gene.

Agents which modulate expression, by virtue of the fact that they control the amount of PD-L2 in a cell, also modulate the total amount of PD-L2 activity in a cell.

In one embodiment, the agent the modulates PD-L2 stimulates one or more PD-L2 activities. Examples of such stimulatory agents include active PD-L2 polypeptide and a nucleic acid molecule encoding PD-L2 that has been introduced into the cell. In another embodiment, the agent inhibits one or more PD-L2 activities. In a preferred embodiment, the agent inhibits or enhances the interaction of PD-L2 with its natural binding partner(s). In a particularly preferred embodiment, the binding partner is PD-1. Examples of such inhibitory agents include antisense PD-L2 nucleic acid molecules, anti-PD-L2 antibodies, PD-L2 inhibitors, and compounds identified in the subject screening assays. In a further preferred embodiment, an inhibitory agent is a combination of an anti-PD-L2 antibody and an anti-PD-L1 antibody.

These modulatory methods can be performed *in vitro* (e.g., by contacting the cell with the agent) or, alternatively, by contacting an agent with cells *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a condition or disorder that would benefit from up- or down-modulation of a PD-L2 polypeptide, e.g., a disorder characterized by unwanted, insufficient, or aberrant expression or activity of a PD-L2 polypeptide or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) PD-L2 expression or activity. In another embodiment, the method involves administering a PD-L2 polypeptide or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted PD-L2 expression or activity.

Stimulation of PD-L2 activity is desirable in situations in which PD-L2 is abnormally downregulated and/or in which increased PD-L2 activity is likely to have a beneficial effect. Likewise, inhibition of PD-L2 activity is desirable in situations in which PD-L2 is abnormally upregulated and/or in which decreased PD-L2 activity is likely to have a beneficial effect.

Exemplary agents for use in downmodulating PD-L2 (*i.e.*, PD-L2 antagonists) include, e.g., antisense nucleic acid molecules, antibodies that recognize and block PD-L2, combinations of antibodies that recognize and block PD-L2 and antibodies that recognize and block PD-L1, and compounds that block the interaction of PD-L2 with its naturally occurring binding partner(s) on an immune cell (e.g., soluble, monovalent PD-L2 molecules; soluble forms of PD-L2 molecules that do not bind Fc receptors on antigen presenting cells; soluble forms of PD-L2 binding partners; and compounds

identified in the subject screening assays). In a preferred embodiment, the binding partner is PD-1. Exemplary agents for use in upmodulating PD-L2 (*i.e.*, PD-L2 agonists) include, *e.g.*, nucleic acid molecules encoding PD-L2 polypeptides, multivalent forms of PD-L2, compounds that increase the expression of PD-L2, 5 compounds that enhance the interaction of PD-L2 with its naturally occurring binding partners (*e.g.*, PD-1) and cells that express PD-L2.

3. Downregulation of Immune Responses

There are numerous embodiments of the invention for upregulating the inhibitory function of a PD-L2 polypeptide to thereby downregulate immune responses. Downregulation can be in the form of inhibiting or blocking an immune response already in progress, or may involve preventing the induction of an immune response. The functions of activated immune cells can be inhibited by downregulating immune cell responses or by inducing specific anergy in immune cells, or both.

15 For example, in embodiments where PD-L2 binds to an inhibitory receptor, *e.g.*, PD-1, forms of PD-L2 that bind to the inhibitory receptor, *e.g.*, multivalent PD-L2 on a cell surface, can be used to downmodulate the immune response. Likewise, the PD-L2-PD-1 interaction can also be enhanced by the use of an additional agent, *e.g.*, an agent that blocks the interaction of PD-L1 with PD-1, that can further downmodulate the 20 immune response.

In one embodiment of the invention, an activating antibody used to stimulate PD-L2 activity is a bispecific antibody. For example, such an antibody can comprise a PD-L2 binding site and another binding site which targets a cell surface receptor on an immune cell, *e.g.*, a T cell, a B cell, or a myeloid cell. In one embodiment, such an 25 antibody, in addition to comprising a PD-L2 binding site, can further comprise a binding site which binds to a B cell antigen receptor, a T cell antigen receptor, or an Fc receptor, in order to target the molecule to a specific cell population. Selection of this second antigen for the bispecific antibody provides flexibility in selection of cell population to be targeted for inhibition.

30 Agents that promote a PD-L2 activity or which enhance the interaction of PD-L2 with its natural binding partners, *e.g.*, PD-1 (*e.g.*, PD-L2 activating antibodies or PD-L2 activating small molecules) can be identified by their ability to inhibit immune cell proliferation and/or effector function, or to induce anergy when added to an *in vitro* assay. For example, cells can be cultured in the presence of an agent that stimulates

signal transduction via an activating receptor. A number of art-recognized readouts of cell activation can be employed to measure, *e.g.*, cell proliferation or effector function (*e.g.*, antibody production, cytokine production, phagocytosis) in the presence of the activating agent. The ability of a test agent to block this activation can be readily
 5 determined by measuring the ability of the agent to effect a decrease in proliferation or effector function being measured. In one embodiment, at low antigen concentrations, PD-L2-PD-1 interactions inhibit strong B7-CD28 signals. In another embodiment, at high antigen concentrations, PD-L2-PD-1 interactions reduce cytokine production but do not inhibit T cell proliferation. Accordingly, the ability of a test compound to block
 10 activation can be determined by measuring cytokine production and/or proliferation at different concentrations of antigen.

In one embodiment of the invention, tolerance is induced against specific antigens by co-administering an antigen with a PD-L2 agonist. For example, tolerance can be induced to specific polypeptides. In one embodiment, immune responses to
 15 allergens or foreign polypeptides to which an immune response is undesirable can be inhibited. For example, patients that receive Factor VIII frequently generate antibodies against this clotting factor. Co-administration of an agent that stimulates PD-L2 activity or interaction with its natural binding partner, *e.g.*, PD-1, with recombinant factor VIII (or physically linking PD-L2 to Factor VIII, *e.g.*, by cross-linking) can result in immune
 20 response downmodulation.

In one embodiment, a PD-L2 agonist and another agent that can block activity of costimulatory receptors on an immune cell can be used to downmodulate immune responses. Exemplary molecules include: agonists forms of other PD-1 ligands (*e.g.*, PD-L1), soluble forms of CTLA-4, anti-B7-1 antibodies, anti-B7-2 antibodies, or
 25 combinations thereof. Alternatively, two separate peptides (for example, a PD-L2 polypeptide with blocking forms of B7-2 and/or B7-1 polypeptides), or a combination of antibodies (*e.g.*, activating antibodies against a PD-L2 polypeptide with blocking anti-B7-2 and/or anti-B7-1 monoclonal antibodies) can be combined as a single composition or administered separately (simultaneously or sequentially) to downregulate immune
 30 cell mediated immune responses in a subject. Furthermore, a therapeutically active amount of one or more peptides having a PD-L2 polypeptide activity, along with one or more polypeptides having B7-1 and/or B7-2 activity, can be used in conjunction with other downmodulating reagents to influence immune responses. Examples of other immunomodulating reagents include antibodies that block a costimulatory signal (*e.g.*,

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against CD28 or ICOS), antibodies that activate an inhibitory signal via CTLA4, and/or antibodies against other immune cell markers (*e.g.*, against CD40, CD40 ligand, or cytokines), fusion proteins (*e.g.*, CTLA4-Fc or PD-1-Fc), and immunosuppressive drugs (*e.g.*, rapamycin, cyclosporine A, or FK506).

5 The PD-L2 polypeptides may also be useful in the construction of therapeutic agents which block immune cell function by destruction of cells. For example, portions of a PD-L2 polypeptide can be linked to a toxin to make a cytotoxic agent capable of triggering the destruction of cells to which it binds.

For making cytotoxic agents, polypeptides of the invention may be linked, or
10 operatively attached, to toxins using techniques that are known in the art, *e.g.*, via crosslinking or recombinant DNA techniques. The preparation of immunotoxins is, in general, well known in the art (see, *e.g.*, US Patent 4,340,535 and EP 44167, both incorporated herein by reference). Numerous types of disulfide bond-containing linkers are known which can successfully be employed to conjugate the toxin moiety with a
15 polypeptide. In one embodiment, linkers that contain a disulfide bond that is sterically "hindered" are to be preferred, due to their greater stability *in vivo*, thus preventing release of the toxin moiety prior to binding at the site of action.

A wide variety of toxins are known that may be conjugated to polypeptides or antibodies of the invention. Examples include: numerous useful plant-, fungus- or even
20 bacteria-derived toxins, which, by way of example, include: various A chain toxins, particularly ricin A chain; ribosome inactivating proteins such as saporin or gelonin; alpha-sarcin; aspergillin; restrictocin; and ribonucleases such as placental ribonuclease, angiogenic, diphtheria toxin, or pseudomonas exotoxin. A preferred toxin moiety for use in connection with the invention is toxin A chain which has been treated to modify
25 or remove carbohydrate residues, deglycosylated A chain. (US Patent 5,776,427).

Infusion of one or a combination of such cytotoxic agents (*e.g.*, PD-L2 ricin (alone or in combination with PD-L1-ricin), into a patient may result in the death of immune cells, particularly in light of the fact that activated immune cells that express higher amounts of PD-L2 binding partners, *e.g.*, PD-1. For example, because PD-1 is
30 induced on the surface of activated lymphocytes, a PD-L2 polypeptide can be used to target the depletion of these specific cells by Fc-R dependent mechanisms or by ablation by conjugating a cytotoxic drug (*e.g.*, ricin, saporin, or calicheamicin) to the PD-L2 polypeptide. In one another embodiment, the toxin can be conjugated to an anti-PD-L2 antibody in order to target for death PD-L2-expressing antigen-presenting cell. In a

further embodiment, the PD-L2-antibody-toxin can be a bispecific antibody. Such bispecific antibodies are useful for targeting a specific cell population, *e.g.*, using a marker found only on a certain type of cell, *e.g.*, B lymphocytes, monocytes, dendritic cells, or Langerhans cells.

5 Downregulating immune responses by activating PD-L2 activity or the PD-L2-PD-1 interaction (and thus stimulating the negative signaling function of PD-1) is useful in downmodulating the immune response, *e.g.*, in situations of tissue, skin and organ transplantation, in graft-versus-host disease (GVHD), or allergies, or in autoimmune diseases such as systemic lupus erythematosus and multiple sclerosis. For example, blockage of immune cell function results in reduced tissue destruction in tissue
10 transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by immune cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which promotes the activity of PD-L2 or the interaction of PD-L2 with its natural binding partner(s), *e.g.*, PD-1, on
15 immune cells (such as a soluble, multimeric form of a PD-L2 polypeptide) alone or in conjunction with another downmodulatory agent prior to or at the time of transplantation can inhibit the generation of a costimulatory signal. Moreover, promotion of PD-L2 activity or PD-L2-PD-1 interaction (and thus, a PD-1 inhibitory signal) may also be sufficient to anergize the immune cells, thereby inducing tolerance in a subject.

20 Induction of long-term tolerance by promoting a PD-1-mediated inhibitory signal may avoid the necessity of repeated administration of these activating reagents.

