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(54) 【発明の名称】 トランスジェニック XENOMOUSE (登録商標) から獲得される P E U D O M O N A S A E R U G I N O S A L P S に対するヒト抗体

(57) 【要約】

本明細書中に記載される発明により、非ヒト動物において産生された、Pseudomonas aeruginosa のリポ多糖 (LPS) に特異的に結合するヒト抗体が提供される。本発明はさらに、非ヒト動物における抗体の作製方法、およびハイブリドーマおよび組換え宿主細胞系を含む細胞株における抗体の発現方法を提供する。抗体を含むキットおよび薬学的組成物、ならびに本明細書中に記載される薬学的組成物を患者に投与することにより、シュードモナス感染を処置または予防する方法もまた、提供される。

S20 Monoclonal Antibody Gene Usage

Heavy Chain Gene Segments

<u>VH</u>	<u>D</u>	<u>JH</u>
V _H 3/V3-33	D2-8	J _H 4b

Light Chain Gene Segments

<u>VL</u>	<u>JL</u>
V _κ 2/A2	J _κ 1

【特許請求の範囲】

【請求項 1】

非ヒト動物で発現され、そして *Pseudomonas aeruginosa* LPS に特異的に結合する、単離されたヒト抗体またはその抗原結合部分。

【請求項 2】

前記ヒト抗体がモノクローナル抗体である、請求項 1 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 3】

前記抗体またはその一部が、*Pseudomonas aeruginosa* 細胞についてオプソニン作用性である、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。 10

【請求項 4】

前記抗体またはその一部が、*Pseudomonas aeruginosa* 細胞の食作用を促進する、請求項 3 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 5】

前記抗体またはその一部が、*Pseudomonas aeruginosa* に対する免疫応答を強化する、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 6】

前記抗体またはその一部が、*Pseudomonas aeruginosa* 細胞の死滅を促進する、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。 20

【請求項 7】

前記 *Pseudomonas aeruginosa* 細胞の死滅の促進が、該 *Pseudomonas aeruginosa* 細胞に対して致死性である試薬の送達を含む、請求項 6 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 8】

前記 *Pseudomonas aeruginosa* 細胞の死滅の促進が、該 *Pseudomonas aeruginosa* に対する免疫応答の強化を含む、請求項 6 に記載の単離されたヒト抗体またはその抗原結合部分。 30

【請求項 9】

前記抗体またはその抗原結合部分が、*Pseudomonas aeruginosa* 感染を阻害する、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 10】

前記抗体またはその抗原結合部分が、*Pseudomonas aeruginosa* LPS に対して、 5×10^{-7} M から 1×10^{-7} M の K_d で結合する、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 11】

前記抗体またはその抗原結合部分が、*Pseudomonas aeruginosa* LPS に対して、 1×10^{-7} M から 5×10^{-8} M の K_d で結合する、請求項 10 に記載の単離されたヒト抗体またはその抗原結合部分。 40

【請求項 12】

前記抗体またはその抗原結合部分が、*Pseudomonas aeruginosa* LPS に対して、 5×10^{-8} M から 1×10^{-8} M の K_d で結合する、請求項 10 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 13】

前記 *Pseudomonas aeruginosa* LPS が、06ad、011、Habs16、170003、および PA01 Hallaway からなる群より選択される *Pseudomonas aeruginosa* 株由来である、請求項 1 または請求項 50

2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 1 4】

前記抗体またはその抗原結合部分が、1 時間から 3 0 日の間のインビボでの半減期を有する、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 1 5】

前記抗体またはその抗原結合部分が、1 6 日から 3 0 日の間のインビボでの半減期を有する、請求項 1 4 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 1 6】

前記抗体またはその抗原結合部分が、1 時間から 1 5 日の間のインビボでの半減期を有する、請求項 1 4 に記載の単離されたヒト抗体またはその抗原結合部分。

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【請求項 1 7】

前記抗体が、免疫グロブリン G (I g G)、I g M、I g E、I g A、および I g D からなる群より選択される重鎖イソ型を有する免疫グロブリン分子であるかまたは該免疫グロブリン分子に由来する、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 1 8】

前記 I g G が、I g G 1、I g G 2、I g G 3、および I g G 4 からなる群より選択されるサブタイプである、請求項 1 7 に記載の単離されたヒト抗体。

【請求項 1 9】

前記抗体またはその一部が標識されている、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

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【請求項 2 0】

前記標識が、放射標識、酵素標識、蛍光標識、毒素、磁気因子、2 次抗体、親和性標識、エピトープタグ、抗生物質、補体タンパク質、およびサイトカインからなる群より選択される、請求項 1 9 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 2 1】

κ 軽鎖およびそのフレームワーク配列を含む、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 2 2】

前記 κ 軽鎖の前記フレームワーク配列が、ヒト V k 2 / A 2 遺伝子によってコードされる、請求項 2 1 に記載の単離されたヒト抗体またはその抗原結合部分。

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【請求項 2 3】

前記 κ 軽鎖が、生殖細胞系 V k 2 / A 2 遺伝子によってコードされる κ 軽鎖からの、7 個から 1 5 個の間のアミノ酸変化を含む、請求項 2 1 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 2 4】

前記 κ 軽鎖が、生殖細胞系 V k 2 / A 2 遺伝子によってコードされる κ 軽鎖からの、6 個未満のアミノ酸変化を含む、請求項 2 1 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 2 5】

前記 κ 軽鎖が、生殖細胞系 V k 2 / A 2 遺伝子によってコードされる κ 軽鎖からの、3 個未満のアミノ酸変化を含む、請求項 2 4 に記載の単離されたヒト抗体またはその抗原結合部分。

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【請求項 2 6】

前記 κ 軽鎖が配列番号 4 のアミノ酸配列を含む、請求項 2 1 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 2 7】

前記軽鎖が配列番号 3 の核酸配列によってコードされる、請求項 2 1 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 2 8】

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λ 軽鎖を含む、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 29】

可変領域（V）、多様性領域（D）、および結合領域（J）から構成され、そしてそのフレームワーク配列から構成される重鎖を含む、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 30】

前記重鎖の前記可変領域がヒト $V_H 3 / V 3 - 3 3$ 遺伝子によってコードされる、請求項 29 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 31】

前記重鎖の前記多様性領域がヒト $D 2 - 8$ 遺伝子によってコードされる、請求項 29 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 32】

前記重鎖の前記連結領域がヒト $J_H 4 b$ 遺伝子によってコードされる、請求項 29 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 33】

前記可変領域が、生殖細胞系 $V_H 3 / V 3 - 3 3$ 遺伝子によってコードされる可変領域からの、7 個から 15 個の間アミノ酸変化を含む、請求項 29 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 34】

前記重鎖が、生殖細胞系 $V_H 3 / V 3 - 3 3$ 遺伝子によってコードされる可変領域からの、6 個未満のアミノ酸変化を含む、請求項 29 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 35】

前記重鎖が、生殖細胞系 $V_H 3 / V 3 - 3 3$ 遺伝子によってコードされる可変領域からの、3 個未満のアミノ酸変化を含む、請求項 29 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 36】

前記重鎖が配列番号 2 のアミノ酸配列を含む、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 37】

前記重鎖が配列番号 1 の核酸配列によってコードされる、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 38】

F a b フラグメント、F (a b ')₂ フラグメント、および F_v フラグメントからなる群より選択される抗原結合ドメインを含む、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 39】

前記抗体が単鎖抗体である、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 40】

前記抗体がキメラ抗体である、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 41】

異なるヒト抗体由来のフレームワーク領域および C D R 領域を含む、請求項 40 に記載のキメラ抗体。

【請求項 42】

前記キメラ抗体が二重特異的である、請求項 40 に記載のキメラ抗体。

【請求項 43】

前記キメラ抗体が、P s e u d o m o n a s a e r u g i n o s a L P S および以下

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からなる群より選択される標識について二重特異的である、請求項 4 2 に記載のキメラ抗体：放射標識分子、酵素標識、蛍光標識、毒素、磁気因子、2 次抗体、親和性標識、エピトープタグ、抗生物質、補体タンパク質、およびサイトカイン。

【請求項 4 4】

前記抗体またはその一部が誘導体化されている、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 4 5】

前記抗体またはその一部が、ポリエチレングリコール、少なくとも 1 つのメチル基もしくはエチル基、または少なくとも 1 つの炭水化物部分で誘導体化されている、請求項 4 4 に記載の単離されたヒト抗体またはその抗原結合部分。

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【請求項 4 6】

請求項 1 または請求項 2 に記載の抗体またはその抗原結合部分、および薬学的に受容可能なキャリアを含有する、薬学的組成物。

【請求項 4 7】

請求項 1 または請求項 2 に記載の抗体またはその抗原結合部分、薬学的に受容可能なキャリア、および容器を備える、キット。

【請求項 4 8】

使用のための説明書をさらに備える、請求項 4 7 に記載のキット。

【請求項 4 9】

請求項 1 に記載の単離されたヒト抗体を、*Pseudomonas aeruginosa* 感染の危険があるかまたは *Pseudomonas aeruginosa* に現在感染している患者に投与する工程を包含する、*Pseudomonas aeruginosa* 感染を処置または予防するための方法。

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【請求項 5 0】

前記ヒト抗体がモノクローナル抗体である、請求項 4 9 に記載の方法。

【請求項 5 1】

前記投与が、注射手段、経粘膜手段、経口手段、吸入手段、眼内手段、直腸手段、長期作用性移植手段、リポソーム手段、乳濁剤手段、クリーム剤手段、局所的手段、または徐放手段によって実施される、請求項 4 9 に記載の方法。

【請求項 5 2】

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前記抗体が第 2 のタンパク質との融合体である、請求項 4 9 に記載の方法。

【請求項 5 3】

前記抗体が、放射標識、毒素、補体タンパク質、サイトカイン、または抗生物質で標識されている、請求項 4 9 に記載の方法。

【請求項 5 4】

前記第 2 のタンパク質が、毒性ペプチド部分、補体タンパク質、放射標識タンパク質、サイトカイン、または抗生物質タンパク質からなる群より選択される、請求項 5 2 に記載の方法。

【請求項 5 5】

前記患者が、熱傷患者、手術患者、プロテゼレシピエント、呼吸患者、癌患者、嚢胞性繊維症患者、または免疫無防備状態の患者である、請求項 4 9 に記載の方法。

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【請求項 5 6】

前記薬学的組成物がさらに、毒素、補体タンパク質、放射標識タンパク質、サイトカイン、抗生物質、またはそれらの任意の組合せを含む、請求項 4 9 に記載の方法。

【請求項 5 7】

請求項 1 または請求項 2 に記載の抗体を産生する、単離された細胞株。

【請求項 5 8】

前記細胞株がハイブリドーマである、請求項 5 7 に記載の細胞株。

【請求項 5 9】

以下の工程を包含する、*Pseudomonas aeruginosa* LPS に特異

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的に結合する単離されたヒト抗体またはその抗原結合部分を産生する、方法：

a) 該抗体が産生される条件下で該抗体を産生することができる非ヒト細胞を培養する工程：

b) 該細胞培養物から該抗体を単離する工程。

【請求項 60】

前記細胞がハイブリドーマである、請求項 59 に記載の方法。

【請求項 61】

前記細胞が、前記ヒト抗体またはその抗原結合部分をコードする単離された核酸分子で形質転換され、そして該細胞が、細菌細胞、酵母細胞、昆虫細胞、両生類細胞、および哺乳動物細胞からなる群より選択される、請求項 59 に記載の方法。

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【請求項 62】

前記哺乳動物細胞が、ヒト細胞、マウス細胞、ラット細胞、イヌ細胞、サル細胞、ヤギ細胞、ブタ細胞、ウシ細胞、およびハムスター細胞からなる群より選択される、請求項 61 に記載の方法。

【請求項 63】

前記哺乳動物細胞が、HeLa細胞、NIH 3T3細胞、CHO細胞、BHK細胞、VERO細胞、CV-1細胞、NS/O細胞、およびCOS細胞からなる群より選択される、請求項 61 に記載の方法。

【請求項 64】

以下の工程を包含する、Pseudomonas aeruginosa LPS に特異的に結合する単離されたヒト抗体またはその抗原結合部分を作製するための、方法：

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a) ヒト免疫グロブリン遺伝子座が組み込まれた非ヒト動物を、Pseudomonas aeruginosa の抗原性組成物で免疫する工程；

b) 該非ヒト動物の、該抗原性組成物に対する体液性応答を増大させる工程；および

c) 該非ヒト動物から該ヒト抗体を単離する工程。

【請求項 65】

Pseudomonas aeruginosa LPS に特異的に結合するヒト抗体の重鎖またはその抗原結合部分をコードする、非ヒト動物から単離された核酸分子。

【請求項 66】

前記核酸分子が前記ヒト抗体を産生するハイブリドーマから単離される、請求項 65 に記載の単離された核酸分子。

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【請求項 67】

配列番号 _____ のヌクレオチド配列を有する Pseudomonas aeruginosa LPS に特異的に結合する、ヒト抗体の重鎖またはその抗原結合部分をコードする、単離された核酸分子またはそのフラグメント。