To achieve sufficient immunosuppression or tolerance in a subject, it may also be desirable to block the costimulatory function of other molecules. For example, it may be desirable to block the function of B7-1 and B7-2 by administering a soluble
25 form of a combination of peptides having an activity of each of these antigens or blocking antibodies against these antigens (separately or together in a single composition) prior to or at the time of transplantation. Alternatively, it may be desirable to promote inhibitory activity of PD-L2 and inhibit a costimulatory activity of B7-1 and/or B7-2. Other downmodulatory agents that can be used in connection with the
30 downmodulatory methods of the invention include, for example, agents that transmit an inhibitory signal via CTLA4, soluble forms of CTLA4, antibodies that activate an inhibitory signal via CTLA4, blocking antibodies against other immune cell markers, or soluble forms of other receptor ligand pairs (*e.g.*, agents that disrupt the interaction

between CD40 and CD40 ligand (*e.g.*, anti CD40 ligand antibodies)), antibodies against cytokines, or immunosuppressive drugs.

For example, activating PD-L2 activity or the interaction of PD-L2 with its natural binding partner(s), *e.g.*, PD-1 (and thus the inhibitory function of said binding partner(s)), may also be useful in treating autoimmune disease. Many autoimmune disorders are the result of inappropriate activation of immune cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive immune cells may reduce or eliminate disease symptoms. Administration of agents that promote activity of PD-L2 or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, may induce antigen-specific tolerance of autoreactive immune cells which could lead to long-term relief from the disease. Additionally, co-administration of agents which block costimulation of immune cells by disrupting receptor-ligand interactions of B7 molecules with costimulatory receptors may be useful in inhibiting immune cell activation to prevent production of autoantibodies or cytokines which may be involved in the disease process. The efficacy of reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythematosus in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., *Fundamental Immunology*, Raven Press, New York, 1989, pp. 840-856).

Inhibition of immune cell activation is useful therapeutically in the treatment of allergies and allergic reactions, *e.g.*, by inhibiting IgE production. An agent that promotes PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, can be administered to an allergic subject to inhibit immune cell-mediated allergic responses in the subject. Stimulation PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, can be accompanied by exposure to allergen in conjunction with appropriate MHC molecules. Allergic reactions can be systemic or local in nature, depending on the route of entry of the allergen and the pattern of deposition of IgE on mast cells or basophils. Thus, immune cell-mediated allergic responses can be inhibited locally or systemically by administration of an agent that promotes PD-L2 activity or PD-L2-PD-1 interaction.

Inhibition of immune cell activation through stimulation of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, may also be important therapeutically in pathogenic infections of immune cells (*e.g.*, by viruses or bacteria). For example, in the acquired immune deficiency syndrome (AIDS), viral replication is stimulated by immune cell activation. Stimulation of PD-L2 activity or PD-L2-PD-1 interaction may result in inhibition of viral replication and thereby ameliorate the course of AIDS.

Downregulation of an immune response via stimulation of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, may also be useful in promoting the maintenance of pregnancy. PD-L2 is normally highly expressed in placental trophoblasts, the layer of cells that forms the interface between mother and fetus and may play a role in preventing maternal rejection of the fetus. Females at risk for spontaneous abortion (*e.g.*, those identified by screening for PD-L2 activity, as described in the "Prognostic Assays" section, those who have previously had a spontaneous abortion or those who have had difficulty conceiving) because of immunologic rejection of the embryo or fetus can be treated with agents that stimulate the activity of PD-L2 or its interaction with its natural binding partner(s), *e.g.*, PD-1.

Downregulation of an immune response via stimulation of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, may also be useful in treating an autoimmune attack of autologous tissues. For example, PD-L2 is normally highly expressed in the heart and protects the heart from autoimmune attack. This is evidenced by the fact that the Balb/c PD-1 knockout mouse exhibits massive autoimmune attack on the heart with thrombosis. Thus, conditions that are caused or exacerbated by autoimmune attack (*e.g.*, in this example, heart disease, myocardial infarction or atherosclerosis) may be ameliorated or improved by increasing PD-L2 activity or PD-L2 binding to its natural binding partner, *e.g.*, PD-1. It is therefore within the scope of the invention to modulate conditions exacerbated by autoimmune attack, such as autoimmune disorders (as well as conditions such as heart disease, myocardial infarction, and atherosclerosis) by stimulating PD-L2 activity or PD-L2 interaction with PD-L1.

In an additional embodiment, in performing any of the methods described herein, it is within the scope of the invention to downregulate an immune response by stimulating the activities of both PD-L2 and PD-L1. PD-L1 is an additional PD-1 ligand which is described in the co-pending U.S. Application Serial No. 09/644,934;

International Publication WO 01/14557; Dong, H. *et al.* (1999) *Nat. Med.* 5:1365-1369; and Freeman, G.J. *et al.* (2000) *J. Exp. Med.* 192:1027-1034; the contents of each of which are incorporated herein by reference. In a further additional embodiment, in performing any of the methods described herein, it is within the scope of the invention to downregulate an immune response by administering one or more additional agents. For example, the use of other agents known to downregulate the immune response can be used in conjunction with an agent that stimulates PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1.

10 4. Upregulation of Immune Responses

Inhibition of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, as a means of upregulating immune responses is also useful in therapy. Upregulation of immune responses can be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through inhibition of PD-L2 activity or PD-L2-PD-1 interaction is useful in cases of infections with microbes, *e.g.*, bacteria, viruses, or parasites, or in cases of immunosuppression. For example, in one embodiment, an agent that inhibits PD-L2 activity or PD-L2-PD-1 interaction, *e.g.*, a non-activating antibody (*i.e.*, a blocking antibody) against PD-L2, a combination of non-activating antibodies against PD-L2 and PD-L1, or a soluble form of PD-L2, is therapeutically useful in situations where upregulation of antibody and cell-mediated responses, resulting in more rapid or thorough clearance of a virus, bacterium, or parasite, would be beneficial. These conditions include viral skin diseases such as Herpes or shingles, in which case such an agent can be delivered topically to the skin. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of such agents systemically. In certain instances, it may be desirable to further administer other agents that upregulate immune responses, for example, forms of B7 family members that transduce signals via costimulatory receptors, in order further augment the immune response.

30 Alternatively, immune responses can be enhanced in an infected patient by removing immune cells from the patient, contacting immune cells *in vitro* with an agent that inhibits the PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, and reintroducing the *in vitro*-stimulated immune cells into the patient. In another embodiment, a method of enhancing immune responses involves isolating

infected cells from a patient, *e.g.*, virally infected cells, transfecting them with a nucleic acid molecule encoding a form of PD-L2 that cannot bind its natural binding partner(s), *e.g.*, PD-1, such that the cells express all or a portion of the PD-L2 molecule on their surface, and reintroducing the transfected cells into the patient. The transfected cells
 5 may be capable of preventing an inhibitory signal to, and thereby activating, immune cells *in vivo*.

A agent that inhibits PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, can be used prophylactically in vaccines against various polypeptides, *e.g.*, polypeptides derived from pathogens. Immunity against a pathogen,
 10 *e.g.*, a virus, can be induced by vaccinating with a viral polypeptide along with an agent that inhibits PD-L2 activity or PD-L2-PD-1 interaction, in an appropriate adjuvant. Alternately, a vector comprising genes which encode for both a pathogenic antigen and a form of PD-L2 that blocks PD-L2-PD-1 interaction can be used for vaccination. Nucleic acid vaccines can be administered by a variety of means, for example, by
 15 injection (*e.g.*, intramuscular, intradermal, or the biolistic injection of DNA-coated gold particles into the epidermis with a gene gun that uses a particle accelerator or a compressed gas to inject the particles into the skin (Haynes *et al.* (1996) *J. Biotechnol.* 44:37)). Alternatively, nucleic acid vaccines can be administered by non-invasive means. For example, pure or lipid-formulated DNA can be delivered to the respiratory
 20 system or targeted elsewhere, *e.g.*, Peyer's patches by oral delivery of DNA (Schubbert (1997) *Proc. Natl. Acad. Sci. USA* 94:961). Attenuated microorganisms can be used for delivery to mucosal surfaces (Sizemore *et al.* (1995) *Science* 270:29).

In another embodiment, the antigen in the vaccine is a self-antigen. Such a vaccine is useful in the modulation of tolerance in an organism. Immunization with a
 25 self antigen and an agent that blocks PD-L2 activity or PD-L2 interaction with its natural binding partner (*e.g.*, PD-1) can break tolerance (*i.e.*, interfere with tolerance of a self antigen). Such a vaccine may also include adjuvants such as alum or cytokines (*e.g.*, GM-CSF, IL-12, B7-1, or B7-2).

In one embodiment, an agent which inhibits PD-L2 activity or PD-L2 interaction
 30 with its natural binding partner(s), *e.g.*, PD-1, can be administered with class I MHC polypeptides by, for example, a cell transfected to coexpress a PD-L2 polypeptide or blocking antibody and MHC class I α chain polypeptide and β_2 microglobulin to result in activation of T cells and provide immunity from infection. For example, viral

pathogens for which vaccines are useful include: hepatitis B, hepatitis C, Epstein-Barr virus, cytomegalovirus, HIV-1, HIV-2, tuberculosis, malaria and schistosomiasis.

In another application, inhibition of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, can be useful in the treatment of tumor immunity.

- 5 Tumor cells (*e.g.*, sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, or carcinoma) can be transfected with a nucleic acid molecule that inhibits PD-L2 activity or PD-L2-PD-1 interaction. These molecules can be, *e.g.*, nucleic acid molecules which are antisense to PD-L2, or can encode non-activating anti-PD-L2 antibodies or combinations of anti-PD-L2 and anti-PD-L1 antibodies. These molecules can also be the variable region of an anti-PD-L2 antibody and/or an anti-PD-L1 antibody. If
10 desired, the tumor cells can also be transfected with other polypeptides which activate costimulation (*e.g.*, B7-1 or B7-2). The transfected tumor cells are returned to the patient, which results in inhibition (*e.g.*, local inhibition) of PD-L2 activity or PD-L2-PD-1 interaction. Alternatively, gene therapy techniques can be used to target a tumor
15 cell for transfection *in vivo*.

- Stimulation of an immune response to tumor cells can also be achieved by inhibiting PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, by treating a patient with an agent that inhibits PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1. Preferred examples of such
20 agents include, *e.g.*, antisense nucleic acid molecules, antibodies that recognize and block PD-L2, a combination of antibodies that recognize and block PD-L2 and antibodies that recognize and block PD-L1, and compounds that block the interaction of PD-L2 with its naturally occurring binding partner(s) on an immune cell (*e.g.*, soluble, monovalent PD-L2 molecules; soluble forms of PD-L2 molecules that do not bind to Fc
25 receptors on antigen presenting cells; soluble forms of PD-L2 binding partner(s); and compounds identified in the subject screening assays). In a most preferred embodiment, the PD-L2 binding partner is PD-1.

- In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to express sufficient amounts of MHC class I or MHC class II molecules, can
30 be transfected with nucleic acid encoding all or a portion of (*e.g.*, a cytoplasmic-domain truncated portion) of an MHC class I α chain polypeptide and β_2 microglobulin polypeptide or an MHC class II α chain polypeptide and an MHC class II β chain polypeptide to thereby express MHC class I or MHC class II polypeptides on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with an

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PD-L2 inhibiting polypeptide or antisense nucleic acid induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II-associated polypeptide, such as the invariant chain, can also be cotransfected with a DNA encoding a PD-L2 inhibiting polypeptide or antisense nucleic acid to promote presentation of tumor associated antigens and induce tumor specific immunity. Expression of B7-1 by B7-negative murine tumor cells has been shown to induce T cell mediated specific immunity accompanied by tumor rejection and prolonged protection to tumor challenge in mice (Chen, L. *et al.* (1992) *Cell* 71:1093-1102; Townsend, S.E. and Allison, J.P. (1993) *Science* 259:368-370; Baskar, S. *et al.* (1993) *Proc. Natl. Acad. Sci.* 90:5687-5690). Thus, the induction of an immune cell-mediated immune response in a human subject can be sufficient to overcome tumor-specific tolerance in the subject.

In another embodiment, the immune response can be stimulated by the inhibition of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, such that preexisting tolerance is overcome. For example, immune responses against antigens to which a subject cannot mount a significant immune response, *e.g.*, tumor-specific antigens, can be induced by administering an agent that inhibits the activity of PD-L2 activity or the ability of PD-L2 to bind to its natural binding partner, *e.g.*, PD-1. PD-1 antagonists can be used as adjuvants to boost responses to foreign antigens in the process of active immunization.

In one embodiment, immune cells are obtained from a subject and cultured *ex vivo* in the presence of an agent that inhibits PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1, to expand the population of immune cells. In a further embodiment the immune cells are then administered to a subject. Immune cells can be stimulated to proliferate *in vitro* by, for example, providing the immune cells with a primary activation signal and a costimulatory signal, as is known in the art. Various forms of PD-L2 polypeptides or agents that inhibit PD-L2 activity or PD-L2-PD-1 and/or PD-L1/PD-1 interaction can also be used to costimulate proliferation of immune cells. In one embodiment, immune cells are cultured *ex vivo* according to the methods described in PCT Application No. WO 94/29436. The costimulatory molecule can be soluble, attached to a cell membrane or attached to a solid surface, such as a bead.

In an additional embodiment, in performing any of the methods described herein, it is within the scope of the invention to upregulate an immune response by administering one or more additional agents. For example, the use of other agents known to stimulate the immune response, such as cytokines, adjuvants, or stimulatory forms of costimulatory molecules or their ligands can be used in conjunction with an agent that inhibits PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1. For example, an agent that inhibits PD-L1 (*e.g.*, a blocking antibody against PD-L1) can be used in conjunction with an agent that inhibits PD-L2 (*e.g.*, a blocking antibody against PD-L2). PD-L1 is an additional PD-1 ligand which is described in the co-pending U.S. Application Serial No. 09/644,934; International Publication WO 01/14557; Dong, H. *et al.* (1999) *Nat. Med.* 5:1365-1369; and Freeman, G.J. *et al.* (2000) *J. Exp. Med.* 192:1027-1034; the contents of each of which are incorporated herein by reference.

E. Identification of Cytokines Modulated by Modulation of PD-L2 Activity or PD-L2-PD-1 Interaction

The PD-L2 molecules described herein can be used to identify cytokines which are produced by or whose production is enhanced or inhibited in immune cells in response to modulation of PD-L2 activity or PD-L2 interaction with its natural binding partner(s), *e.g.*, PD-1. Immune cells expressing PD-1 can be suboptimally stimulated *in vitro* with a primary activation signal, for example, T cells can be stimulated with phorbol ester, anti-CD3 antibody or preferably, antigen, in association with an MHC class II molecule, and given a costimulatory signal, *e.g.*, by a stimulatory form of B7 family antigen, for instance by a cell transfected with nucleic acid encoding a B7 polypeptide and expressing the peptide on its surface, or by a soluble, stimulatory form of the peptide. The cells can then be contacted with cells expressing PD-L2 and/or treated with agents which inhibit PD-L2-PD-1 interaction (*e.g.*, antibodies against PD-L2, or combinations of antibodies against PD-L2 and antibodies against PD-L1). Known cytokines released into the media can be identified by ELISA or by the ability of an antibody which blocks the cytokine to inhibit immune cell proliferation or proliferation of other cell types that are induced by the cytokine. For example, an IL-4 ELISA kit is available from Genzyme (Cambridge, MA), as is an IL-7 blocking antibody. Blocking antibodies against IL-9 and IL-12 are available from Genetics

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Institute (Cambridge, MA). The effect of stimulating or blocking PD-L2 activity or the interaction of PD-L2 and PD-1 on the cytokine profile can then be determined.

An *in vitro* immune cell costimulation assay as described above can also be used in a method for identifying novel cytokines which can be modulated by modulation of PD-L2 activity or PD-L2-PD-1 interaction. For example, where stimulation of the CD28/CTLA4 pathway seems to enhance IL-2 secretion, stimulation of the ICOS pathway seems to enhance IL-10 secretion (Hutloff *et al.* (1999) *Nature* 397:263). If a particular activity induced upon costimulation, *e.g.*, immune cell proliferation, cannot be inhibited by addition of blocking antibodies to known cytokines, the activity may result from the action of an unknown cytokine. Following costimulation, this cytokine can be purified from the media by conventional methods and its activity measured by its ability to induce immune cell proliferation.

To identify cytokines which may play a role the induction of tolerance, an *in vitro* T cell costimulation assay as described above can be used. In this case, T cells would be given the primary activation signal and contacted with a selected cytokine, but would not be given the costimulatory signal. After washing and resting the immune cells, the cells would be rechallenged with both a primary activation signal and a costimulatory signal. If the immune cells do not respond (*e.g.*, proliferate or produce cytokines) they have become tolerized and the cytokine has not prevented the induction of tolerance. However, if the immune cells respond, induction of tolerance has been prevented by the cytokine. Those cytokines which are capable of preventing the induction of tolerance can be targeted for blockage *in vivo* in conjunction with reagents which block B lymphocyte antigens as a more efficient means to induce tolerance in transplant recipients or subjects with autoimmune diseases. For example, one could administer a cytokine blocking antibody to a subject along with an agent that promotes PD-L2 activity or PD-L2-PD-1 interaction.

This invention is further illustrated by the following examples, which should not be construed as limiting. The contents of all references, patents, and published patent applications cited throughout this application, as well as the Figures and Sequence Listing, are incorporated herein by reference.

EXAMPLES

The following Materials and Methods were used in Examples 1-9.

5 Materials and Methods

Mice

Hoffman-La Roche, Nutley, NJ, provided mice expressing a TCR transgene (DO11.10) which is specific for OVA peptide (323-339) in association with IA^d. Balb/c mice were obtained from Taconic Farms (Germantown, NY). All mice were used between the ages of 6-12 weeks. The mice were cared for in accordance with institutional guidelines.

Molecular cloning

15 A BLAST search of the non-redundant database of the National Center for Biotechnology Information (NCBI) using the human PD-L1 protein sequence as a query identified GenBank Accession No. AF142780 (also referred to herein as mouse PD-L2) as having 38% amino acid identity with PD-L1. PD-L1 is a PD-1 ligand described in the co-pending U.S. Application Serial No. 09/644,934; International Publication WO
20 01/14557; Dong, H. *et al.* (1999) *Nat. Med.* 5:1365-1369; and Freeman, G.J. *et al.* (2000) *J. Exp. Med.* 192:1027-1034; the contents of each of which are incorporated herein by reference. The AF142780 coding region was amplified by PCR using as primers 5'-dGTCGACCACCATGCTGCTCCTGCTGCCGATA-3' (SEQ ID NO:7) and 5'-dGTCGACTCACTAGATCCTCTTTCTCTGGATTATCAC-3' (SEQ ID NO:8) and
25 cloned into pEF6 (Invitrogen, Carlsbad, CA). A search of the NCBI EST database identified 2 human fetal heart ESTs (GenBank Accession Nos. AA247117 and AA247128) with homology to murine PD-L2. A 101 bp region was amplified by PCR using as primers 5'-dGTACATAATAGAGCATGGCAGCA-3' (SEQ ID NO:9) and 5'-dCCACCTTTTGCAAAGCTGGCTGT-3' (SEQ ID NO:10). The PCR product was
30 biotin-labeled and used to isolate a full-length cDNA by Cloncapture (Clontech, Palo Alto, CA) from a human placenta cDNA library in the pAXEF vector (Freeman, G.J. *et al.* (2000) *J. Exp. Med.* 192:1027-1034). The full-length human PD-L2 cDNA sequence has been deposited with GenBank (accession number to be assigned).

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Fusion proteins and cell transfections

The Ig fusion proteins consist of the complete extracellular region of a receptor linked to the hinge-CH2-CH3 domains of murine Ig γ 2a (with four point mutations blocking Fc receptor and complement binding) to give Ig(γ 2a) fusions (Duncan, A.R. *et al.* (1988) *Nature* 332:563-564; Morgan, A. *et al.* (1995) *Immunology* 86:319-324). Control.Ig consists of the Oncostatin-M leader sequence linked to murine Ig γ 2a. These recombinant proteins were produced in stably transfected CHO cell lines and purified from conditioned media using protein A-Sepharose. The murine PD-L2 cDNA in pEF6 was linearized with ScaI and co-electroporated into CHO.I-A^d, or CHO.I-A^d.mB7-2 cells with a plasmid construct containing a puromycin resistance gene under the control of a phosphoglycerate kinase gene promoter. The human PD-L2 cDNA in pAXEF was linearized with ApaLI and co-electroporated into CHO.K1 cells with a puromycin-resistance gene under the control of a phosphoglycerate kinase gene promoter. Transfectants were selected in 10 μ g/ml puromycin, stained with hPDI.Ig, sorted, and cloned by limiting dilution.

Northern blot analysis

Mouse and human multiple tissue northern blots (Clontech, Palo Alto, CA) were probed with ³²P-dCTP radiolabeled cDNA probes in QuikHyb (Stratagene, La Jolla, CA) according to manufacturer's instructions. The human PD-L2 probe consisted of a 1.2 kb XbaI fragment spanning the coding region and 3' UTR sequence. The mouse PD-L2 probe consisted of a 444 kb KpnI/EcoRV cDNA fragment spanning the coding region. Actin probes were supplied by Clontech. Blots were washed twice at room temperature in 2X SSC, 0.1% SDS, followed by 0.1X SSC, 0.1% SDS at 65°C, and examined by autoradiography.

Flow cytometry

For detection of PD-L2, 5x10⁴ transfected CHO cells were incubated with 5 μ g/ml of human PD-Ig (hPD-I.Ig) (Genetics Institute, Cambridge, MA) and developed with goat anti-mouse IgG2a-phycoerythrin (PE) (Southern Biotechnology Associates Inc, Birmingham, AL). In addition, cells were stained separately with 5 μ g/ml anti-IA^d-PE or B7.2-PE (Pharmingen, San Diego, CA).

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CD4⁺ T cells were incubated with biotinylated anti-PD-1 (or biotinylated anti-CD28 (Pharmingen) or biotinylated anti-CD25 (Pharmingen) and developed with streptavidin-PE (Pharmingen). All isotype controls were obtained from Pharmingen. Following each step, cells were washed three times with PBS/1% BSA/0.02% sodium azide. After the final incubation, cells were fixed with 1% paraformaldehyde. Ten thousand events were analyzed on a FACSCalibur (Becton Dickinson, Mountain View, CA). All isotype controls were all obtained from Pharmingen.