【請求項 68】

前記フラグメントが、前記ヒト抗体の 1 個から 3 個の CDR 領域をコードする配列を含む、請求項 67 に記載の単離された核酸分子。

【請求項 69】

請求項 65～68 のいずれか 1 項に記載の核酸分子またはそのフラグメントを含有する、ベクター。

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【請求項 70】

前記ベクターがさらに、前記核酸に作動可能に連結された発現制御配列を含む、請求項 69 に記載のベクター。

【請求項 71】

Pseudomonas aeruginosa LPS に特異的に結合するヒト抗体の軽鎖またはその抗原結合部分をコードする、非ヒト動物から単離された核酸分子。

【請求項 72】

前記核酸分子が、前記ヒト抗体を産生するハイブリドーマから単離される、請求項 71 に記載の単離された核酸分子。

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【請求項 73】

配列番号 3 のヌクレオチド配列を有する *Pseudomonas aeruginosa* LPS に特異的に結合するヒト抗体の軽鎖またはその抗原結合部分をコードする、単離された核酸分子またはそのフラグメント。

【請求項 74】

前記フラグメントが、前記ヒト抗体の 1 個から 3 個の CDR 領域をコードする配列を含む、請求項 71 に記載の単離された核酸分子。

【請求項 75】

請求項 71 ～ 74 のいずれか 1 項に記載の核酸分子を含有する、ベクター。

【請求項 76】

前記ベクターがさらに、前記核酸に作動可能に連結された発現制御配列を含む、請求項 75 に記載のベクター。

【請求項 77】

以下を含有する、単離された宿主細胞：

a) 非ヒト動物から単離され、そして *Pseudomonas aeruginosa* LPS に特異的に結合する、ヒト抗体の軽鎖またはその抗原結合部分をコードする、核酸分子；あるいは

b) 該核酸分子を含有する、ベクター。

【請求項 78】

以下を含有する、単離された宿主細胞：

a) 非ヒト動物から単離され、そして *Pseudomonas aeruginosa* LPS に特異的に結合する、ヒト抗体の重鎖またはその抗原結合部分をコードする、核酸分子；あるいは

b) 該核酸分子を含有する、ベクター。

【請求項 79】

以下を含有する、単離された宿主細胞：

a) 非ヒト動物から単離されそして重鎖またはその抗原結合部分をコードする核酸分子、および *Pseudomonas aeruginosa* LPS に特異的に結合するヒト抗体の軽鎖またはその抗原結合部分をコードする単離された核酸分子；あるいは

b) 該核酸分子を含有する、単数または複数のベクター。

【請求項 80】

前記細胞が、ハイブリドーマ細胞、細菌細胞、酵母細胞、昆虫細胞、両生類細胞、および哺乳動物細胞からなる群より選択される、請求項 77 ～ 79 のいずれか 1 項に記載の単離された宿主細胞。

【請求項 81】

前記哺乳動物細胞が、ヒト細胞、マウス細胞、ラット細胞、イヌ細胞、サル細胞、ヤギ細胞、ブタ細胞、ウシ細胞、およびハムスター細胞からなる群より選択される、請求項 80 に記載の宿主細胞。

【請求項 82】

前記哺乳動物細胞が、HeLa細胞、NIH 3T3細胞、CHO細胞、BHK細胞、VERO細胞、CV-1細胞、NS/O細胞、およびCOS細胞からなる群より選択される、請求項 80 に記載の方法。

【請求項 83】

請求項 77 ～ 81 のいずれか 1 項に記載の宿主細胞を、前記核酸分子が発現される条件下で培養する工程を包含する、非ヒト動物から同定されそして *Pseudomonas aeruginosa* LPS に特異的に結合するヒト抗体の、重鎖またはその抗原結合部分、軽鎖またはその抗原結合部分、あるいは軽鎖および重鎖の両方またはそれらの抗原結合部分を組換え産生する、方法。

【請求項 84】

請求項 67 に記載の核酸分子によってコードされるか、または請求項 78 に記載の宿主細胞

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胞から単離される、請求項 1 または請求項 2 に記載の抗体から得られた、単離された重鎖またはその抗原結合部分。

【請求項 85】

請求項 73 に記載の核酸分子によってコードされるか、または請求項 77 に記載の宿主細胞から単離される、請求項 1 または請求項 2 に記載の抗体から得られた、単離された軽鎖またはその抗原結合部分。

【請求項 86】

請求項 67 に記載の核酸分子を含有する非ヒトトランスジェニック動物であって、該非ヒトトランスジェニック動物が前記核酸分子を発現する、非ヒトトランスジェニック動物。

【請求項 87】

請求項 73 に記載の核酸分子を含有する非ヒトトランスジェニック動物であって、前記非ヒトトランスジェニック動物が前記核酸分子を発現する、非ヒトトランスジェニック動物。

【請求項 88】

Pseudomonas aeruginosa LPS に特異的に結合するヒト抗体の重鎖またはその抗原結合部分をコードする単離された核酸分子、および該ヒト抗体の軽鎖またはその抗原結合部分をコードする単離された核酸分子を含有する非ヒトトランスジェニック動物であって、ここで、該動物が該核酸分子を発現する、非ヒトトランスジェニック動物。

【請求項 89】

前記動物が、マウス、ラット、ハムスター、ウシ、ヒツジ、霊長類、ウマ、およびブタからなる群より選択される、請求項 86～88 のいずれか 1 項に記載の、非ヒトトランスジェニック動物。

【請求項 90】

前記単離された核酸分子またはその一部の発現によって生じたヒト抗体が、前記動物の B リンパ球細胞またはその後代由来の細胞の表面上で発現される、請求項 86～88 のいずれか 1 項に記載の非ヒトトランスジェニック動物。

【請求項 91】

前記単離された核酸分子またはその一部の発現によって生じたヒト抗体が、前記動物のリンパ液、血液、乳汁、唾液、または腹水中に分泌される、請求項 86～88 のいずれか 1 項に記載の非ヒトトランスジェニック動物。

【請求項 92】

請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分、および第 2 のポリペプチド配列を含有する、融合タンパク質。

【請求項 93】

前記第 2 のポリペプチド配列が、エピトープタグ、親和性タグ、毒性ポリペプチド、抗生物質、酵素、2 次抗体配列、補体タンパク質、およびサイトカインからなる群より選択される、請求項 92 に記載の融合タンパク質。

【請求項 94】

前記抗体の前記重鎖イソ型が、 μ 、 γ 、 δ 、 ε 、または α である、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 95】

前記抗体の血清半減期を増大させる 1 個から 10 個の間のアミノ酸置換を含有する、請求項 84 に記載の単離された重鎖またはその抗原結合部分。

【請求項 96】

前記抗体の血清半減期を増大させる 1 個から 10 個の間のアミノ酸置換を含有する、請求項 85 に記載の単離された軽鎖またはその抗原結合部分。

【請求項 97】

以下の工程を包含するプロセスによって産生される、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分：

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a) ヒト免疫グロブリン遺伝子座を含有する非ヒト動物を、単離された *Pseudomonas aeruginosa* LPS 調製物、雄性 *Pseudomonas aeruginosa* 細胞調製物、弱毒化 *Pseudomonas aeruginosa* 細胞調製物、および死滅させた *Pseudomonas aeruginosa* 細胞調製物からなる群より選択される抗原で免疫する工程；

b) 該非ヒト動物の、該抗原に対する免疫応答を増大させる工程；および

c) 該非ヒト動物から該抗体を単離する工程。

【請求項 98】

ヒトの生体材料が混入していない動物または細胞から単離される、請求項 1 または請求項 2 に記載の単離されたヒト抗体またはその抗原結合部分。

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【請求項 99】

前記生体材料が、ウイルス、酵素、ホルモン、サイトカイン、レセプター、レセプターリガンド、免疫グロブリン、補体、核タンパク質、および細胞質シグナル伝達タンパク質からなる群より選択される、請求項 99 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 100】

前記ウイルスが、エプスタインバーウイルスまたはレトロウイルスである、請求項 100 に記載の単離されたヒト抗体またはその抗原結合部分。

【請求項 101】

アメリカンタイプカルチャーコレクション登録番号 _____ を有する、S20 mAb を産生するハイブリドーマ細胞株。

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【請求項 102】

請求項 101 に記載のハイブリドーマ細胞株によって産生される、モノクローナル抗体。

【請求項 103】

Pseudomonas aeruginosa LPS の O 特異的側鎖に特異的に結合する、単離されたヒト抗体またはその抗原結合部分。

【請求項 104】

前記 *Pseudomonas aeruginosa* 株が、06ad、011、Habs16、17003、および PA01 Halloway からなる群より選択される、請求項 103 に記載の抗体またはその抗原結合部分。

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【請求項 105】

モノクローナルである、請求項 103 または 104 に記載の抗体またはその抗原結合部分。

【請求項 106】

約 1.0 の相対的な結合力を有する、請求項 103 ～ 105 のいずれか 1 項に記載の抗体または抗原結合部分。

【請求項 107】

1 つの株の LPS の O 特異的側鎖に結合し、そして他の 4 個の株の LPS の O 特異的側鎖には結合しない、請求項 104 に記載の抗体または抗原結合部分。

【請求項 108】

Pseudomonas aeruginosa PA01 株の LPS の O 特異的側鎖に特異的に結合する、ヒトモノクローナル抗体またはその抗原結合部分。

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【請求項 109】

IgM 抗体である、請求項 108 に記載のヒトモノクローナル抗体または抗原結合部分。

【請求項 110】

ATCC 登録番号 # _____ または ATCC 登録番号 # _____ を有するハイブリドーマ細胞株によって産生される、請求項 108 に記載のヒトモノクローナル抗体または抗原結合部分。

【請求項 111】

Pseudomonas aeruginosa 170003 株の LPS の O 特異的側鎖に特異的に結合する、ヒトモノクローナル抗体またはその抗原結合部分。

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【請求項 1 1 2】

I g M抗体である、請求項 1 1 1 に記載のヒトモノクローナル抗体または抗原結合部分。

【請求項 1 1 3】

A T C C登録番号# _____を有するハイブリドーマ細胞株によって産生される、請求項 1 1 1 に記載のヒトモノクローナル抗体またはその抗原結合部分。

【請求項 1 1 4】

請求項 1 0 8 ～ 1 1 3 のいずれか 1 項に記載の抗体の結合を阻害する、ヒトモノクローナル抗体またはその抗原結合部分。

【請求項 1 1 5】

P s e u d o m o n a s a e r u g i n o s a L P S の O 特異的側鎖に特異的に結合し、図 9 に示される C D R 1、C D R 2、および C D R 3 の 1 つ以上を含有する重鎖を含む、抗体またはその抗原結合部分。 10

【請求項 1 1 6】

前記重鎖が、図 9 に示される C D R 3 を含む、請求項 1 1 5 に記載の抗体または抗原結合部分。

【請求項 1 1 7】

配列番号 2 に示されるアミノ酸配列を含有する、請求項 1 1 5 に記載の抗体または抗原結合部分。

【請求項 1 1 8】

図 1 0 に示される C D R 1、C D R 2、および C D R 3 の 1 つ以上を含有する軽鎖を含む、P s e u d o m o n a s a e r u g i n o s a L P S の O 特異的側鎖に特異的に結合する、抗体またはその抗原結合部分。 20

【請求項 1 1 9】

前記重鎖が、図 1 0 に示される C D R 3 を含む、請求項 1 1 8 に記載の抗体または抗原結合部分。

【請求項 1 2 0】

配列番号 4 に示されるアミノ酸配列を含有する、請求項 1 1 8 に記載の抗体または抗原結合部分。

【請求項 1 2 1】

請求項 1、請求項 1 0 2、請求項 1 0 3、および請求項 1 1 5 ～ 1 2 0 に記載の抗体または一部からなる群より選択される、1 つ以上のヒトモノクローナル抗体またはその抗原結合部分を含有し、P s e u d o m o n a s a e r u g i n o s a 感染の予防または阻害のための、受動性ワクチン。 30

【請求項 1 2 2】

2 つ以上のヒトモノクローナル抗体またはその抗原結合部分を含有しており、ここで、該ヒトモノクローナル抗体または一部が、P s e u d o m o n a s a e r u g i n o s a の異なる株に特異的に結合する、請求項 1 2 1 に記載の受動性ワクチン。