Activation of T cells

To generate activated antigen-specific T cells, splenocytes were prepared from DO11.10 mice and treated with Tris-NH₄Cl to deplete erythrocytes. Cells were cultured with 1 µg/ml of OVA peptide for 72 hours (Analytical Biotechnology Services, Boston, MA) in RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS (Sigma, St Louis, MO), 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, 250 ng/ml amphotericin B, 10 mM Hepes, 50 pM 2-ME (all from Life Technologies) and 15 mg/ml of gentamicin (BioWhittaker, Walkersville, MD). CD4⁺ T cells were purified by positive selection using magnetic-activated cell sorting separation columns (Miltenyi Biotec, Auburn, CA) with resulting purity of >98%. Cells were rested overnight before re-stimulation.

Bead Stimulation of T cells

Anti-CD3 Ab (2C11; Pharmingen, La Jolla, CA), mPD-L2.Ig, and control.Ig were covalently attached to polyurethane-coated tosyl-activated Dynabeads (Dyna, Lake Success, NY). Beads were prepared with a constant sub-optimal anti-CD3 Ab concentration (60% of the total bound protein) and mPD-L2.Ig or control Ig (40% of total bound protein). Beads have a protein binding capacity of 5 µg/10⁷ beads. T cells were purified from Balb/c lymph nodes by negative selection using cell enrichment columns (R & D Systems, Minneapolis, MN). 0.5-1x10⁵ T cells were cultured per well in 96 well plates with a 2:1 ratio of the indicated coated tosyl beads:cells. Proliferation was measured by [³H]-thymidine incorporation for the last 10 hours of a 72 hr culture.

CHO cell stimulation of T cells

Proliferation of transfected CHO cells was inhibited by incubation with 50 µg/ml of mitomycin C (Bristol Laboratories, Princeton, NJ) or 16 hours at 37°C. At the end of

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the incubation period, the cells were harvested with 10 mM EDTA in PBS, washed twice and left on ice for 1 hour. The cells were subsequently washed three times and resuspended in culture medium. 10^5 previously activated $CD4^+$ T cells were cultured with varying concentrations of OVA peptide and 10^4 mitomycin C-treated CHO transfectants in 96 well plates. To assay proliferation, cultures were incubated for 48 hrs and pulsed with 1 μ Ci/well of [^{3} H] thymidine (New England Nuclear, Boston, MA) for the last 6 hours of the incubation period.

Cytokine ELISA

Aliquots of supernatants were harvested at various times after initiation of cultures. IL-2, IL-4, IFN- γ and IL-10 levels were analyzed using mAbs and recombinant cytokine standards from Pharmingen. Detection limits were as follows: IL-2: 20 pg/ml; IL-4: 40 pg/ml; IFN- γ : 100 pg/ml; and IL-10: 200 pg/ml.

RNAase protection assay (RPA)

$CD4^+$ T cells were restimulated with various CHO cell transfectants and 0.01 μ g/ml OVA peptide. After 48 hours, cells were harvested and mRNA was isolated using TRIzol[®] reagent (Life Technologies, Grand Island, NY). 5 μ g mRNA was analyzed for cytokine levels by RNAase protection assay using RiboQuant multiprobe kit mCK1 according to manufacturer's instructions (Pharmingen, San Diego, CA).

Cell Cycle Analysis

$CD4^+$ T cells were restimulated with 0.01 μ g/ml peptide and CHO transfectants as described above. After 36 hours of culture, cells were recovered and stained with anti-CD4-FITC. Cells were washed in PBS, fixed in 70% ethanol for 1 hour on ice and then resuspended in PBS containing 10 μ g/ml RNase (Sigma, St. Louis, MO) and 50 μ g/ml propidium iodide (Sigma). Analysis was performed within an hour of staining using a FACSCalibur.

EXAMPLE 1: IDENTIFICATION AND CHARACTERIZATION OF HUMAN AND MOUSE PD-L2 cDNA

In this example, the identification and characterization of the genes encoding human PD-L2 and mouse PD-L2 is described.

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Isolation of the human PD-L2 cDNA

The invention is based, at least in part, on the discovery of human genes encoding novel polypeptides, referred to herein as human PD-L2. The human PD-L2 cDNA was isolated from a human placental cDNA library using as a probe the human
 5 EST AA247117. The entire sequence of human PD-L2 was determined and found to contain an open reading frame termed human "PD-L2"

The nucleotide sequence encoding the human PD-L2 is shown in Figure 1 and is set forth as SEQ ID NO:1. The polypeptide encoded by this nucleic acid comprises about 273 amino acids and has the amino acid sequence shown in Figure 1 and set forth
 10 as SEQ ID NO:2. The coding region (open reading frame) of SEQ ID NO:1 is set forth as SEQ ID NO:3.

The nucleotide sequence encoding the mouse PD-L2 is shown in Figure 2 and is set forth as SEQ ID NO:4. The polypeptide encoded by this nucleic acid comprises about 247 amino acids and has the amino acid sequence shown in Figure 2 and set forth
 15 as SEQ ID NO:5. The coding region (open reading frame) of SEQ ID NO:4 is set forth as SEQ ID NO:6.

Analysis of the Human and Mouse PD-L2 Molecules

Each of the amino acid sequences of human and mouse PD-L2 was analyzed by
 20 comparison to other B7 family members for the presence of a signal peptide. These analyses resulted in the identification of a signal peptide domain in the amino acid sequence of the native human PD-L2 (SEQ ID NO:2) at about residues 1-19 (Figure 3). These analyses further identified a signal peptide domain in the amino acid sequence of the native mouse PD-L2 (SEQ ID NO:5) at about residues 1-19 (Figure 3).

25 Each of the amino acid sequences of human and mouse PD-L2 was also analyzed by comparison to other B7 family members for the presence of an IgV domain. These analyses resulted in the identification of an IgV domain in the amino acid sequence of the native human PD-L2 (SEQ ID NO:2) at about residues 20-120 (Figure 3), and at about residues 1-101 in the predicted mature polypeptide. These analyses further
 30 identified an IgV domain in the amino acid sequence of the native mouse PD-L2 (SEQ ID NO:5) at about residues 20-120 (Figure 3), and at about residues 1-101 in the predicted mature polypeptide.

Additionally, each of the amino acid sequences of human and mouse PD-L2 was analyzed by comparison to other B7 family members for the presence of an IgC domain. These analyses resulted in the identification of an IgC domain in the amino acid sequence of the native human PD-L2 (SEQ ID NO:2) at about residues 121-219 (Figure 3), and at about residues 102-200 in the predicted mature polypeptide. These analyses further identified an IgC domain in the amino acid sequence of the native mouse PD-L2 (SEQ ID NO:5) at about residues 121-219 (Figure 3), and at about residues 102-200 in the predicted mature polypeptide.

Each of the amino acid sequences of human and mouse PD-L2 was further analyzed by comparison to other B7 family members for the presence of an extracellular domain. These analyses resulted in the identification of an extracellular domain in the amino acid sequence of the native human PD-L2 (SEQ ID NO:2) at about residues 1-219 (Figure 1), and at about residues 1-200 in the predicted mature polypeptide. These analyses further identified an extracellular domain in the amino acid sequence of the native mouse PD-L2 (SEQ ID NO:5) at about residues 1-219 (Figure 2), and at about residues 1-200 in the predicted mature polypeptide.

Each of the amino acid sequences of human and mouse PD-L2 was also analyzed by comparison to other B7 family members for the presence of a transmembrane domain. These analyses resulted in the identification of a transmembrane domain in the amino acid sequence of the native human PD-L2 (SEQ ID NO:2) at about residues 220-243 (Figure 3), and at about residues 201-224 in the predicted mature polypeptide. These analyses further identified a transmembrane domain in the amino acid sequence of the native mouse PD-L2 (SEQ ID NO:5) at about residues 220-242 (Figure 3), and at about residues 201-223 in the predicted mature polypeptide.

Each of the amino acid sequences of human and mouse PD-L2 was further analyzed by comparison to other B7 family members for the presence of a cytoplasmic domain. These analyses resulted in the identification of a cytoplasmic domain in the amino acid sequence of the native human PD-L2 (SEQ ID NO:2) at about residues 244-273 (Figure 3), and at about residues 225-254 in the predicted mature polypeptide. These analyses further identified a cytoplasmic domain in the amino acid sequence of the native mouse PD-L2 (SEQ ID NO:5) at about residues 243-247 (Figure 3), and at about residues 224-228 in the predicted mature polypeptide.

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The mouse PD-L2 protein has 38% amino acid identity with mouse PD-L1 (see Figure 6). Mouse and human PD-L2 have 70% amino acid identity (Figure 6), which is higher than the 46-50% identity between human and murine B7-1 or B7-2.

5 **EXAMPLE 2: EXPRESSION OF RECOMBINANT PD-L2 POLYPEPTIDE IN BACTERIAL CELLS**

In this example, human PD-L2 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, PD-L2 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-PD-L2 fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

20 **EXAMPLE 3: BINDING OF PD-L2 TO PD-1**

COS cells were transfected with an expression plasmid containing AF142780, the mouse PD-L2 cDNA, or a control mouse PD-1 ligand. After 72 hours, the transfected COS cells were detached by incubation in PBS containing 0.5 mM EDTA for 30 min. at 37°C.

The ability of COS cells expressing PD-L2 to bind to various Ig fusion proteins was tested. FACS analysis of binding of IgG2a (control Ig), ICOS-IgG, and PD-1-Ig by PD-L2-transfected COS cells showed that neither IgG2a or ICOS-IgG was bound by PD-L2 or the control PD-1 ligand. PD-1-Ig, however, was shown to bind to PD-L2 and to the control PD-1 ligand (Figure 4).

Experiments using the human PD-L2 cDNA yielded results similar to those obtained using the mouse PD-L2 cDNA.

Experiments were also performed using CHO cells. Flow cytometry studies indicate that hPD-1-Ig recognizes CHO cells stably transfected with PD-L2 (Fig. 7). This staining was specific, since there was no binding to control transfectants (CHO-IA^d, CHO-IA^d/B7.2). CHO cells transfected with PD-L2 showed no staining with CTLA4-Ig, CD28-Ig or ICOS-Ig. An alternatively spliced variant of murine PD-

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L2, wherein the IgV exon is deleted, was also isolated. This variant did not bind PD-1-Ig, indicating that the IgV domain is necessary for PD-L2 binding to PD-1.

EXAMPLE 4: GENERATION OF FULLY HUMAN ANTIBODIES TO PD L2

5 In this example, fully human antibodies against PD-L2 are made in mice that are transgenic for human immunoglobulin framework genes. Transgenic mice are made using standard methods, *e.g.*, according to Hogan *et al.*, *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory, which is incorporated herein by reference, or are purchased commercially. Embryonic stem cells are
10 manipulated according to published procedures (*Teratocarcinomas and embryonic stem cells: a practical approach*, Robertson, E.J. ed., IRL Press, Washington, D.C., 1987; Zijlstra *et al.* (1989) *Nature* 342:435-438; and Schwartzberg *et al.* (1989) *Science* 246:799-803, each of which is incorporated herein by reference). DNA cloning procedures are carried out according to Sambrook, J. *et al.* in *Molecular Cloning: A Laboratory Manual*, 2d ed., 1989, Cold Spring Harbor Laboratory Press, Cold Spring
15 Harbor, N.Y., which is incorporated herein by reference. Oligonucleotides are synthesized, *e.g.*, on an Applied Bio Systems oligonucleotide synthesizer according to specifications provided by the manufacturer or are purchased commercially.

Transgenic mice are immunized using a purified or recombinant PD-L2 or a
20 fusion protein comprising at least an immunogenic portion of the extracellular domain of PD-L2. Approximately four hundred µg of PD-L2 in 100 µL of phosphate buffered saline (PBS) is injected intraperitoneally into each mouse. Serum samples are collected approximately six days later by retro-orbital sinus bleeding.

Antibody reactivity and specificity for PD-L2 are assessed using an indirect
25 enzyme-linked immunosorbent assay (ELISA). Several immunoglobulin superfamily molecules are tested as controls (*e.g.*, CTLA4 and CD28) to analyze the antibody specificity of the antibody for PD-L2. Antibodies having human framework regions which bind to PD-L2 are detected by enzyme conjugates specific for human IgM and human IgG sub-classes with no cross reactivity to mouse immunoglobulin. Briefly,
30 PVC microtiter plates are coated with PD-L2 by coating wells overnight at 37°C with 5 µg/mL PD-L2 in PBS. Serum samples are diluted in PBS, 5% serum, 0.5% Tween-20 and are incubated in the wells for 1 hour at room temperature, followed by anti-human IgG Fc and IgG F(ab')₂-horseradish peroxidase or anti-human IgM Fc-horseradish peroxidase in the same diluent. After 1 hour at room temperature enzyme activity is

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assessed by addition of ABTS substrate (Sigma, St. Louis, Mo.) and read after 30 minutes at 415-490 nm. In pre-immunization serum samples from the same mice, titers of human antibodies to the same target antigens are also tested.

Spleen cells isolated from mice having appropriate antibody titers are harvested.

- 5 The spleen cells are fused to appropriate fusion partners (*e.g.*, myeloma cells) to make hybridomas. Hybridomas and antibodies are manipulated according to *Antibodies: A Laboratory Manual*, Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988), which is incorporated herein by reference.

10 **EXAMPLE 5: GENERATION OF ANTIBODIES TO PD-L2**

Anti-human PD-L2 antibodies

Anti-PD-L2 antibodies can also be prepared by cDNA immunization of mice.

- For production of anti-human PD-L2 antibodies, female Balb/c mice (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were prepared for cDNA immunization by injecting 50 μ l of 10 mM cardiotoxin (Sigma Chemical Company, St. Louis, MO) in 0.9% saline into
- 15 μ l of 10 mM cardiotoxin (Sigma Chemical Company, St. Louis, MO) in 0.9% saline into the tibialis anterior muscle of each hind limb. Five days later, 50 μ l of 1 mg/ml purified hPD-L2 cDNA in the pAXEF mammalian expression vector in 0.9% saline was injected into each regenerating anterior tibialis anterior muscle of each mouse. The cDNA immunization was repeated twice at 2 week intervals. The mice were then rested for
- 20 four weeks, followed by three boosts of 100 μ g cDNA per mouse, spread 3-4 weeks apart. Five days before fusion, a mouse selected for fusion was boosted with cDNA intramuscularly. Spleen cells were fused with SP2/0 myeloma cells, cloned, and the hybridomas screened by ELISA for reactivity with hPD-L2-mIgG2a fusion protein, followed by cell surface staining of hPD-L2 transfected 300.19 and COS cells and for
- 25 lack of reactivity with untransfected cells and hPD-L1 transfected cells.

- Nine mouse anti-human PD-L2 monoclonal antibodies (mAbs) were isolated. In order to determine the capacity of these antibodies to block the PD-1/PD-L2 interaction, the anti-PD-L2 mAbs were preincubated with PD-L2 transfected cells. Inhibition of the interaction was measured as the capacity of the mAb to reduce the binding of
- 30 biotinylated PD-1-Ig to PD-L2 transfected cells. The best mAbs were antibodies 24F.10C12 (also referred to herein as 10C12) and 24F7G12 (also referred to herein as 7G12). The results are as follows:

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		<u>mean fluorescence intensity of biotinylated</u>
		<u>hPD-1-Ig bound to 300-hPD-L2 cells</u>
	<u>mAb</u>	
	7G12	0.110
	10C12	0.109
5	positive control	2.57

Anti-mouse PD-L2 antibodies

For production of anti-mouse PD-L2 antibodies, female Lewis strain rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were prepared for cDNA immunization by injecting 100 μ l of 10 mM cardiotoxin (Sigma Chemical Company, St. Louis, MO) in 0.9% saline into the tibialis anterior muscle of each hind limb. Five days later, 100 μ l of 1 mg/ml purified murine PD-L2 cDNA in the pEF6 mammalian expression vector in 0.9% saline was injected into each regenerating anterior tibialis anterior muscle of each rat. The cDNA immunization was repeated three times at 2-3 week intervals. The rats were then immunized with 1.5×10^7 CHO-mPD-L2 transfectants four times at 2-5 week intervals. Five days before fusion, a rat selected for fusion was immunized with both cDNA (200 μ g) and cells (5×10^7). Spleen cells were fused with SP2/0 myeloma cells, cloned, and the hybridomas screened by cell surface staining of mPD-L2 transfected 300.19 and COS cells and for lack of reactivity with untransfected cells and mPD-L1 transfected cells. Monoclonal antibodies were produced that specifically recognize mPD-L2.

EXAMPLE 6: EXPRESSION OF PD-L2

To analyze the expression PD-L2, mRNA expression was analyzed by Northern blot hybridization in a variety of tissues and in activated antigen presenting cells. Human and murine PD-L2 mRNAs are expressed highly in normal placenta, and are expressed at low levels in normal spleen, lymph nodes, and thymus. PD-L2 is also expressed in human heart, but is expressed at a low level in mouse heart. Other tissues in which PD-L2 is expressed are human pancreas, lung and liver. PD-L2 mRNA was not detected in unstimulated human monocytes but was upregulated by IFN- γ stimulation. The induction of PD-L2 was slightly delayed in kinetics as compared to the upregulation of PD-L1 (Freeman, G.J. *et al.* (2000) *J. Exp. Med.* 192:1027-1034). Northern blot analysis of murine tumor cell lines revealed PD-L2 mRNA expression in lines of lymphoid origin such as PU5-1.8 (myeloid lymphoma), RAW 264.7

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(macrophage lymphoma), R1.1 (T lymphoma), L1210 (lymphocytic leukemia), P388D1 (monocyte/macrophage lymphoma), and P815 (mastocytoma). PD-L2 was expressed poorly or not at all in fibroblast cell lines (M-MSV Balb/3T3, K-Balb, LM) and the P19 teratocarcinoma cell line. PD-L2 is also expressed in hepatoma (Hepa 1-6) and

5 neuroblastoma (NB41A3) cell lines.

EXAMPLE 7:PD-L2-PD-1 INTERACTION INHIBITS TCR MEDIATED RESPONSES

To investigate the role of the PD-L2-PD-1 pathway in T cell activation, a Dynal bead-based T cell activation system was used. Purified T cells from Balb/c lymph nodes were activated with beads coated with anti-CD3 plus control.Ig or mPD-L2.Ig. Proliferation was measured by ³H-thymidine incorporation. T cells activated with anti-CD3 plus mPD-L2.Ig coated beads showed a marked decrease in proliferation relative to anti-CD3 plus control.Ig activated cells (Figure 8). Thus, engagement of PD-1 on T

10 cells by PD-L2 leads to inhibition of T cell proliferation.

In order to study antigen specific signals, CHO cells co-expressing PD-L2 and I-A^d and CHO cells expressing similar levels of I-A^d alone (Figure 7) were compared in their ability to activate DO11.10 CD4⁺ T cells. Because PD-1 is upregulated after activation, pre-activated DO11.10 T cells were used to enable maximal PD-L2

20 interaction. Pre-activated CD4⁺ T cells were incubated with a range of concentrations of OVA (323-339) peptide and mitomycin-C treated CHO transfectants. As seen in Figure 9, pre-activated DO11.10 CD4⁺ T cells gave a moderate proliferative response to OVA peptide presented by CHO.I-A^d. In contrast, this response was significantly reduced in the presence of PD-L2. Interleukin (IL)-4, IFN- γ and IL-10 productions were also

25 markedly reduced (Fig. 9). IL-2 was not detected under these conditions of activation. These data show that PD-1-PD-L2 interactions can inhibit TCR mediated proliferation and cytokine production. The inhibitory effects of the PD-1-PD-L2 pathway and the expression of PD-L2 in non-lymphoid organs points to a role in controlling autoimmune reactions.

30

EXAMPLE 8:PD-L2 CAN INHIBIT TCR-CD28 SIGNALS

Since optimal T cell clonal expansion requires both TCR and CD28 signals, the interplay between the TCR plus CD28 and PD-L2-PD-1 signals was examined. To address this issue, the following CHO transfectants were used: CHO.I-A^d.B7-2 and

CHO.I-A^d.B7-2.PD-L2. The ability of these CHO transfectants to stimulate previously activated DO11.10 CD4⁺ T cells with OVA peptide was compared. The expression of I-A^d and B7-2 was similarly high on these CHO transfectants (Fig. 7). The level of PD-L2 expression, as measured by PD-1.Ig binding, was high. As expected, introduction of B7-2 led to an increase in proliferative responses by T cells at all antigen concentrations, with the most marked stimulation at low antigen concentrations (Figure 10). Co-expression of PD-L2 on CHO transfectants inhibited TCR and B7-2 mediated proliferative responses at low peptide concentrations (0.01 µg/ml and 0.001 µg/ml) (Figure 10). At 0.01 µg/ml peptide concentration, PD-L2 significantly inhibited TCR-B7-2 mediated cytokine production, consistent with the inhibition of proliferation (Figure 11). At 0.1 µg/ml peptide concentration, where there was only a weak inhibition of proliferation, cytokine production was inhibited when DO11.10 CD4⁺ T cells were cultured with peptide and CHO.I-A^d.B7-2.PD-L2 transfectants (Figure 12). Therefore, PD-1 engagement by PD-L2 can downregulate TCR-CD28 mediated stimulation of cytokine production.

To determine whether the diminished cytokine production was due to reduced mRNA levels, cytokine mRNA levels were measured by RNAase protection assay. Interleukin-4, IL-10, IL-13, IL-2, IL-6 and IFN-γ mRNAs were readily detected in previously activated DO11.10 CD4⁺ T cells after stimulation with 0.01 µg/ml OVA peptide presented by CHO.I-A^d.B7-2. However, the introduction of PD-L2 into the CHO.I-A^d.B7-2 transfectants significantly reduced mRNA levels for both T_H1 and T_H2 cytokines. There was minimal expression of cytokine mRNAs when previously activated T cells were incubated alone or with peptide presented by CHO.I-A^d. These results further demonstrate the capacity of the PD-L2-PD-1 pathways to antagonize a B7-CD28 signal when antigenic stimulation is weak or limiting.

To assess whether the ability of the PD-L2-PD-1 pathway to inhibit at low antigen concentrations was related to variations in levels of CD28 and PD-1 expression at different antigen concentrations, surface expression of PD-1 and CD28 was examined. Table 1 shows the mean fluorescence intensity of PD-1, CD28 and CD25 on DO11.10 CD4⁺ T cells following different activation conditions.