【請求項 1 2 3】

生物学的サンプル中の P s e u d o m o n a s a e r u g i n o s a の存在を検出するための方法であって、該サンプルを、請求項 1、請求項 1 0 2、請求項 1 0 3、または請求項 1 1 5 ～ 1 2 0 のいずれか 1 項に記載の抗体またはその抗原結合部分と接触させる工程を包含する、方法。 40

【発明の詳細な説明】

【0 0 0 1】

(技術分野)

本発明は、P s e u d o m o n a s a e r u g i n o s a 感染およびこのような感染によって引き起こされる状態の処置または予防についての組成物および方法に関する。特に、本発明は、ヒト抗体に関し、この抗体は、P s e u d o m o n a s a e r u g i n o s a リポ多糖類 (L P S) に特異的に結合し、そしてその核酸分子をコードする。本発明は、非ヒト動物におけるこの抗体の作製についての方法、および細胞株 (ハイブリドーマ 50

および組換え宿主細胞系を含む)におけるこの抗体の発現についての方法にさらに関する。本発明はまた、この抗体を含むキットおよび薬学的組成物に関する。本発明は、患者に、本明細書中に記載される組成物のいずれかを投与することによって、Pseudomonas 感染を処置または予防する方法にさらに関する。

【0002】

(発明の背景)

(Pseudomonas aeruginosa 感染)

Pseudomonas aeruginosa は、グラム陰性の鞭毛桿体菌 (flagellated rod bacteria) であり、これは、手術、プロテゼ移植および気道処置から生じる院内感染において、有意な病原体であり続ける。Pseudomonas aeruginosa はまた、癌、嚢胞性線維症、糖尿病、心臓疾患、外耳炎 (otitis externa) (外耳炎 (swimmer's ear))、骨髄炎、角膜潰瘍、毛包炎、乳腺炎、肺炎、髄膜炎、尿路感染症、心内膜炎、腹膜炎および老年患者または免疫無防備状態の患者において見出される他の疾患の病因における日和見病原体である。

【0003】

手術患者は、しばしば、その病気 (例えば、外傷、熱傷、吸息損傷および癌) または処置 (例えば、切開または経皮カテーテル法、気管内挿管、心臓手術および胸郭手術、神経手術ならびに胃腸手術による天然の上皮関門の崩壊) によって、Pseudomonas aeruginosa 感染の危険性の増大にある。抗生物質処置または予防、固形器官移植レシピエントの治療的免疫抑制、あるいは Pseudomonas aeruginosa への環境曝露による天然の腸微生物叢の崩壊は、患者を危険性の増大に置き得る。さらに、多剤耐性株は、入院患者単位ならびにナーシングホームにおける有意な感染を引き起こし得る。

【0004】

手術患者は、院内肺炎によって冒され、これは、しばしば、Pseudomonas aeruginosa によって引き起こされる。発症は、入院の最初の 72 時間後に生じ、そして熱、化膿性痰、白血球増加、および胸部 X 線撮影によって明らかになる新規または変化した肺浸潤によって特徴付けられる。口腔咽頭部は、急速にコロニー形成され、これは、下気道に蔓延し得る。手術患者全体における院内感染の発生率は、約 5%~8% であり、そしてあらゆる危篤患者においては、おそらくより高い。外科集中治療室 (ICU) から報告される肺炎の発生率は、15%~20% であり、そして時折より高い。Barie ら、Am. J. Surgery 179: 2S~7S (2000) を参照のこと。

【0005】

嚢胞性線維症 (CF) 患者は、慢性コロニー形成 (colonization) を患い、狭いが進化性のスペクトルの細菌病原体、Pseudomonas aeruginosa は、主要な CF 病原体のままであり、CF 成人において、80%~90% までの世界的罹患率である。このような感染は、衰弱性の炎症悪化、および進行性の肺損傷の断続的エピソードを導く。顕現性病原体はまた、複数の抗生物質レジメンに耐性である傾向があり、従って、感染制御は、CF 患者の生活の質および平均余命における決定的役割を果たす。

【0006】

慢性コロニー形成の発症は、努力呼気量 (FEV) の促進に関連する。本来のコロニー形成株は、ムコイドコロニー形態に形質転換し、これは、アルギネートとして公知の非常に粘着性のエキソ多糖類 (exopolysaccharide) の豊富な産生に起因する。コロニー形成株は、有意により親ムチン性 (mucinophilic) および走化性となり、そして粘膜せん毛クリアランス障害に関連する。Govan J. Royal Soc. Med. 93 補遺、38: 40~45 (2000) を参照のこと。さらに、CF 患者の肺から単離される Pseudomonas aeruginosa は、LPS 脂肪酸アシル化パターンにおける変化、およびいくつかの陽イオン抗菌ペプチド (CAMP) 50

の殺菌活性に対する耐性の増強の変化を示す。

【0007】

*Pseudomonas aeruginosa*のLPSリピドAにおける変更は、CF隔離集団において見出され、これは、抗菌ペプチドに対する細菌耐性およびLPSが炎症性メディエータを誘導する能力の両方を増大した。CF患者は、血清および痰の両方において、*Pseudomonas aeruginosa* LPSに対する非常に高い抗体力価を有し、これは、インビボでのその生物学的活性（例えば、炎症前メディエータ放出）を中和し得る。Pier Trends Microbiol. 8: 247~251 (2000)を参照のこと。

【0008】

重篤な熱傷創傷患者における罹病率および死亡率の主要な原因は、*Pseudomonas aeruginosa*の感染である。Leeら、Vaccine 18: 1952~1961 (2000)を参照のこと。熱傷創傷は、非常に滲出性であり、細菌コロニー形成について、湿性の栄養豊かな環境を作製する。熱傷創傷は、重篤な組織損傷に起因して、患者の免疫応答および脈管由来の抗生物質に主として到達しがたい。さらに、熱傷創傷は、宿主を免疫無防備状態のままにし、免疫グロブリンγ (IgG)のレベルは、内因性に減少する。処置を受けないと、熱傷創傷感染は、蔓延し得、そして敗血症に発達し得、炎症性サイトカイン（インターロイキン-1 (IL-1)、IL-6および腫瘍壊死因子 (TNF)を含む）の関連した産生が生じる。熱傷創傷感染はまた、治癒の遅延、瘢痕の増大、部分的な肥厚欠陥から全体の肥厚欠陥への転換、および栄養要求の増大をもたらし得る。

【0009】

静脈内免疫グロブリン (IVIg)は、益々使用されて、細菌感染およびウイルス感染、ならびに原発性免疫不全障害および二次性免疫不全障害の両方を処置している。IVIgは、正常ドナー由来のプールされるヒトポリクローナル抗体から構成され、これは、原発性および二次性の抗体欠損症についての補充療法として使用され、そして免疫媒介性疾患（自己免疫炎症状態および全身性炎症状態を含む）を処置するために使用される。免疫グロブリンは、細菌のオプソニン作用および食作用、細菌毒素の中和、微生物付着の阻害、ならびに細菌の補体誘導性溶解を促進する。Feltsら、Burns 25: 415~423 (1999)を参照のこと。

【0010】

防御性免疫グロブリンの創傷部位および熱傷部位への直接送達および局所送達は、熱傷創傷の血管新生、ならびにバイオフィーム障壁の欠如を克服するための合理的手段を表す。予防的および感染後の両方のIgGの局所送達は、*Pseudomonas aeruginosa*感染性の熱傷創傷のマウスモデルにおいて、生存率を改善したことが実証された。Feltsら、Burns 25: 415~423 (1999)を参照のこと。

【0011】

プロテゼデバイスの生体工学における進歩は、無数の患者の寿命を改善した。しかし、この進歩は、移植関連感染によって抑制されており、これは、しばしば、抗生物質処置に耐える。感染性生物（*Pseudomonas aeruginosa*を含む）は、合成の移植材料を選択的に標的化し、重篤かつ犠牲の大きな感染を誘導し、これは、頻繁に、コロニー形成デバイスの除去を必要とする。

【0012】

最初の微生物接着は、生体材料のコロニー形成の主要な決定要素である。なぜなら、最初の接着生物は、しばしば、生体材料表面に接着する成熟したバイオフィームに進行するからである。プロテゼ上のバイオフィーム形成の減少を目指す研究の焦点は、移植材料の表面の修飾またはコーティングに指向されている。しかし、表面化学および抗生物質放出コーティングを利用するアプローチは、完全に首尾良くはない。

【0013】

手術部位は、しばしば、免疫無防備状態であるので、見込みのあるアプローチは、局所創

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傷部位の免疫刺激を伴う。研究は、プールされるポリクローナルヒト抗体が、感染性細菌にオプソニンを作用させ、そしてプールされる抗体は、*Pseudomonas aeruginosa*の接着速度および表面増殖動力学を阻害し得、従って、バイオフィーム形成を減少することを示した。Poelstraら、J. Biomed. Mat. Res. 51:224~232 (2000)を参照のこと。

【0014】

腹膜炎は、しばしば、潰瘍、虫垂炎、憩室炎、イレウス、射創または刺創、腹手術中の障害、および連続携行式腹膜灌流(CAPD)によって引き起こされる。外因性の病原性細菌(*Pseudomonas aeruginosa*を含む)によって引き起こされる院内腹膜炎は、免疫無防備状態集団および老年集団についての特に重大な問題である。

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【0015】

腹膜炎についての現在の処置レジメンは、抗生物質に集中するが、しかし抗生物質耐性が、有意な速度で生じ、そして頻繁に、臨床不全に関連する。IVIgは、腹膜炎において、有望だが、一貫しない結果を示したが、しかし、熱傷創傷に関して、*Pseudomonas aeruginosa*に対するプールされるポリクローナル免疫グロブリンの局所(腹膜)送達は、マウスモデルにおいて、感染を有意に減少することが示された。Barekziら、Antimicrob. Agents Chemotherap. 43:1609~1615 (1999)を参照のこと。

【0016】

抗生物質レジメンを用いた*Pseudomonas aeruginosa*感染の処置は、益々困難になっている。なぜなら、とりわけ、抗生物質耐性株が現れたからである。*Pseudomonas aeruginosa*感染の予防および処置についての受動抗体治療の出現は、有望ではあるが、非ヒト動物抗体のない精製ヒト抗体(これは、臨床量で、*Pseudomonas aeruginosa*に特異的に結合する)の有用性によって抑制されている。

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【0017】

非ヒト抗体調製物(マウスモノクローナル抗体を含む)は、その免疫原性のために、一般的に、ヒト治療について許容されない。ヒトポリクローナル抗体調製物は、ヒト治療に適切ではあるが、*Pseudomonas aeruginosa*についての可変力価の防御性抗体を有し、そして複数のドナー由来の抗体の精製の高いコストを有する。

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【0018】

モノクローナル抗体は、理論上、低いコストで、そして所望の特異性で、無制限の量で作製され得る。しかし、有効なヒトモノクローナル抗体は、作製が困難であり、そしてエプスタインバーウイルスを用いて形質転換されるべき適切な抗体を発現するヒトB細胞を必要とする。この得られるモノクローナル抗体調製物は、ヒトの治療用途に適切ではないようである。さらに、今まで試験されたヒトモノクローナル抗体の大半は、IgMであり、これは、肺組織に十分に浸透せず、そして免疫複合体形成および炎症の増強に関連し得る。

【0019】

従って、*Pseudomonas aeruginosa*に特異的に結合する精製ヒトIgG抗体、その調製および使用についての方法、ならびにその薬学的組成物およびキットについての必要性が存在する。

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【0020】

(発明の要旨)

本発明は、単離ヒト抗体を提供し、この抗体は、*Pseudomonas aeruginosa*リポ多糖類(LPS)に特異的に結合する。本発明は、非ヒト動物におけるこの抗体の作製についての方法、細胞株(ハイブリドーマおよび組換え宿主細胞系を含む)におけるこの抗体の発現についての方法をさらに提供する。本発明はまた、この抗体を含むキットおよび薬学的組成物を提供する。さらに、本発明は、患者に、本明細書中に記載される薬学的組成物を投与することによって、*Pseudomonas*感染を処置または予

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防する方法を提供する。

【0021】

(発明の詳細な説明)

本発明に従って、完全なヒト単離抗体またはその抗原結合部分が提供され、これは、*Pseudomonas aeruginosa* LPSに特異的に結合する。好ましい実施形態において、この完全なヒト単離抗体は、モノクローナルである。他の好ましい実施形態は、抗体の重鎖および軽鎖をコードするヌクレオチド配列、ならびに抗体の重鎖および軽鎖を含むアミノ酸配列、ならびに特に、CDR1～CDR3の連続した重鎖配列および軽鎖配列に対応する特定の配列を含む。さらに、本明細書中に開示される抗体と類似の結合性質を有する抗体、および本明細書中に開示される抗体と類似の機能性を有する抗体（または他のアンタゴニスト）が提供される。このような免疫グロブリン分子およびモノクローナル抗体を発現するハイブリドーマがまた提供される。