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Table 1: MFI of CD28, PD-1, and CD25 on freshly isolated and previously activated DO11.10 CD4⁺ T cells and during restimulation with CHO.I-A^d.B7-2

	Antibody	MFI							
Freshly isolated CD4 ⁺ DO11.10 T cells	CD28	33							
	PD-1	14							
	CD25	27							
	Isotype	16							
Previously activated CD4 ⁺ DO11.10 T cells		Expt. 1							Expt. 2
	CD28	50							117
	PD-1	51							51
	CD25	373							2705
Restimulated CD4 ⁺ DO11.10 T cells	Isotype	12							17
	Peptide Conc. (μ g/ml)	0.001	0.01	0.1	1.0	0.001	0.01	0.1	1.0
		Expt. 1							Expt. 2
	CD28	77	106	123	165	77	121	338	516
	PD-1	78	56	41	39	117	92	84	55
	CD25	334	417	560	1154	2507	2602	2971	7021
	Isotype	11	14						

CD4⁺ T cells were incubated with biotinylated anti-PD-1, biotinylated anti-CD28, biotinylated anti-CD25, or biotinylated isotype control and developed with streptavidin-PE.

Freshly isolated DO11.10 CD4⁺ T cells expressed CD28, but neither PD-1 nor
 5 CD25. T cells stimulated with 1 μ g/ml of peptide presented by splenic APCs
 upregulated CD28 modestly and strongly upregulated PD-1 and CD25. After
 restimulation of previously activated DO11.10 CD4⁺ T cells with various concentrations
 of peptide presented by CHO.I-A^d.B7.2, CD28 and CD25 expression increased on CD4⁺
 T cells with higher peptide concentration. In contrast, PD-1 expression was highest on
 10 T cells activated at low peptide concentration and decreased at higher peptide
 concentrations. The higher expression of PD-1 at lower antigen doses further suggests a
 mechanism whereby the PD-L2-PD-1 pathway can attenuate weak antigen responses.

EXAMPLE 9: MECHANISM OF ACTION OF THE PD-1-PD-L2 PATHWAY

15 Cross-linking of CTLA-4 has been shown to inhibit cell cycle progression in
 naive T cells (Krummel, M.F. and Allison, J.P. (1996) *J. Exp. Med.* 183:2533-2540;

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Walunas, T.L. *et al.* (1996) *J. Exp. Med.* 183:2541-2550). As PD-1 was isolated from murine cell lines undergoing apoptosis, a possible mechanism of action of the PD-1:PD-L2 pathway might be to increase programmed cell death (e.g., activation-induced cell death or AICD). To address this issue, DO11.10 CD4⁺ T cells were

5 restimulated with 0.01 µg/ml peptide and various CHO transfectants, and cell cycle progression was analyzed. After 48 hours, cells were recovered, stained with CD4-FTTC, permeabilized, and incubated with propidium iodide to analyze the G₀/G₁, S/G₂ and sub-diploid populations. CD4⁺ T cells restimulated with peptide presented by CHO-IA^d or CHO-IA^d/PD-L2 both have a large proportion of cells in the sub-diploid

10 population, indicative of apoptosis (Figure 13). These results were confirmed by annexin staining. In cultures where CD4⁺ T cells were stimulated by peptide presented by CHO-IA^d (1 µg/ml), there were increased number of cells in the S/G₂ phase, indicating that the cells were in cycle. The introduction of PD-L2 into IA^d transfectants led to an increased number of cells in the G₀/G₁ phase, suggesting cell cycle arrest.

15 Cell cycle progression in the presence of B7-2 and PD-L2 signals was then compared. CD4⁺ T cells stimulated by 0.01 µg/ml peptide presented by CHO-IA^d.B7-2 showed an increased number of cells in the S/G₂ phase and a decreased number in the sub-diploid population indicating cells were in cycle and rescued from apoptosis by B7-CD28 costimulation (Figure 14). As seen with the CHO-IA^d transfectants at higher

20 peptide concentrations, introduction of PD-L2 into B7-2 transfectants led to an increased number of cells in the G₀/G₁ phase and a corresponding decrease in those in S phase. There was no difference in the proportion of apoptotic cells between B7-2 and B7-2.PD-L2 stimulated T cells, indicating that PD-1 crosslinking did not lead to an increase in cell death.

25

EXAMPLE 10: STIMULATION OF T CELL ACTIVATION BY INHIBITION OF PD-1:PD-LIGAND INTERACTION

As shown above, signaling via PD-1 is dominated by strong TCR/CD28 costimulatory signals; the PD-1 signaling pathway inhibits moderate TCR/CD28

30 costimulatory signals, with cytokine production being reduced first without a decrease in T cell proliferation. As the TCR/CD28 costimulatory signals weaken, the PD-1 pathway dominates, with a great reduction in cytokine production accompanied by a reduction in proliferation. Accordingly, in order to determine whether inhibition of the PD-1 pathway via inhibition of the interaction with PD-L1 or PD-L2 would enhance T cell

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activation, mixed lymphocyte reactions (MLRs) were performed using weakly functioning antigen presenting cells (APCs) (*i.e.*, antigen presenting cells with weak TCR/CD28 costimulation).

5 Mature dendritic cells are potent APCs. However, treatment with IL-10 reduces their potency. Previous reports indicate that IL-10 greatly reduces dendritic cell APC potency, and this has been attributed to a reduction in the expression of MHC, B7-1, and B7-2. However, experiments herein indicate that the reduction is modest; moreover, IL-10 treated dendritic cells express PD-L1 and PD-L2.

10 Immature myeloid dendritic cells were isolated by culturing human peripheral blood monocytes in IL-4 and GM-CSF. Exposure of immature dendritic cells to an inflammatory cocktail of IL-1 β , TNF- α , IL-6, and PGE₂ elicits the development of mature dendritic cells that function as APCs. However, the addition of IL-10 to the inflammatory cytokines given during the maturation phase results in APCs that function only 1/6 to 1/3 as well.

15 T cell activation assays (MLRs) were performed as described generally above, using IL-10 treated dendritic cells as APCs, in the presence of antibodies to PD-L1 and/or PD-L2, or control antibodies. The addition of anti-PD-L1 or PD-L2 mAb to cultures of IL-10 treated dendritic cells plus allogeneic T cells resulted in a 3-fold increase in T cell proliferation, as compared to control IgG treated cultures (Figure 15).
20 A combination of anti-PD-L1 and anti-PD-L2 antibodies resulted in an increase in stimulation greater than that seen with either antibody alone (Figure 16). This stimulation is consistent with the result expected from blockade of the immunoinhibitory signal mediated by PD-L1 and/or PD-L2 binding to PD-1.

25 Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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What is claimed:

1. A method of identifying a compound which modulates T cell activation at a first and second antigen concentration comprising:
 - 5 a) contacting a T cell expressing a PD-L2 target molecule with a test compound at a first antigen concentration;
 - b) determining the ability of the test compound to modulate T cell proliferation or cytokine production at the first antigen concentration;
 - c) contacting a T cell expressing a PD-L2 target molecule with the
 - 10 test compound at a second antigen concentration; and
 - d) determining the ability of the test compound to modulate T cell proliferation or cytokine production at the second antigen concentration,thereby identifying a compound which modulates T cell activation at a first and second antigen concentration.
- 15 2. The assay of claim 1, wherein the PD-L2 target molecule is PD-1.

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FIG. 1

Human PD-L2 nucleotide sequence

1 GCAAACCTTAAGCTGAATGAACAACCTTTCTTCTCTTGAATATATCTTAACGCCAA
ATTTTGAGTGTCTTTTGTACCCATCCTCATATGTCCCAGCTGGAAAAGATCCTG
GGTTGGAGCTACTGCATGTTGATTGTTTGTCTTTTCTTTTGGCTGTTCAATTTGG
TGGCTACTATAAGGAAATCTAACACAAACAGCAACTGTTTTTGTGTTTACTTTT
GCATCTTTACTTGTGGAGCTGTGGCAAGTCCCTCATATCAAATACAGAACATGATCT
TCCTCCTGCTAATGTTGAGCCTGGAAATGCAGCTTCACCAGATAGCAGCTTTATTC
ACAGTGACAGTCCCTAAGGAACGTACATAATAGAGCATGGCAGCAATGTGACCCCT
GGAAATGCAACTTTGACACTGGGAAGTCATGTGAACCTTGGAGCAATAACAGCCAGTT
TGCAAAGGTGGAAAATGATACATCCCCACACCGTGAAAGAGCCACTTTGCTGGAG
GAGCAGCTGCCCTAGGGGAGGCCCTCGTTCACATACCTCAAGTCCAAGTGAGGGA
CGAAGGACAGTACCAATGCATAATCATCTATGGGGTCGCCCTGGGACTACAAGTACC
TGACTCTGAAAAGTCAAAGCTTCCCTACAGGAAAATAAACACTCACATCCTAAAGGTT
CCAGAAACAGATGAGGTAGAGCTCACCTGCCAGGCTACAGTTATCCTCTGGCAGA
AGTATCTGGCCAAACGTACAGCGTTCTGCCAACACAGCCACTCCAGGACCCCTG
AAGGCCTCTACCAAGTCAACAGTGTCTGCGCTAAAGCCACCCCTGGCAGAAAC
TTCAGCTGTGTGTTCTGGAATACTCACGTGAGGGAACCTTACTTTGGCCAGCATTTGA
CCTTCAAAGTCAGATGGAACCCAGGACCCATCCAACTTGGCTGCTTACATTTTCA
TCCCCTCCTGCATCATTTGCTTTTCAATTTTCATAGCCACAGTGATAGCCCTAAGAAAA
CAACTCTGTCAAAGCTGTATTCTTCAAAGACACACAAAAAGACCTGTCAACAC
AACAAAGGGGAAGTGAACAGTGTCTGTAACCTGTGGTCTTGGGAGCCAGGGTG
ACCTGATATGACATCTAAGAAAGCTTCTGGACTCTGAACAAGATTGGTGGCCCTG
CAGAGCTTGCCATTTGCACTTTTCAAATGCCCTTTGGATGACCCAGCA 1223

Human PD-L2 amino acid sequence

1 MIFLLIMLSLEQLHQIAALEFTVTPKELYIIHGNSVTLECNFTGSHVNLGAI
ASLQKVENDTSPHREERATLLEEQLPLGKASFHIPQVQVRDEGQYQCIIYGVANDY
KYLTLKVKASYRKINTHLKVPETDEVELTCQATGYPLAEVSWPNVSVFANTSHSR
TPEGLYQVTSVLRLEKPPGRNFSVFNTHVRELTLASIDLQSQMEPRTHPTWLLH
IFIPSCIIAFIFIATVIALRKQLCQKLYSSKDTTKRPVTTTKREVNSAI 273

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FIG. 2

Mouse PD-L2 nucleotide sequence

1 GAATTCGGCACGAGGTCAAATGTGGCATACTTTTGTGTCTCCTTCTGTCTCCAA
 CTAGAGAGAACACACTTACGGCTCCTGTCCCGGGCAGGTTTGGTTGTGCGGTGTGAT
 TGGCTTCCAGGGAACCTGATACAGGAGCAACTGTGTGCTGCTTTTCTGTGTCTT
 TGCTTGAGGAGCTGTGTGCTGGGTGCTGATATTGACACAGACCATGCTGCTCCTGCTG
 CCGATACTGAACCTGAGCTTACAACCTTCATCCTGTAGCAGCTTTATTACCCGTGAC
 AGCCCTTAAAGAAGTGTACCCGTAGACGTGCGGAGCAGTGTGAGCCTGGAGTGG
 ATTTTGACCGCAGAGAATGCACTGAACCTGGAAGGGATAAGAGCCAGTTTGCAAG
 GTAGAAAATGATACGTCTCTGCAAGTGAAAGAGCCACCTGTGAGGAGAGCAGCT
 GCCCTGGGAAAGGCTTTGTCCACATCCCTAGTGTCCAAGTGAGAGATTCCGGGC
 AGTACCGTGTGCTGGTCACTGTGCGGGGCCCTGGGACTACAAGTACCTGACGGTG
 AAAGTCAAAGCTTCTTACATGAGGATAGACACTAGGATCCTGGAGGTTCCAGGTAC
 AGGGGAGGTGCAGCTTACCTGCCAGGCTAGAGGTTATCCCTAGCAGAGTGTCTCT
 GGCAAAATGTCACTGTCTCTGCCAACCAAGCCACATCAGGACCCCGAAGGCCCTC
 TACCAAGTCAACAGTGTCTCTGCCCTCAAGCCTCAGCCTAGCAGAACTTCAGCTG
 CATGTTCTGGAATGCTCACATGAAGGAGCTGACTTCAGCCATCATTGACCTCTGA
 GTCGGATGGAAACCCAAAGTCCCAAGACGTGGCCACTTCATGTTTTCATCCCGGCC
 TGCACCATCGCTTTGATCTTCTCGGCCATAGTGATAATCCAGAGAAAGAGGATCTA
 GGGGAAGCTGTATTACGGAAGAAGTGGTCTCTCTTCCAGATCTGGACCTGCGGT
 CTTGGGAGTTGGAAGGATCTGATGGGAAACCTCAAGAGACTTCTGGACTCAAAGT
 GAGAATCTTGCAAGACCTGCCATTGCACTTTGAACCTTTGGACGGTGACCCAG
 GGCTCCGAAGAGGAGCTTGTAAAGACTGACAATCTTCCCTCTGTCTCAAGACTCTCT
 GAACAGCAAGACCCCAATGGCACTTTAGACTTACCCCTGGGATCTTGGACCCAGT
 GAGGGCCTAAGGCTCCTAATGACTTTTCAAGGAGAGAACAAAGGAATTGCTCTCCG
 CCCCACCCACCTCCTGCTTTCGCGAGGGAGACATGGAAATTCACGTTACTAAA
 ATAGATTGTCAATAGAGTTATTTATAGCCCTCATTTCTCCGGGGAAGTTGGAAGT
 TCAGACAGGGGTTTTTCATAAACAAAGTCATAACTGATGTGTTTACAGCATCCTAG
 AATCTGGCAGCCTCTGAAGTCTAATTAAGTGAAGCATTTAAGCAACAGTCAA
 GTGCCCTGCTGTGGTATTGTTCTACTTTTCTGTTTTTAAAGTGTGAGTCACAA
 GGTAAATTGTTGTAACCTGTGATATCACTGTTTCTGTGTCTCTTTTCAACTACA
 TCTTTTAAACAAAAA

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Mouse PD-L2 amino acid sequence

1 MLLLLPILNLSQLHEVAALFTVAPKEVYTVDVSSVSLECFDRETELEGIR
 ASLQKVENDTSLQSERATLLEEQLPLGKALFHIPSVQVRDSGQYRCLVICGAANDY
 KYLTVKVASYMRIDTRILEVPGTEVQLTCQARGYPLAEVSWQNVSPANTSHIR
 TPEGLYQVTSVLRLEKQPSRNFSCMFWNHMKELTSALIDPLSRMEPKVPRTWPLH
 VEIPACTIALIFLAIVIIQRRI

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FIG. 3

PD-L2

Domains Order: signal, IgV, IgC, transmembrane,
cytoplasmic

Murine 247 aa

Signal MLLLLPIINLSLQLHPVAA
Peptide

IgV LETVTAPKEVYTVDVGSSVSLECDFDRECTELEGRASLQKVENTSLQS
ERATLLEEQLPLGKALFHIPSVQVRDSGQYRCLVICGAAWDYKYLTVKVK

IgC ASYMRIDTRILVEVPGTGEVQLTCQARGYPLAEVSWQNVSPANTSHIRTPE
GLYQVTSVLRLKPPQSRNFSMFWNAHMKELTSIIDPLSRMEPKVPR

Trans- TWPLHVFIPACTIALIFLAIVII
membrane

Cyto- QRKRI
plasmic

Extracellular

Human 273 aa

Signal MIFLLMLSLQLQLHQIAA
peptide

IgV LETVTVPKELYIIHGSSNVTLECNFTGSHVNLGAIASLQKVENTSPHR
ERATLLEEQLPLGKASFHIPQVVRDEGQYQCIIYGVANDYKYLTLKVK

IgC ASYRKINTHILKVPETDEVELTCQATGYPLAEVSWPNVSPANTSHSRTPE
GLYQVTSVLRLKPPPGRNFSVFWNTHVRELTLASIDLQSQMEPRTHP

Trans- TWLLHIFIPSCIIAFIFIATVIAL
membrane

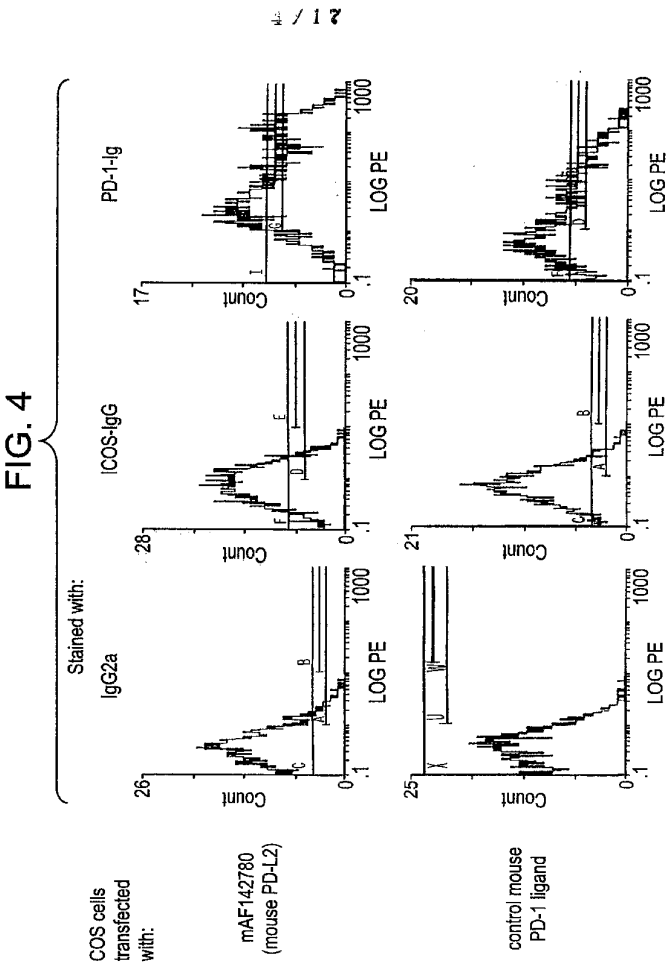
Cyto- RKQLCQKLYSSKDTTKRPVTTTKREVNSAI
plasmic

Extracellular

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FIG. 5

murine PD-L2 vs human PD-L2

70% identity

mPD-L2: 15 HPVAALFTVTAPKEVYTVDVGSSVLECDPDRRECTELEGIRASLQKVENDTSLQSERAT 74
H +AALEFTVT PKE+Y ++ GS+VLESC+FD L I ASLQKVENDTSLQSERAT 74

hPD-L2: 15 HQIAALFTVTVPKEELYIIIEHGSNVTLECNFTGSHVNLGALTASLQKVENDTSPHRRERAT 74

mPD-L2: 75 LLEEQLPLGKALFHIPVQVRDVGQYRCIVICGAAMDYKYLTVKVKASYMRIDTRILEVP 134
LLEEQLPLGKA FHIP VQVRD GQY+C++I G AMDYKYLTVKVKASY +I+T IL+VP

hPD-L2: 75 LLEEQLPLGKASFHIPVQVRDEGQYQCIIYGVAMDYKYLTVKVKASYRKINTHILKVP 134

mPD-L2: 135 GTGEVQLTCQARGYFLAEVSNQVSVNPANTSHIRTPEGLYQVTSVLRLKQPSPRNFSQMF 194
T EV+LTCQA GYFLAEVSN NVSVNPANTSH RTPEGLYQVTSVLRLK P RNFSC+P

hPD-L2: 135 ETDEVELTCQATGYFLAEVSNVSNVNPANTSHRSRTPEGLYQVTSVLRLKPPPGRNFSQVF 194

mPD-L2: 195 WNAHMKELTSAIIDPLSRMEPKVPTWFLHVFIPACTIALIFLAIVIIQKKRI 247
WN H++ELT A ID S+MEP+ TW LH+FIP+C IA IF+A VI RK++

mPD-L2: 195 WNTHVRELTLASIDLSQSQMEPRTHPTWLLHIFIFIPSCIIAFIFITATVIALRKQL 247

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FIG. 6

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mPD-L2 MLLLLPIINLSLOLEPVRALEFTVTPKEVYTTDVGSSTVSECCDEDRRECTIELEGI....
hPD-L2 MIFLLIMSLELOLBQHAALFTVTVPRELYITIEGSGNVTLECNEDTGSHTVNLGAI.....
mPD-L1 ~MRIFAGIIFACCHLIRAFITAPKDLVVVEYGSNVTMECREFEVERELDLALVVYWE
hPD-L1 ~MRIFAVETFTYWHILNAFITVTWPKDLVVVEYGSNVTMECKEPEVEKQLDLAALIVYWE
1.....10.....20.....30.....40.....50.....

mPD-L2 ...RASLCKV...ENNTSLG...SERATILLEHQLPLCKALFHPSQVVRDSQVRCCLVIC
hPD-L2 ...TASLCKV...ENNTSEH...RERATILLEHQLPLCKASFHPSQVVRDSQVRCCLVIC
mPD-L1 KEEEQVIEFVAGFEELKPECHSNFRERASEKDKQLKGNALQITDVKLQDAQVYCCILSY
hPD-L1 MEKKNIIIEFHSEELKQHSYBGRARLKLQSLGNAALQITDVKLQDAQVYRCMLSY
61.....70.....80.....90.....100.....110.....

mPD-L2 GAWDYKYLIVKVRASMRIDTRITETPETCEVQLTQQAQGYELAEVSWCN.....VSV
hPD-L2 GAWDYKYLIVKVRASMRKINHHILKVPEETCEVQLTQQAQGYELAEVSWCN.....VSV
mPD-L1 GGA.DYKRILKVNAPYRKINQRIASVDPATSEHEITQQAQGYELAEVSWCN.....VSV
hPD-L1 GGA.DYKRILKVNAPYRKINQRIASVDPATSEHEITQQAQGYELAEVSWCN.....VSV
121.....130.....140.....150.....160.....170.....

mPD-L2 PANTSRIETEGLYQVTSVLRLEKPEGRNFSQMFVNAHMRDLTSA...IIDELSRMEKVP
hPD-L2 PANTSRIETEGLYQVTSVLRLEKPEGRNFSQMFVNAHMRDLTSA...IIDELSRMEKVP
mPD-L1 KRSVTSRTTEGMILNVTSLERVNATANDVYCTEWRSCQGNHTAELIPEIPATHPPON
hPD-L1 KTTITNSKREKLEFNVTSLERNTTHHEIEYCTEWRRLDEENHTAELIPEIPATHPPON
181.....190.....200.....210.....220.....230.....