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【0022】

本明細書中の用語は、一般的に、当業者によって理解されるようなその通常の意味を有する。以下の用語は、本明細書中で使用される場合、以下の一般的な意味を有することが意図される：

「Bリンパ球性細胞またはその後代」は、Bリンパ球性系統由来の任意の細胞またはBリンパ球性系統に運命付けられる任意の細胞をいう。例は、最初期Bリンパ球幹細胞から記憶B細胞に開始するB細胞発生経路におけるすべてのBリンパ球、形質細胞およびインビトロで作製される任意のハイブリドーマを含むがこれらに限定されない。

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【0023】

「二重特異性抗体」は、2つの別々の抗原についての親和性を有する1つの抗体である。例えば、二重特異性抗体は、重鎖および軽鎖の1つの組み合わせを用いて、*Pseudomonas aeruginosa* LPSを認識し得、そして第一の組み合わせに付着した重鎖および軽鎖の第二の組み合わせを用いて、白血球細胞の表面マーカーを認識し得る。McCormickら、J. Immunol. 158:3474～3482 (1997)を参照のこと。

【0024】

「キメラ抗体」は、その本来の形態から変更されて、別のタンパク質由来のアミノ酸配列を含む抗体である。キメラ抗体は、本来の抗体アミノ酸配列の少なくとも一部分、代表的には、抗原結合領域(F_{ab})を含む部分を保持する。キメラ抗体の例としては、二重特異性抗体、および他の非免疫グロブリンタンパク質配列との融合が挙げられるがこれらに限定されない。

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【0025】

「サイトカイン」は、一般的に、免疫系のシグナリング分子をいう。サイトカインとしては、インターロイキン(IL)、トランスフォーミング増殖因子(TGF)、腫瘍壊死因子(TNF)、リンホトキシン(LT)、インターフェロン、顆粒球-マクロファージコロニー刺激因子(GM-CSF)、マクロファージCSF、顆粒球CSFおよび遊走阻害因子が挙げられるがこれらに限定されない。

【0026】

「誘導体化する」は、非免疫グロブリン因子の免疫グロブリン分子への接着のプロセスをいう。誘導体化因子の例としては、毒素、補体、抗生物質、ペプチド、多糖類、脂質、有機ポリマー、放射能標識および無機化合物が挙げられるがこれらに限定されない。

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【0027】

「発現制御配列」は、特定の条件下、または特定の細胞において、遺伝子配列の誘導性発現または構成発現を可能にする配列をいう。発現制御配列を調節する細胞プロセスの例としては、遺伝子転写、タンパク質翻訳、メッセンジャーRNAスプライシング、免疫グロブリンアイソタイプスイッチ、タンパク質グリコシル化、タンパク質開裂、タンパク質分泌、細胞内タンパク質局在および細胞外タンパク質ホーミングが挙げられるがこれらに限定されない。

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「融合タンパク質」は、2つ以上の異なるタンパク質のアミノ酸配列を含むキメラタンパク質をいう。代表的には、融合タンパク質は、当該分野で周知のインビトロ組換え技術から得られる。しかし、融合タンパク質は、インビボ交差、または他の組換え事象から得られ得る。

【0028】

「ヒト免疫グロブリン分子」は、ヒト免疫グロブリン遺伝子配列によってコードされる免疫グロブリンタンパク質をいう。この免疫グロブリン遺伝子配列は、任意の非ヒト動物において発現され得る。

【0029】

「ヒトモノクローナル抗体」は、同一の特異性を有するヒト抗体の集団のメンバーである抗体をいう。このヒト抗体の集団は、ハイブリドーマまたは他の不死化細胞株において、ならびに外因性ヒト抗体遺伝子配列を発現する組換え細胞株において産生され得る。 10

【0030】

「免疫無防備状態の患者」は、外来抗原または因子に対する免疫応答が、他の状態（例えば、器官移植患者）についての処置に関連して、疾患（例えば、AIDS）、侵襲性手術または薬物治療のいずれかによって損なわれる患者をいう。

【0031】

「標識」は、本発明の免疫グロブリンに、普通にはこの免疫グロブリンに関連しない機能的特性を付着する任意の分子をいう。標識は、免疫グロブリンの化学修飾を介して、二重特異性免疫グロブリンの2つのF_ab領域のうちの1つによるこの標識の認識を介して、 20
第三の因子についての親和性（例えば、アビジン／ビオチン（biogen）相互作用）を介して、放射能標識を介して、または組換え発現される融合タンパク質として付着され得る。標識は、臨床目的または研究目的についての分子タグまたは放射性タグとして、あるいはPseudomonas aeruginosa感染との闘争についての因子（例えば、毒素または補体タンパク質）として機能し得る。標識の他の例としては、酵素、蛍光分子、磁気標識、エピトープタグ（例えば、H. インフルエンザ赤血球凝集素（hemagglutinin）、抗生物質、補体タンパク質およびサイトカインが挙げられ得る。

【0032】

「呼吸患者」は、いくつかの他の医療処置に関連して、呼吸器系の疾患について処置されているか、または呼吸ケア（例えば、挿管または換気）を受けているかのいずれかの任意 30
の患者をいう。

【0033】

「手術患者」は、侵襲性手術手順（代表的には、真皮の穿刺または切開を伴う）に供される任意の患者をいう。

【0034】

「毒素」は、細胞の殺傷の目的について、抗体に付着され得るタンパク質または非タンパク質化合物をいい、この細胞に、抗体が付着している。毒素の例としては、補体、抗生物質、ペプチド、多糖類、脂質、有機ポリマー、放射能標識および無機化合物が挙げられるがこれらに限定されない。

【0035】

「ベクター」は、目的のDNA配列のクローニング、増殖、組換え、変異またはそのネイティブ細胞の外側への発現を可能にするDNA分子をいう。しばしば、ベクターは、発現制御配列を有し、これは、特定の条件下、または特定の細胞において、遺伝子配列の誘導性発現または構成発現を可能にする。ベクターの例としては、プラスミド、酵母人工染色体（YAC）、ウイルス、バクテリオファージおよびファージミドが挙げられるがこれらに限定されない。 40

【0036】

「Xenomouse（登録商標）」は、相同に標的される内因性の免疫グロブリン遺伝子座を保有するマウスをいい、内因性のマウス免疫グロブリンの発現を不可能にするが、ヒト免疫グロブリン遺伝子座の実質的部分を保有するようにする。Xenomouse（登 50

録商標) 系統のマウスは、ヒト免疫グロブリン遺伝子の体細胞再配列、ヒト可変遺伝子の超変異 (hypermutation) および免疫グロブリンアイソタイプスイッチを可能にする。従って、Xenomouse (登録商標) 系統のマウスは、ヒト免疫グロブリン遺伝子配列を利用して、抗原性チャレンジに対する有効な体液性応答の開始を可能にする。この得られる抗体は、完全にヒトであり、そしてその動物自身から、この動物から抽出される培養細胞から、そしてXenomouse (登録商標) Bリンパ球性系統またはその後代から作製されるハイブリドーマから単離され得る。さらに、特定の抗原性チャレンジに対して惹起される免疫グロブリンをコードする、再配列されるヒト遺伝子配列は、当該分野で周知の組換え手段によって単離され得る。

【0037】

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(抗体構造)

基本的な抗体構造単位は、テトラマーを含むことが知られている。各々のテトラマーは、ポリペプチド鎖の2つの同一の対から構成され、各々の対は、1つの「軽」鎖 (約25 kDa) および1つの「重」鎖 (約50~70 kDa) を有する。各々の鎖のアミノ末端部分は、主に抗原認識を担う、およそ100~110以上のアミノ酸の可変領域を含む。各々の鎖のカルボキシ末端部分は、主に、エフェクター機能を担う定常領域を規定する。ヒト軽鎖は、 κ 軽鎖および λ 軽鎖として分類される。重鎖は、 μ 、 δ 、 γ 、 α または ϵ として分類され、そしてそれぞれ、IgM、IgD、IgG、IgA および IgE として抗体のアイソタイプを規定する。軽鎖および重鎖の内部において、可変領域および定常領域は、約12以上のアミノ酸の「J」領域によって結合され、重鎖はまた、約10以上のアミノ酸の「D」領域を含む。一般的に、Fundamental Immunology、第7章 (Paul, W. 編、第2版、Raven Press, N. Y. (1989)) (すべての目的について、その全体において参考として援用される) を参照のこと。各々の軽/重鎖対の可変領域は、抗体結合部位を形成する。

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【0038】

従って、インタクトなIgG抗体は、2つの結合部位を有する。二重特異性抗体 (bifunctional antibody) または二重特異性抗体 (bispecific antibody) においてを除いて、この2つの結合部位は、同じである。

【0039】

この鎖はすべて、3つの超可変領域 (また、相補性決定領域またはCDRと呼ばれる) によって結合される、同じ一般的構造の比較的保存される枠組み構造領域 (FR) を示す。この各々の対の2つの鎖由来のCDRは、枠組み構造領域によって整列され、特定のエプトープへの結合を可能にする。N末端からC末端へ、軽鎖および重鎖の両方は、FR1ドメイン、CDR1ドメイン、FR2ドメイン、CDR2ドメイン、FR3ドメイン、CDR3ドメインおよびFR4ドメインを含む。各々のドメインへのアミノ酸の割り当ては、Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md (1987 および 1991))、または Chothia および Lesk J. Mol. Biol. 196:901~917 (1987); Chothia ら、Nature 342:878~883 (1989) の定義に従う。

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【0040】

二重特異性抗体 (bispecific antibody) または二重特異性抗体 (bifunctional antibody) は、2つの異なる重/軽鎖対、および2つの異なる結合部位を有する人工のハイブリッド抗体である。二重特異性抗体は、種々の方法 (ハイブリドーマの融合または Fab' フラグメントの結合を含む) によって産生され得る。例えば、Songsivilai および Lachmann Clin. Exp. Immunol. 79:315~321 (1990)、Kostelny ら、J. Immunol. 148:1547~1553 (1992) を参照のこと。さらに、二重特異性抗体は、「diabodies」 (Holliger ら、「Diabodies」: small bivalent and bispecific antibody frag

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ments」PNAS USA 90:6444~6448 (1993)) または「Janusins」(Trauneckerら、「Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells」EMBO J 10:3655~3659 (1991) およびTrauneckerら、「Janusins: new molecular design for bispecific reagents」Int J Cancer Suppl 7:51~52 (1992)) として形成され得る。二重特性抗体の産生は、従来の抗体の産生と比較して、比較的労働集約的なプロセスであり得、そして精製の収率および程度は、二重特異性抗体について、一般的により低い。二重特異性抗体は、1つの結合部位を有するフラグメントの形態 (例えば、Fab、Fab' およびFv) には存在しない。

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【0041】

(非ヒト動物由来のヒト抗体)

ヒト抗体は、マウスまたはラットの可変領域および／または定常領域を有する抗体に関連する問題のいくらかを回避する。このようなマウスまたはラット由来のタンパク質の存在は、抗体の迅速な除去を導き得るか、または患者による抗体に対する免疫応答の作製を導き得る。マウスまたはラット由来の抗体の利用を回避するために、ヒト化抗体を開発し得るか、または齧歯類へのヒト抗体機能の導入を通じて、完全なヒト抗体を作製し、その結果、この齧歯類が、完全なヒト抗体を産生することが要求されている。

【0042】

YACにおいて、メガベースサイズのヒト遺伝子座をクローニングおよび再構築する能力、ならびにその遺伝子座をマウス生殖系列に導入する能力は、極めて大きい。または不十分にマッピングされる遺伝子座の機能的成分の解明、ならびにヒト疾患の有用なモデルの作製に対する強力なアプローチを提供する。さらに、マウス遺伝子座のそのヒト等価物での置換についてのこのようなテクノロジーの利用は、発生の間のヒト遺伝子産物の発現および制御、他の系とのその伝達、ならびに疾患誘導および進行におけるその関与への独特の洞察を提供し得る。

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【0043】

このようなストラテジーの重要な実際の適用は、マウスの体液性免疫系の「ヒト化」である。ヒト免疫グロブリン(Ig)遺伝子座のマウス(このマウスにおいて、内因性のIg遺伝子は、不活化されている)への導入は、プログラム発現および抗体のアセンブリ、ならびにB細胞発生におけるその役割の基礎をなす機構を研究する機会を提供する。さらに、このようなストラテジーは、完全なヒトモノクローナル抗体(Mab)の産生(ヒト疾患における抗体治療の保証の履行に対する重要な画期的出来事)についての理想的供給源を提供し得る。完全なヒト抗体は、マウスまたはマウス由来のMabに内因性の免疫原性応答およびアレルギー応答を最小化することが期待され、従って、投与される抗体の効能および安全性を増大することが期待される。完全なヒト抗体の使用は、慢性ヒト疾患および再発性ヒト疾患(例えば、炎症、自己免疫、癌および細菌感染)の処置において、実質的利点を提供することが期待され得、この疾患は、潜在的に、度重なる抗体投与を必要とする。

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【0044】

この目的に向けた1つのアプローチは、マウス抗体産生に欠陥のあるマウス系統を、ヒトIg遺伝子座の大きなフラグメントを用いて操作することであり、これは、このようなマウスが、マウス抗体の非存在下において、ヒト抗体の大きなレパートリーを産生することを見越していた。大きなヒトIgフラグメントは、大きな可変性の遺伝子多様性、ならびに抗体産生および発現の適切な調節を保持する。抗体多様化および選択、ならびにヒトタンパク質に対する免疫学的寛容の欠如についてのマウス機構の利用によって、これらのマウス系統における再生ヒト抗体レパートリーは、目的の任意の抗原(ヒト抗原を含む)に対する高親和性抗体を生じるはずである。ハイブリドーマテクノロジーを用いて、所望の特異性を有する抗原特異的ヒトMabは、容易に産生および選択され得る。

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【0045】

この一般的なストラテジーは、1994年に公表されるように、第一のXenomouse（登録商標）系統の作製と関連して実証された。Greenら、Nature Genetics 7:13~21（1994）を参照のこと。このXenomouse（登録商標）系統は、酵母人工染色体（YAC）（ヒト重鎖遺伝子座およびκ軽鎖遺伝子座のそれぞれ245 kbおよび190 kbサイズの生殖系列配置フラグメントを含む）を用いて操作され、これは、中心的な可変領域配列および定常領域配列を含んだ。ヒトIg含有YACは、抗体の再配列および発現の両方について、このマウス系統に適合することが証明され、そして不活化マウスIg遺伝子について置換し得た。これは、B細胞発生を誘導する能力、完全なヒト抗体の成熟様ヒトレパートリーを産生する能力、および抗原特異的ヒトMabを作製する能力によって実証された。これらの結果はまた、より多数のV遺伝子、さらなる調節エレメントおよびヒトIg定常領域を含むヒトIg遺伝子座のより大きな部分の導入が、実質的に完全なレパートリーを要約し得ることを示唆し、これは、感染および免疫に対するヒト体液性応答の特徴である。Greenらの研究は、それぞれヒト重鎖遺伝子座およびκ軽鎖遺伝子座のメガベースサイズの生殖系列配置のYACフラグメントの導入を通じて、ヒト抗体レパートリーの約80%を超える導入に最近拡張されて、Xenomouse（登録商標）マウスを産生した。Mendezら、Nature Genetics 15:146~156（1997）、GreenおよびJakobovits J. Exp. Med. 188:483~495（1998）、ならびに1996年12月3日に出願された米国特許出願番号08/759,620（この開示は、本明細書によって、参考として援用される）を参照のこと。

【0046】

このようなアプローチは、さらに、1990年1月12日に出願された米国特許出願番号07/466,008、1990年11月8日に出願された同07/610,515、1992年7月24日に出願された同07/919,297、1992年7月30日に出願された同07/922,649、1993年3月15日に出願された同08/031,801、1993年8月27日に出願された同08/112,848、1994年4月28日に出願された同08/234,145、1995年1月20日に出願された同08/376,279、1995年4月27日に出願された同08/430,938、1995年6月5日に出願された同08/464,584、1995年6月5日に出願された同08/464,582、1995年6月5日に出願された同08/463,191、1995年6月5日に出願された同08/462,837、1995年6月5日に出願された同08/486,853、1995年6月5日に出願された同08/486,857、1995年6月5日に出願された同08/486,859、1995年6月5日に出願された同08/462,513、1996年10月2日に出願された同08/724,752および1996年12月3日に出願された同08/759,620において議論および詳細に概説される。また、Mendezら、Nature Genetics 15:146~156（1997）ならびにGreenおよびJakobovits J. Exp. Med. 188:483~495（1998）を参照のこと。また、1996年6月12日に特許を取った後に公開された欧州特許第EP 0 463 151 B1号、1994年2月3日に公開された国際特許出願第WO 94/02602、1996年10月31日に公開された国際特許出願第WO 96/34096および1998年6月11日に公開された同第WO 98/24893を参照のこと。上記で引用される特許、出願および参考文献の各々の開示は、本明細書によって、その全体において参考として援用される。

【0047】

本発明に従った抗体は、好ましくは、トランスジェニックマウスの利用を通じて調製され、このマウスは、ヒト抗体を産生する挿入ゲノムの実質的部分を有するが、内因性のマウス抗体の産生においては不完全にする。次いで、このようなマウスは、ヒト免疫グロブリン分子および抗体を産生し得、そしてマウス免疫グロブリン分子および抗体の産生は不完全である。同じことの達成について利用されるテクノロジーは、本明細書中に開示される

特許、出願および参考文献に開示される。

【0048】

このようなテクノロジーの使用を通じて、*Pseudomonas aeruginosa* LP Sに対する完全なヒトモノクローナル抗体またはその抗原結合部分が産生された。本質的に、本発明者らは、マウスのXen oMouse（登録商標）システムを、熱殺傷した*Pseudomonas aeruginosa*を用いて免疫し、*Pseudomonas aeruginosa* LP S抗体を発現するマウスから脾臓細胞およびリンパ節細胞（例えば、B細胞）を回収し、このような回収される細胞を、非分泌性骨髓腫細胞に融合して、不死化ハイブリドーマ細胞株を調製し、そしてハイブリドーマ細胞株をスクリーニングして、目的の抗原に特異的な抗体を産生する細胞株を同定した。

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【0049】

認識されるように、本発明に従った抗体は、ハイブリドーマ細胞株ではない細胞株において発現され得る。特定の抗体をコードする配列は、適切な宿主細胞の形質転換について使用され得る。形質転換は、ポリヌクレオチドの宿主細胞への導入（例えば、ポリヌクレオチドのウイルス（またはウイルスベクター）へのパッケージング、およびウイルス（またはベクター）を用いた宿主細胞の形質導入を含む）についての任意の公知の方法、あるいは米国特許第4,399,216号、同第4,912,040号、同第4,740,461号および同第4,959,455号（これらの特許は、本明細書によって、本明細書中において参考として援用される）によって例示されるような当該分野で公知のトランスフェクション手順によって行われ得る。使用されるこの形質転換手順は、形質転換されるべき宿主に依存する。異種ポリヌクレオチドの哺乳動物細胞への導入についての方法は、当該分野で周知であり、そしてデキストラン媒介性トランスフェクション、リン酸カルシウム沈殿、ポリブレン媒介性トランスフェクション、プロトプラスト融合、エレクトロポレーション、リポソームへのポリヌクレオチドのカプセル化、およびDNAの核への直接マイクロインジェクションが挙げられる。

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【0050】

発現についての宿主として利用可能な哺乳動物細胞株は、当該分野で周知であり、そして以下を含むがこれらに限定されないAmerican Type Culture Collection（ATCC）から入手可能な多くの不死化細胞株を含む：チャイニーズハムスター卵巣（CHO）細胞、NS/O、HeLa細胞、ベビーハムスター腎臓（BHK）細胞、サル腎臓細胞（COS）、ヒト肝細胞癌腫細胞（例えば、Hep G2）および多くの他の細胞株。特定の好みの細胞株が、どの細胞株が、高い発現レベルを有するか、そして構成的な*Pseudomonas aeruginosa* LP S結合性質を有する抗体を産生するかの決定を通じて選択される。

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【0051】

さらに、産生細胞株からの本発明の抗体（またはその抗体由来の他の部分）の発現は、多くの公知の技術を用いて増強され得る。例えば、発現の増強は、ジヒドロ葉酸レダクターゼ（DHFR）またはグルタミンシンテターゼ遺伝子発現系（GS系）を利用して、同時増幅発現系によって実現され得る。例えば、KaufmanおよびSharp J. Mol. Biol. 159:601~621（1982）；欧州特許第0 216 846号、同第0 256 055号および同第0 323 997号；ならびに欧州特許出願第89303964.4を参照のこと。

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【0052】

本発明の抗体はまた、哺乳動物または植物（これは、目的の免疫グロブリン重鎖配列および軽鎖配列についてトランスジェニックである）の作製、およびその動物または植物から回収可能な形態にある抗体の産生を通じて産生され得る。哺乳動物におけるトランスジェニック産生に関連して、抗体は、ヤギ、ウシまたは他の哺乳動物の乳汁において産生され得、そしてこれらの乳汁から回収され得る。例えば、米国特許第5,827,690号、同第5,756,687号、同第5,750,172号および同第5,741,957号を参照のこと。

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【0053】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、非ヒト動物において発現され、そして *Pseudomonas aeruginosa* LPS に特異的に結合する。好ましい実施形態において、この単離ヒト抗体またはその抗原結合部分は、モノクローナル抗体である。

【0054】

本発明は、さらに、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* 細胞についてオプソニン作用性である。好ましい実施形態において、この単離ヒト抗体またはその抗原結合部分は、*Pseudomonas aeruginosa* 細胞の食作用を促進する。

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【0055】

本発明はまた、この単離ヒト抗体またはその抗原結合部分が、*Pseudomonas aeruginosa* に対する免疫応答を増強することを意図する。好ましい実施形態において、この単離ヒト抗体またはその抗原結合部分は、*Pseudomonas aeruginosa* 細胞の殺傷を促進する。より好ましい実施形態において、この単離ヒト抗体またはその抗原結合部分は、*Pseudomonas aeruginosa* 細胞に致死的な因子の送達によって、*Pseudomonas aeruginosa* 細胞の殺傷を促進する。

【0056】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPS に特異的に結合し、ここで、この抗体またはその抗原結合部分は、*Pseudomonas aeruginosa* 感染を阻害する。

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【0057】

本発明はまた、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPS に特異的に結合し、ここで、この抗体またはその抗原結合部分は、*Pseudomonas aeruginosa* LPS に、 5×10^{-7} M 以下、好ましくは、 5×10^{-7} M $\sim$ 1×10^{-7} M の解離定数 (K_d) で結合する。より好ましい実施形態において、この抗体またはその抗原結合部分は、*Pseudomonas aeruginosa* LPS に、 1×10^{-7} M $\sim$ 5×10^{-8} M の K_d で結合する。より好ましい実施形態において、この抗体またはその抗原結合部分は、*Pseudomonas aeruginosa* LPS に、 5×10^{-8} M $\sim$ 1×10^{-8} M の K_d で結合する。

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【0058】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPS に特異的に結合し、そして1時間以上のインビボ半減期を有する。好ましい実施形態において、この抗体またはその抗原結合部分は、1時間と30日との間のインビボ半減期を有する。より好ましい実施形態において、この抗体またはその抗原結合部分は、16日と30日との間のインビボ半減期を有する。別のより好ましい実施形態において、この抗体またはその抗原結合部分は、1時間と15日との間のインビボ半減期を有する。

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【0059】

本発明の *Pseudomonas aeruginosa* LPS に特異的に結合するこの単離ヒト抗体またはその抗原結合部分は、免疫グロブリン G (IgG)、IgM、IgE、IgA および IgD であり得る。好ましい実施形態において、この IgG は、IgG1 サブタイプ、IgG2 サブタイプ、IgG3 サブタイプまたは IgG4 サブタイプであり得る。

【0060】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPS に特異的に結合し、そして標識される。好ましい実施形態において、この標識は、放射性標識、酵素標識、蛍光標識、毒素、磁気因子、二次

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抗体、親和性標識、エピトープタグ、抗生物質、補体タンパク質またはサイトカインである。

【0061】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、そして κ 軽鎖およびその枠組み構造配列を含む。好ましい実施形態において、この κ 軽鎖の枠組み構造配列は、ヒトV κ 2/A2遺伝子によってコードされる。好ましい実施形態において、この κ 軽鎖は、生殖系列V κ 2/A2遺伝子によってコードされる κ 軽鎖由来の7と15との間の変化を含む。より好ましい実施形態において、この κ 軽鎖は、生殖系列V κ 2/A2遺伝子によってコードされる κ 軽鎖由来の6以下のアミノ酸変化を含む。より好ましい実施形態において、この κ 軽鎖は、生殖系列V κ 2/A2遺伝子によってコードされる κ 軽鎖由来の3以下のアミノ酸変化を含む。