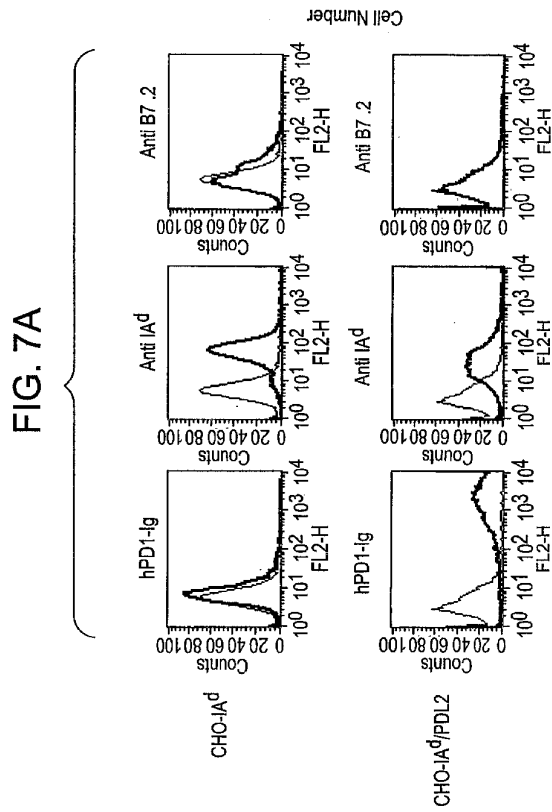
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hPD-L2 RTVPLHVFIPACTIALIEPAIVITQRREI~~~~~
mPD-L1 RTVPLHVFIPACTIALIEPAIVITQRREI~~~~~
hPD-L1 RTVPLHVFIPACTIALIEPAIVITQRREI~~~~~
241.....250.....260.....270.....280.....290.....

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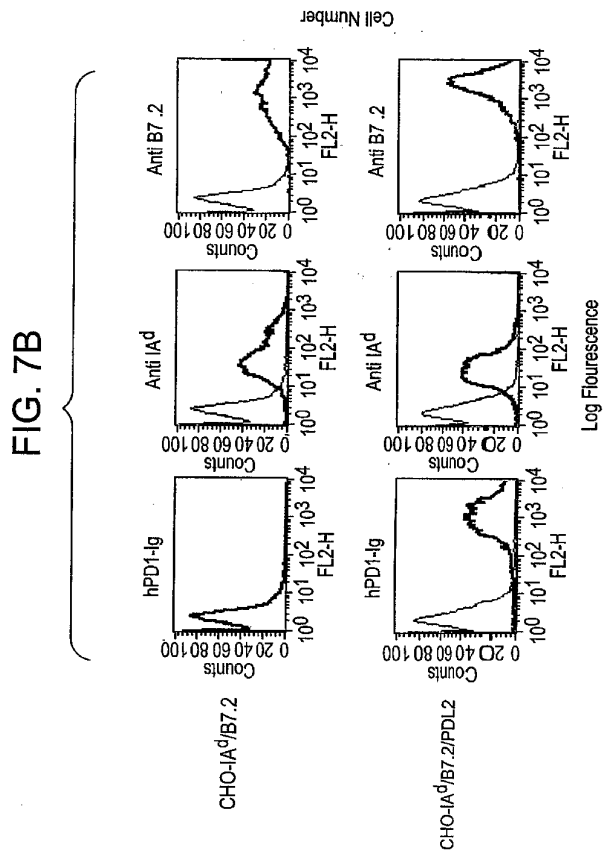
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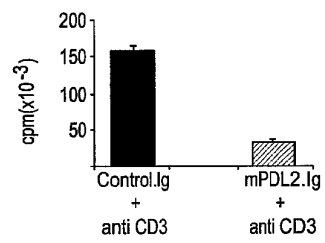
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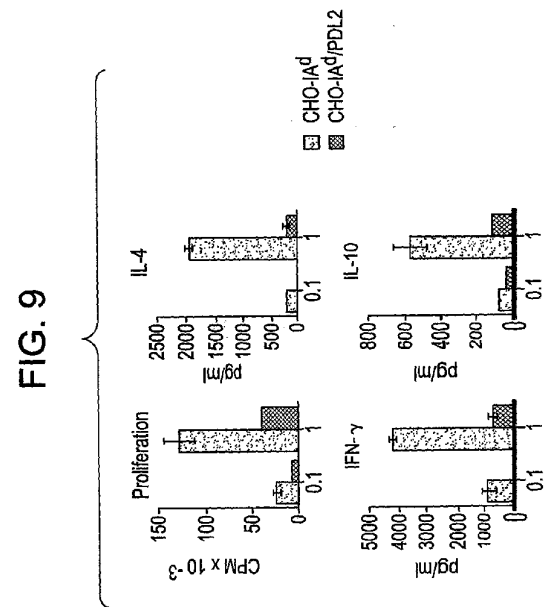
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FIG. 8



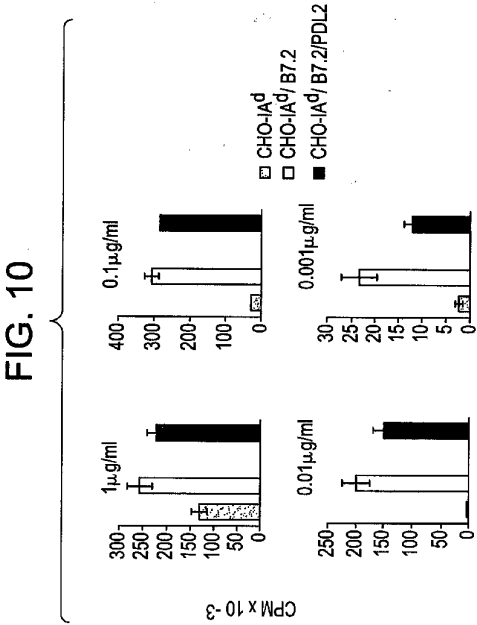
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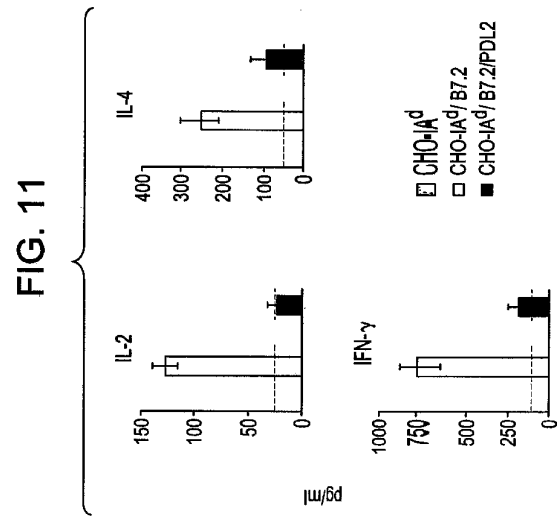


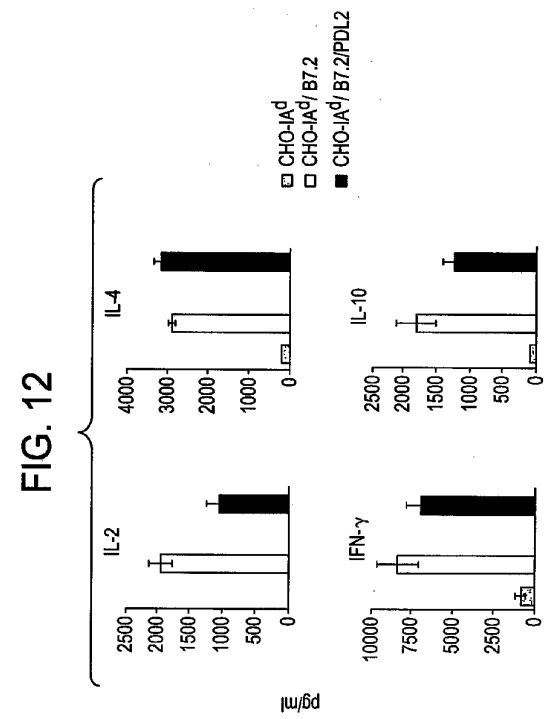
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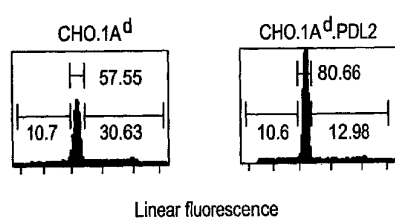


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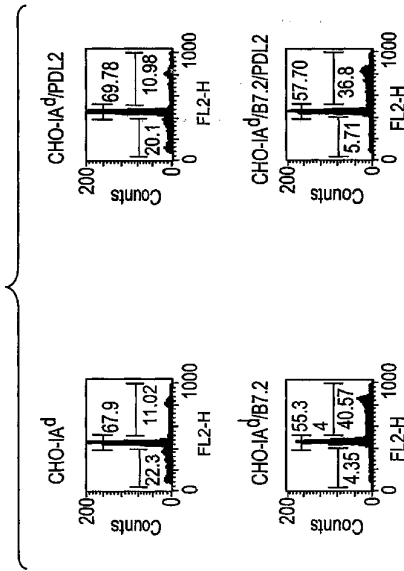
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FIG. 13



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FIG. 14

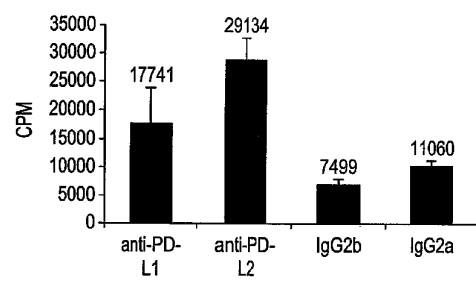


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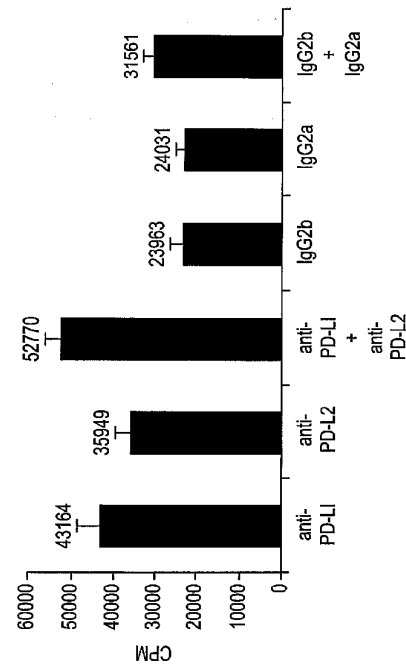
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FIG. 15



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FIG. 16



【国際調査報告】

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A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12N15/12 C07K14/705 G01N33/50		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N G01N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, BIOSIS		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
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☒ Further documents are listed in the continuation of box C. ☒ Patent family members are listed in annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another claim or other special reason (as specified) "O" document relating to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "Z" document member of the same patent family		
Date of the actual completion of the international search 30 October 2002		Date of mailing of the international search report 07/11/2002
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentboom 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax. (+31-70) 340-2015		Authorized officer Maddox, A

International Application No.
PCT/US 01/20858

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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(81)指定国 AP(GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE,TR),OA(BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG),AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CO,CR,CU,CZ,DE,DK,DM,DZ,EC,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,MZ,NO,NZ,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW

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