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【0062】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、そして配列番号4のアミノ酸配列を有する κ 軽鎖を含む。本発明は、さらに、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、そして配列番号3の核酸配列によってコードされる κ 軽鎖を含む。本発明はまた、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、そして λ 軽鎖を含む。

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【0063】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、可変(V)領域、多様性(D)領域および結合(J)領域から構成され、そしてその枠組み構造配列から構成される重鎖を含む。好ましい実施形態において、この重鎖の可変領域は、ヒトV_H3/V3~33遺伝子によってコードされる。別の好ましい実施形態において、この重鎖の多様性領域は、ヒトD2~8遺伝子によってコードされる。別の好ましい実施形態において、この重鎖の結合領域は、ヒトJ_H4b遺伝子によってコードされる。より好ましい実施形態において、この可変領域は、生殖系列V_H3/V3~33遺伝子によってコードされる可変領域由来の7と15との間のアミノ酸変化を含む。より好ましい実施形態において、この重鎖は、生殖系列V_H3/V3~33遺伝子によってコードされる可変領域由来の6以下のアミノ酸変化を含む。より好ましい実施形態において、この重鎖は、生殖系列V_H3/V3~33遺伝子によってコードされる可変領域由来の3以下のアミノ酸変化を含む。

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【0064】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、そして配列番号2のアミノ酸配列を有する重鎖を含む。

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、そして配列番号1の核酸配列によってコードされる。

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【0065】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、そしてFabフラグメント、F(ab')₂フラグメントおよびF_rフラグメントからなるリストから選択される抗原結合ドメインを含む。

【0066】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、そしてこの抗体は、単鎖抗体である。

【0067】

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本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、そしてこの抗体は、キメラ抗体である。好ましい実施形態において、このキメラ抗体は、異なるヒト抗体由来の枠組み構造領域およびCDR領域を含む。より好ましい実施形態において、このキメラ抗体は、二重特異性である。より好ましい実施形態において、このキメラ抗体は、*Pseudomonas aeruginosa* LPS、および以下からなるリストから選択される標識について二重特異性である：放射性標識分子、酵素標識、蛍光標識、毒素、磁気因子、二次抗体、親和性標識、エピトープタグ、抗生物質、補体タンパク質およびサイトカイン。

【0068】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、そしてこの抗体またはその一部分は、誘導体化される。好ましい実施形態において、この抗体またはその一部分は、ポリエチレングリコール、少なくとも1つのメチル基またはエチル基、あるいは少なくとも1つの炭水化物部分を用いて誘導体化される。

【0069】

本発明は、*Pseudomonas aeruginosa* LPSに特異的に結合する単離ヒト抗体またはその抗原結合部分、および薬学的に受容可能なキャリアを含む薬学的組成物を意図する。本発明は、その抗体またはその抗原結合部分、それについての薬学的に受容可能なキャリア、および容器を含むキットをさらに意図する。好ましい実施形態において、このキットは、使用についての指示書をさらに含む。

【0070】

本発明は、*Pseudomonas aeruginosa*感染の処置または予防についての方法を意図し、この方法は、薬学的組成物を、*Pseudomonas aeruginosa*に感染されている危険性の患者または*Pseudomonas aeruginosa*に現在感染した患者に投与する工程を包含する。

【0071】

好ましい実施形態において、このヒト抗体は、モノクローナル抗体である。別の好ましい実施形態において、この薬学的組成物は、注入、経粘膜（*transmucosal*）、経口、吸入、目、直腸、持続性作用のある移植、リポソーム、乳剤、クリーム、局所放出手段または徐放性手段を介して投与される。別の好ましい実施形態において、この抗体は、第二のタンパク質との融合である。より好ましい実施形態において、この第二のタンパク質は、以下からなるリストから選択される：毒素ペプチド部分、補体タンパク質、放射性標識タンパク質、サイトカインまたは抗生物質タンパク質。別の好ましい実施形態において、この抗体は、放射性標識、毒素、補体タンパク質、サイトカインまたは抗生物質で標識される。別の好ましい実施形態において、この患者は、熱傷患者、手術患者、プロテゼレシピエント、呼吸患者、癌患者、嚢胞性線維症患者または免疫無防備状態の患者である。別の好ましい実施形態において、この薬学的組成物は、毒素、補体タンパク質、放射性標識タンパク質、サイトカイン、抗生物質またはそれらの任意の組み合わせをさらに含む。

【0072】

本発明は、単離細胞株を意図し、これは、ヒト抗体またはその抗原結合部分を産生し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合する。好ましい実施形態において、この細胞株は、ハイブリドーマである。

【0073】

本発明は、*Pseudomonas aeruginosa* LPSに特異的に結合する単離ヒト抗体またはその抗原結合部分の産生方法を意図し、この方法は、以下の工程：

- a) 抗体が産生される条件下で、抗体を産生し得る非ヒト細胞を培養する工程；
 - b) この細胞培養物から抗体を単離する工程、
- を包含する。

【0074】

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好ましい実施形態において、*Pseudomonas aeruginosa* LPSに特異的に結合する単離ヒト抗体またはその抗原結合部分の産生方法は、ハイブリドーマを利用する。別の好ましい実施形態において、この方法は、ヒト抗体またはその抗原結合部分をコードする単離核酸分子を用いて形質転換される細胞を利用し、そしてこの細胞は、以下からなるリストから選択される：細菌細胞、酵母細胞、昆虫細胞、両生動物細胞および哺乳動物細胞。より好ましい実施形態において、この哺乳動物細胞は、以下からなるリストから選択される：ヒト細胞、マウス細胞、ラット細胞、イヌ細胞、サル細胞、ヤギ細胞、ブタ細胞、ウシ細胞およびハムスター細胞。より好ましい実施形態において、この哺乳動物細胞は、以下からなるリストから選択される：HeLa細胞、NIH 3T3細胞、CHO細胞、BHK細胞、VERO細胞、CV-1細胞、NS/O細胞およびCOS細胞。 10

【0075】

本発明は、*Pseudomonas aeruginosa* LPSに特異的に結合する単離ヒト抗体またはその抗原結合部分の作製についての方法を意図し、この方法は、以下の工程：

- a) 非ヒト動物にヒト免疫グロブリン遺伝子座を組み込まれた非ヒト動物を、*Pseudomonas aeruginosa* 抗原性組成物を用いて免疫する工程；
- b) この非ヒト動物が、この抗原性組成物に対する体液性応答を開始することを可能にする工程；および
- c) この非ヒト動物からヒト抗体を単離する工程、 20

【0076】

本発明は、非ヒト動物から単離される核酸分子を意図し、この核酸分子は、ヒト抗体重鎖またはその抗原結合部分をコードし、これは、*Pseudomonas aeruginosa* LPSに特異的に結合する。好ましい実施形態において、この核酸分子は、ヒト抗体を産生するハイブリドーマから単離される。

【0077】

本発明は、ヒト抗体重鎖またはその抗原結合部分をコードする単離核酸分子またはそのフラグメントを意図し、これは、配列番号1のヌクレオチド配列を有する*Pseudomonas aeruginosa* LPSに特異的に結合する。好ましい実施形態において、この単離核酸分子は、ヒト抗体の1と3との間CDR領域をコードする配列を含む。 30

【0078】

本発明は、ヒト抗体重鎖またはその抗原結合部分をコードする核酸分子またはそのフラグメントを含むベクターを意図し、これは、*Pseudomonas aeruginosa*に特異的に結合する。好ましい実施形態において、このベクターは、この核酸に作動可能に結合される発現制御配列をさらに含む。

【0079】

本発明は、非ヒト動物から単離される核酸分子を意図し、この核酸分子は、ヒト抗体軽鎖またはその抗原結合部分をコードし、これは、*Pseudomonas aeruginosa* LPSに特異的に結合する。好ましい実施形態において、この核酸分子は、ヒト抗体を産生するハイブリドーマから単離される。 40

【0080】

本発明は、ヒト抗体軽鎖またはその抗原結合部分をコードする単離核酸分子またはそのフラグメントを意図し、これは、配列番号3のヌクレオチド配列を有する*Pseudomonas aeruginosa* LPSに特異的に結合する。好ましい実施形態において、この単離核酸分子は、ヒト抗体の1と3との間CDR領域をコードする配列を含む。

【0081】

本発明は、ヒト抗体軽鎖またはその抗原結合部分をコードする核酸分子またはそのフラグメントを含むベクターを意図し、これは、*Pseudomonas aeruginosa*に特異的に結合する。好ましい実施形態において、このベクターは、この核酸に作動可 50

能に結合される発現制御配列をさらに含む。

【0082】

本発明は、単離宿主細胞を意図し、これは、以下：

a) 非ヒト動物から単離され、そして *Pseudomonas aeruginosa* LPS に特異的に結合するヒト抗体の軽鎖またはその抗原結合部分をコードする核酸分子；あるいは

b) この核酸分子を含むベクター、を含む。

【0083】

本発明は、単離宿主細胞を意図し、これは、以下：

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a) 非ヒト動物から単離され、そして *Pseudomonas aeruginosa* LPS に特異的に結合するヒト抗体の重鎖またはその抗原結合部分をコードする核酸分子；あるいは

b) この核酸分子を含むベクター、を含む。

【0084】

本発明は、単離宿主細胞を意図し、これは、以下：

a) 非ヒト動物から単離され、そして *Pseudomonas aeruginosa* LPS に特異的に結合するヒト抗体の重鎖またはその抗原結合部分をコードする核酸分子、およびそのヒト抗体の軽鎖またはその抗原結合部分をコードする単離核酸分子；あるいは

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b) この核酸分子を含むベクター、を含む。

【0085】

好ましい実施形態において、上記の単離宿主細胞は、以下からなるリストから選択される：ハイブリドーマ細胞、細菌細胞、酵母細胞、昆虫細胞、両生動物細胞および哺乳動物細胞。より好ましい実施形態において、この哺乳動物細胞は、以下からなるリストから選択される：ヒト細胞、マウス細胞、ラット細胞、イヌ細胞、サル細胞、ヤギ細胞、ブタ細胞、ウシ細胞およびハムスター細胞。より好ましい実施形態において、この哺乳動物細胞は、以下からなるリストから選択される：HeLa細胞、NIH 3T3細胞、CHO細胞、BHK細胞、VERO細胞、CV-1細胞、NS/O細胞およびCOS細胞。

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【0086】

本発明は、非ヒト動物から同定され、そして *Pseudomonas aeruginosa* LPS に特異的に結合する、ヒト抗体の重鎖またはその抗原結合部分、軽鎖またはその抗原結合部分、あるいは軽鎖および重鎖の両方またはそれらの抗原結合部分を組換え産生する方法を意図し、この方法は、この核酸分子が発現される条件下で、上記の宿主細胞を培養する工程を包含する。

【0087】

本発明は、*Pseudomonas aeruginosa* LPS に特異的に結合する単離ヒト抗体重鎖またはその抗原結合部分を意図し、これは、上記の重鎖をコードする核酸分子のいずれかによってコードされるか、または上記の宿主細胞のいずれかから単離される。好ましい実施形態において、この単離ヒト抗体重鎖またはその抗原結合部位は、抗体の血清半減期を増大する1と10との間のアミノ酸置換を含む。

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【0088】

本発明は、*Pseudomonas aeruginosa* LPS に特異的に結合する単離ヒト抗体軽鎖またはその抗原結合部分を意図し、これは、上記の重鎖をコードする核酸分子のいずれかによってコードされるか、または上記の宿主細胞のいずれかから単離される。好ましい実施形態において、この単離ヒト抗体軽鎖またはその抗原結合部位は、抗体の血清半減期を増大する1と10との間のアミノ酸置換を含む。

【0089】

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本発明は、上記の核酸分子のいずれかを含む非ヒトトランスジェニック動物を意図する。好ましい実施形態において、この非ヒトトランスジェニック動物は、核酸分子を発現する。より好ましい実施形態において、この非ヒトトランスジェニック動物は、*Pseudomonas aeruginosa* LPSに特異的に結合するヒト抗体の重鎖またはその抗原結合部分をコードする単離核酸分子、およびそのヒト抗体の軽鎖またはその抗原結合部分をコードする単離核酸分子を含み、そしてこの非ヒト動物は、両方の核酸分子を発現する。より好ましい実施形態において、この非ヒト動物は、以下からなるリストから選択される：マウス、ラット、ハムスター、ウシ、ヒツジ、霊長類、ウマおよびブタ。より好ましい実施形態において、この単離核酸分子またはその一部分の発現から生じるヒト抗体は、動物のBリンパ球性細胞またはその後代由来の細胞の表面上に発現される。別の好ましい実施形態において、この単離核酸分子またはその一部分の発現から生じるヒト抗体は、動物のリンパ、血液、乳汁、唾液または腹水に分泌される。 10

【0090】

本発明は、*Pseudomonas aeruginosa* LPSに特異的に結合する単離ヒト抗体またはその抗原結合部分、および第二のポリペプチド配列を含む融合タンパク質を意図する。好ましい実施形態において、この第二のポリペプチド配列は、以下からなるリストから選択される：エピトープタグ、親和性タグ、毒素ポリペプチド、抗生物質、酵素、第二の抗体配列、補体タンパク質およびサイトカイン。

【0091】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、ここで、この抗体の重鎖のアイソタイプは、 μ 、 γ 、 δ 、 ϵ または α である。 20

【0092】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、ここで、この抗体またはその抗原結合部分は、以下の工程：

- a) ヒト免疫グロブリン遺伝子座を含む非ヒト動物を、以下からなる群より選択される抗原を用いて免疫する工程：単離*Pseudomonas aeruginosa* LPS調製物、雄性*Pseudomonas aeruginosa*細胞調製物、弱毒化*Pseudomonas aeruginosa*細胞調製物、および死菌*Pseudomonas aeruginosa*細胞調製物； 30
- b) この非ヒト動物が、この抗原に対する免疫応答を開始することを可能にする工程；ならびに
- c) この非ヒト動物からこの抗体を単離する工程、を包含するプロセスによって産生される。

【0093】

本発明は、単離ヒト抗体またはその抗原結合部分を意図し、これは、*Pseudomonas aeruginosa* LPSに特異的に結合し、ここで、この*Pseudomonas aeruginosa* LPSは、以下からなるリストから選択される*Pseudomonas aeruginosa*株に由来する：06ad、011、Habs16、170003およびPA01 Halloway。 40

本発明は、汚染ヒト生体材料を含まない動物または細胞から単離される単離ヒト抗体またはその抗原結合部分を意図する。好ましい実施形態において、この生体材料は、ウイルス、酵素、ホルモン、サイトカイン、レセプター、レセプターリガンド、免疫グロブリン、補体、核タンパク質および細胞質シグナリングタンパク質である。より好ましい実施形態において、このウイルスは、エプスタインバーウイルスまたはレトロウイルスである。

【0094】

薬学的組成物は、以下の手段によって製造され得る：従来の混合工程、溶解工程、粒状化工程、糖剤作製工程、湿式粉碎工程、乳化工程、カプセル化工程、捕捉工程または凍結乾燥工程。

【0095】

従って、本発明に従った使用についての薬学的組成物は、賦形剤および補助剤を含む1つ以上の生理学的に受容可能なキャリアを用いて、従来の様式で処方され得、この賦形剤および補助剤は、活性化合物の薬学的に使用され得る調製物へのプロセッシングを容易にする。適切な処方は、選択される投与経路に依存する。

【0096】

注入について、本発明の因子は、水溶液、好ましくは、生理学的に適合性の緩衝液（例えば、ハanks液、リンガー液または生理的食塩水緩衝液）に処方され得る。経粘膜投与について、浸透されるべき障壁に適切な浸透剤が、この処方において使用される。このような浸透剤は、一般的に当該分野で公知である。目投与について、適切な生理食塩水溶液における懸濁液は、当該分野で周知のように使用される。 10

【0097】

経口投与について、この化合物は、この活性化合物を、当該分野で周知の薬学的に受容可能なキャリアと組み合わせることによって容易に処方され得る。このようなキャリアは、本発明の化合物を、処置されるべき患者による経口摂取について、錠剤、丸剤、糖剤、カプセル、液体、ゲル、シロップ、スラリー、懸濁液などとして処方されることを可能にする。経口使用についての薬学的調製物は、固体賦形剤として獲得され得、これは、所望であれば、適切な補助剤の添加の前に、必要に応じて、得られる混合物を研磨し、そしてこの顆粒混合物をプロセッシングして、錠剤または糖剤のコアを得る。適切な賦形剤としては、以下が挙げられる：フィラー（例えば、ラクトース、スクロース、マンニトールまたはソルビトールを含む糖）；セルロース調製物（例えば、トウモロコシデンプン、コムギデンプン、イネデンプン、ジャガイモデンプン、ゼラチン、トラガカントゴム、メチルセルロース、ヒドロキシプロピルメチルセルロース、カルボキシメチルセルロースナトリウムおよび／またはポリビニルピロリドン（PVP）のような）。所望であれば、崩壊剤（例えば、架橋ポリビニルピロリドン、寒天またはアルギン酸もしくはその塩（例えば、アルギン酸ナトリウム））が添加され得る。 20

【0098】

糖衣錠の中核は、適切なコーティングとともに提供される。この目的のためには、濃縮された糖溶液が使用され得る。これは、必要に応じて、アラビアゴム、タルク、ポリビニルピロリドン、カルボポールゲル（carbopol gel）、ポリエチレングリコール、および／または二酸化チタン、ラッカー溶液、および適切な有機溶媒、または溶媒混合物を含み得る。染料または色素が、活性な化合物の用量の異なる組合せを同定するかまたはそれを特徴付けるために、錠剤または糖衣錠のコーティングに添加され得る。 30

【0099】

経口によって使用され得る薬学的調製物には、ゼラチンから作られたプッシュフィット（push-fit）カプセル、ならびにゼラチンおよび可塑剤（例えば、グリセロールまたはソルビトール）から作られた軟らかいシールされたカプセルが含まれる。プッシュフィットカプセルは、ラクトースのような増量剤、澱粉のような結合剤、および／またはタルクもしくはステアリン酸マグネシウムのような潤滑剤、ならびに必要に応じて安定剤とともに、混合物中に有効成分を含み得る。軟らかいカプセル中では、活性な化合物は、適切な液体（例えば、脂肪油、液体パラフィン、または液体のポリエチレングリコール）中に溶解させられるかまたは懸濁され得る。さらに、安定剤が添加させられ得る。経口投与のための全ての処方物は、このような投与に適切な投与量であるべきである。 40

【0100】

舌下投与のためには、組成物は、従来の様式で処方された錠剤またはトローチ剤の形態をとり得る。

【0101】

吸入による投与のためには、本発明に従って使用される化合物は、加圧されたパック、または噴霧器からのエアゾールスプレーの噴出の形態で、適切な発射剤（例えば、ジクロロジフルオロメタン、トリクロロフルオロメタン、ジクロロテトラフルオロエタン、二酸化 50

炭素、または他の適切な気体)の使用とともに、便利に送達される。加圧されたエアゾールの場合には、投与量単位は、測定された量を送達する値を提供することによって、決定され得る。吸入器または通気器での使用のためには、例えば、ゼラチンのカプセルおよびカートリッジは、化合物の粉末混合物およびラクトースまたは澱粉のような適切な散剤基剤を含有して処方され得る。

【0102】

化合物は、注射による(例えば、ボーラス注射または持続的な注入による)非経口投与のために処方され得る。注射用の処方物は、添加された防腐剤とともに、例えば、アンプルまたは複数投薬容器中に単位投与量形態で提示され得る。組成物は、油性または水性のビヒクル中で懸濁物、溶液、または乳濁物のような形態をとり得、そして懸濁剤、安定剤、および/または分散剤のような処方剤を含み得る。

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【0103】

全身的投与のための薬学的処方物は、水溶性の形態で活性な化合物の水溶液を含む。さらに、活性な化合物の懸濁物が、適切な油性の注射用懸濁物として調製され得る。適切な親油性溶媒またはビヒクルには、ゴマ油のような脂肪油、またはオレイン酸エチルもしくはトリグリセリドのような合成の脂肪酸エステル、またはリポソームが含まれる。水性の注射用懸濁物は、懸濁物の粘性を増大させる物質(例えば、カルボキシメチルセルロースナトリウム、ソルビトール、またはデキストラン)を含み得る。必要に応じて、懸濁物はまた、適切な安定剤、または高度に濃縮された溶液の調製を可能にする化合物の可溶性を増大させる試薬を含み得る。

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【0104】

あるいは、有効成分は、適切なビヒクル(例えば、滅菌の発熱物質をふくまない水)との使用前の構成のために粉末形態であり得る。

【0105】

化合物はまた、例えば、ココアバターまたは他のグリセリドのような従来の坐剤基剤を含有する、坐剤または保持浣腸液のような直腸用組成物に処方され得る。

【0106】

上記に記載されている処方物に加えて、化合物はまた、貯蓄用調製物(depot preparation)として処方され得る。このような長期作用性処方物は、移植(例えば、皮下または筋肉内)によって、あるいは筋肉内注射によって投与され得る。従って、例えば、化合物は、適切なポリマー性もしくは疎水性材料(例えば、受容可能な油中の乳濁物として)またはイオン交換樹脂とともに処方されるか、あるいは可溶性の低い誘導体として(例えば、可溶性の低い塩として)処方される場合がある。

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【0107】

本発明の疎水性化合物のための薬学的キャリアは、ベンジルアルコール、非極性界面活性剤、水と混合することができる有機ポリマー、および水相を含有する共溶媒系である。共溶媒系はVPD共溶媒系であり得る。VPDは、3%w/vのベンジルアルコール、8%w/vの非極性界面活性剤ポリソルベート80、および65%w/vのポリエチレングリコール300の溶液であり得、無水エタノールで容量を増大させられる。VPD共溶媒系(VPD:5W)は、水溶液中で5%デキストロースと1:1希釈されたVPDから構成される。この共溶媒系は、疎水性化合物を十分に溶解させ、そしてそれ自体は全身投与の際に低い毒性しか生じない。天然においては、共溶媒系の特性は、その可溶性および毒性の特徴を破壊させることなく相当に変化し得る。さらに、補助溶媒成分の同定は変更され得る:例えば、他の毒性の低い非極性界面活性剤が、ポリソルベート80の代わりに使用され得る;ポリエチレングリコールの画分の大きさが変更され得る;他の生体適合性ポリマー(例えば、ポリビニルピロリドン)によってポリエチレングリコールを置き換え得る;そして他の糖またはポリサッカライドによってデキストロースを置換し得る。

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【0108】

あるいは、疎水性の薬学的化合物のための他の送達系が、使用される場合がある。リポソームおよび乳濁物が、疎水性薬物のための送達ビヒクルまたはキャリアの周知の例である

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。特定の有機溶媒（例えば、ジメチルスルホキシド）もまた使用され得るが、通常は、より大きな毒性を伴う。

【0109】

さらに、化合物は、治療薬を含有する固体の疎水性ポリマーの半透過性マトリックスのような、徐放系を使用して送達され得る。種々の徐放材料が確立されており、そして当業者に周知である。徐放カプセルは、それらの化学的な性質に依存して、数週間から100日を越えて、化合物を放出し得る。

【0110】

治療薬の化学的性質および生物学的安定性に依存して、タンパク質の安定化のためのさらなる戦略が使用され得る。

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【0111】

薬学的組成物はまた、適切な固相またはゲル相キャリアまたは賦形剤を含み得る。このようなキャリアまたは賦形剤の例には、炭酸カルシウム、リン酸カルシウム、種々の糖、澱粉、セルロース誘導体、ゼラチン、およびポリエチレングリコールのようなポリマーが含まれるが、これらに限定されない。

【0112】

本発明の *Pseudomonas aeruginosa* LPS に特異的に結合する単離されたヒト抗体またはその抗原結合部分は、薬学的に適合性の反対イオンを有する塩として提供し得る。薬学的に適合性の塩は、多くの酸（塩酸、硫酸、酢酸、乳酸、酒石酸、リンゴ酸、スクシン酸などを含むがこれらに限定されない）を用いて形成され得る。塩は、対応する遊離塩基の形態の、水性または他のプロトン性溶媒中でより可溶性となる傾向にある。

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【0113】

本発明のキットの成分は、*Pseudomonas aeruginosa* 感染の予防または処置のために本発明の組成物を利用するための説明書を含む。出願人は、最初に、*Pseudomonas aeruginosa* LPS に特異的に結合する単離されたヒト抗体またはその抗原結合部分を用いて、*Pseudomonas aeruginosa* 感染を予防または処置する方法を、本明細書中で開示する。キットに印刷された説明書によって、当業者は、本発明の方法を実施するためにキットを利用することが可能である。

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【0114】

（実施例1）

（*Pseudomonas aeruginosa* LPS に対する完全なヒト抗体を産生するマウスおよびハイブリドーマの作製）

A. *Pseudomonas aeruginosa* 血清型 06 ad

Pseudomonas aeruginosa 血清型 06 ad を、マウスの免疫化、マウスの保護アッセイ、および浸透圧アッセイに使用した。マウスチャレンジアッセイのための細菌を、pseudoselect 寒天（BBL、Becton Dickinson, Sparks, MD）上に新たにプレートし、次いで37℃でインキュベートし、そして1 cfu をLB培地上に接種し、そして 5×10^8 cfu/ml の濃度にまで、水浴を振盪させながら37℃でインキュベートした。細菌を、10,000 rpm で5分間遠心分離し、再懸濁し、そして冷却したリン酸緩衝化生理食塩水（PBS）中で洗浄した。免疫化のために細菌を上記のように増殖させ、そして60℃で1時間熱によって死滅させ、そして使用するまで4℃で保存した。フローサイトメリー浸透圧アッセイで使用した標識した細菌を上記のように増殖させ、そして熱によって死滅させた。しかし、これらの細菌を、 10^9 /ml の濃度を生じるように、アルカリ結合緩衝液（ACB：0.5 M の Na_2CO_3 および0.5 M の NaHCO_3 の1：3の溶液、pH 9.5）中に再懸濁した。0.06%のフルオレセインイソチオシアネートイソマーI（FITC、Amresco、Solon, OH）を含む等量のABCを添加し、そして穏やかに振盪させながら室温で20時間インキュベートした。細菌を、ベローナル緩衝化生理食塩水中で洗浄し、次いで1

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$0.9 / \text{ml}$ で P B S 中に再懸濁し、そして -80°C で保存した。

【0115】

Pseudomonas aeruginosa の 06 ad、001、Habs16、170003、および PA01 Halloway 株の LPS 由来の LPS の O 特異的側鎖の高分子量の多糖部分（高分子量の PS）を、記載するように作成し、そして保存のために凍結乾燥させた。Hatanora、*Infect. Immun.* 62:3608-3616 (1994) を参照のこと。これらの高分子量の PS を、以下に記載するように、酵素結合免疫吸着アッセイ（ELISA）のための 96 ウェルプレートにコーティングするために使用した。06 ad の高分子量の PS はまた、以下に記載するブロッキングおよび結合アッセイにも使用した。

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【0116】

ヒトの重鎖 Ig および軽鎖 Ig についてトランスジェニックであるマウスを交配させ、そして Abgenix Inc., Fremont, CA によって維持した。使用した Xenomouse（登録商標）の株は、Xma2a-3 であった。これは、同時挿入されたヒトの重鎖および軽鎖導入遺伝子を含む YAC で再構築された、Ig 不活化マウスである。マウスを、出荷後、病原体を含まない設備の中でマイクロアイソレータージに閉じ込め、そして食物および水は使用前にオートクレーブした。マウスを、 10^7 個の熱によって死滅させた *Pseudomonas aeruginosa* 06 ad PA で、1 週間に 2 回、腹腔内（ip）で免疫化（P B S 中の 10^7 個の細菌）および／またはフットパットで免疫化（ 10^7 個の細菌および R I B I アジュバント、Sigma, St. Louis, MO）し、そしてそれらの血清を、以下に記載するように ELISA によって抗 *Pseudomonas aeruginosa* 06 ad LPS 抗体についてスクリーニングした。

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【0117】

ハイブリドーマを、以前に記載されたように、免疫化したセロポジティブな Xenomouse（登録商標）動物由来の脾臓および／またはリンパ節細胞と、非分泌型 sp2/0 骨髓腫細胞株との融合によって作製した。Mendezら、*Nat. Gen.* 15:146-156 (1997)；Schreiberら、*J. Immunol.* 146:188-193 (1991) を参照のこと。ハイブリドーマ由来の上清を、以下に記載する ELISA 手順を使用して、ヒト抗 *Pseudomonas aeruginosa* 06 ad LPS の産生についてスクリーニングし、次いで Ig G 抗 LPS 抗体を分泌することを見出したハイブリドーマを、限界希釈によって 3 回クローン化した。1 つの Ig G 2 分泌型クローン（S20）を、固相 *Pseudomonas aeruginosa* 06 ad PS への結合強度の最初の測定に基づいて選択した。

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【0118】

(B. *Pseudomonas aeruginosa* 血清型 PA01 および 170003)

Pseudomonas aeruginosa 血清型 PA01 および 170003 を、マウスの免疫化のために使用した。細菌を pseudoselect 寒天（BBL、Becton Dickinson, Spark, MD）上にプレートし、次いで 37°C でインキュベートし、そして 1 cfu を LB 培地上に接種し、そして $5 \times 10^8 \text{ cfu/ml}$ の濃度にまで、水浴を振盪させながら 37°C でインキュベートした。細菌を、 $10,000 \text{ rpm}$ で 5 分間、遠心分離し、再懸濁し、そして冷却したリン酸緩衝化生理食塩水（P B S）中で洗浄した。免疫化のために細菌を上記のように増殖させ、そして 60°C で 1 時間熱によって死滅させ、そして使用するまで 4°C で保存した。

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【0119】

Pseudomonas aeruginosa の 06 ad、001、Habs16、170003、および PA01 Halloway 株の LPS 由来の LPS の O 特異的側鎖の高分子量の多糖部分（高分子量の PS）を、記載するように作製し、そして保存のために凍結乾燥させた。Hatanora、*Infect. Immun.* 62:3608-36

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16 (1994) を参照のこと。これらの高分子量の P S を、以下に記載するように、酵素結合免疫吸着アッセイ (E L I S A) のための 96 ウェルプレートにコーティングするために使用した。P A 0 1 および 170003 の高分子量の P S はまた、以下に記載するブロッキングアッセイにも使用した。

【0120】

ヒトの重鎖 I g および軽鎖 I g についてトランスジェニックであるマウスを交配させ、そして A b g e n i x I n c . , F r e m o n t , C A に よ っ て 維 持 した。使用した X e n o m o u s e (登録商標) の株は、X m a 2 a - 3 であった。これは、同時挿入されたヒトの重鎖および軽鎖導入遺伝子を含む Y A C で再構築された、I g 不活化マウスである。マウスを、出荷後、病原体を含まない設備の中でマイクロアイソレータゲージに閉じ込め、そして食物および水は使用前にオートクレーブした。マウスを、 10^7 個の熱によって死滅させた *P s e u d o m o n a s a e r u g i n o s a* P A 0 1 または 170003 で、1 週間に 2 回、腹腔内 (i p) で免疫化 (P B S 中の 10^7 個の細菌) および / または フットパッドで免疫化 (10^7 個の細菌および R I B I アジュバント、S i g m a , S t . L o u i s , M O) し、そしてそれらの血清を、以下に記載する E L I S A に よ っ て、抗 *P s e u d o m o n a s a e r u g i n o s a* P A 0 1 または 170003 L P S 抗体について、それぞれスクリーニングした。

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【0121】

ハイブリドーマを、以前に記載されたように、免疫化したセロポジティブな X e n o m o u s e (登録商標) 動物由来の脾臓および / またはリンパ節細胞と、非分泌型 s p 2 / 0 骨髄腫細胞株との融合によって作製した。M e n d e z ら、N a t . G e n . 15 : 146 - 156 (1997) ; S c h r e i b e r ら、J . I m m u n o l . 146 : 188 - 193 (1991) を参照のこと。ハイブリドーマ由来の上清を、以下に記載する E L I S A 手順を使用して、ヒト抗 *P s e u d o m o n a s a e r u g i n o s a* L P S の産生についてスクリーニングし、次いで抗 L P S 抗体を分泌することを見出したハイブリドーマを、限界希釈によって 3 回クローン化した。2 つの I g M 2 分泌型クローン (H 12 および C 3) を、固相 *P s e u d o m o n a s a e r u g i n o s a* P A 0 1 P S への結合強度の最初の測定に基づいて選択した。これらは、上記の脾臓細胞の融合によって誘導された。リンパ節細胞の融合によって誘導された 1 つの I g M 分泌型クローン (L M 1 H 10) を、固相 *P s e u d o m o n a s a e r u g i n o s a* 170003 P S への結合強度の最初の測定に基づいて選択した。

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【0122】

(実施例 2)

(トランスジェニックマウス由来の抗 L P S 抗体からの可変領域遺伝子の特徴付けおよび用法)

ジデオキシ DNA 配列決定を、ヒトモノクローナル抗体の可変領域の配列の決定について以前に記載されているように行った (30、33)。全 RNA を、T R I Z O L 試薬 (G i b c o B R L) を使用して 9 個の異なるクローン由来のハイブリドーマ細胞から単離し、そして P C R の鋳型としての使用のために、ランダムプライムした c D N A に転換した。ヒト重鎖および軽鎖可変領域を、H u m a n I g - P r i m e r S e t (N o v a g e n , M a d i s o n , W I) において提供される縮重リーダーペプチドプライマーおよび定常領域プライマーを使用して増幅した。P C R 産物を、T r i s - 酢酸 - E D T A アガロースゲル上で分析した。ポジティブな P C R 反応産物を、クロロホルムイソアミルアルコール (24 : 1) で抽出し、そして p T 7 B l u e (N o v a g e n) の E c o R I 部位にクローン化した。クローンを、S e q u e n a s e V 2 . 0 DNA 配列決定キット (U S B , C l e v e l a n d , O H) を用いたジデオキシ法に基づいて配列決定した。遺伝子用法分析を、V b a s e データベース (T o m l i n s o n ら、M R C C e n t r e f o r P r o t e i n E n g i n e e r i n g , C a m b r i d g e , U K) を使用して行った。

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【0123】

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PA免疫化トランスジェニックマウス由来の脾臓細胞と非分泌型SP2/O細胞株との融合によって得られたハイブリドーマ由来の可変領域遺伝子をクローン化し、そして可変領域遺伝子の用法を決定するために配列決定した(図1)。ヒトIgトランスジェニックマウスで行った防御型IgG2抗LPSSモノクローナル抗体を、さらなる研究のために選択し、V_H3遺伝子ファミリー由来の遺伝子を利用して、限定されたV_H遺伝子用法との類似性を、種々の細菌の多糖でのヒトの免疫化後に見出した。pseudomonasで免疫化したトランスジェニックマウス由来の脾臓細胞との融合によって作成された9個の他のMabは、DNA配列決定によって決定すると重鎖遺伝子エレメントのV_H3遺伝子ファミリーにも使用される抗体を生じた。より詳細には、DNA配列分析は、V_H3/V3-33およびJH4遺伝子が、防御型抗LPSS Mabに使用されることを示した。同様に、使用した軽鎖遺伝子セグメントはVk2/A2およびJk1であり、これはPSワクチン免疫法の後一般的にヒトで使用される。まとめると、これらのトランスジェニックマウスでのPA免疫化後の抗LPSS抗体の遺伝子用法は、PS免疫化後にヒトで観察されるものと大いに類似するようである。

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【0124】

(実施例3)

(抗Pseudomonas aeruginosa LPSS抗体の検出)

酵素結合免疫吸着アッセイ(ELISA)を、免疫化したマウスの血清中、および本発明者らが以前に記載した(34)ようなハイブリドーマ上清中の、Pseudomonas aeruginosa 06ad LPSSに対する抗体を検出するために使用した。簡潔には、96ウェルマイクロタイターポリスチレンプレート(NUNC、Denmark)を、2μg/mlのPseudomonas aeruginosa 06adの高分子量のPSで4℃にて一晚コーティングし、洗浄し、そしてPBS中の1%のウシ血清アルブミン(BSA; Sigma-Aldrich、ST. Louis, MO)および0.05%のTween 20(Amresco, Solon, OH)でブロックした(200μl/ウェル)。プレートを洗浄し、そしてPBS中の1%のBSA中のS20または血清の連続希釈とともに一晚インキュベートした。プレートを洗浄し、そして結合した抗体を、イソ型特異的アルカリホスファターゼ結合マウス抗ヒトポリクローナル抗体(Southern Biotechnology Associates, Birmingham, AL)の添加によって検出した。プレートを、DEA緩衝液中の100μl/ウェルのp-ニトロフェニルホスフェート(PNPP、Sigma-Aldrich)色素形成物質で発色させた。光学密度を、マイクロプレートリーダー(Biorad, Hercules, CA)を用いて415nmで測定した。S20の特異性を決定するためのブロッキングアッセイを、可溶性のPseudomonas aeruginosa 06adの高分子量のPSまたは種々の濃度のコントロールのOSを、PSでコーティングした96ウェルELISAプレートへの添加の前に、S20に添加したことを除いて、上記と同じ様式で行った。Mabの相対的な結合力を、記載されているように計算した。Chungら、Infect. Immun. 63:4219-4233(1995); Schreiberら、J. Infect. Dis. 167:221-226(1993)を参照のこと。抗体を、高分子量のPSでコーティングしたELISAプレートのウェルに添加し、続いて、Pseudomonas aeruginosa 06adの高分子量のPSの連続希釈物または等量のPBS(ネガティブコントロール)を添加した。H12、C3、およびLN1H10モノクローナル抗体の結合特異性を決定するためのブロッキングアッセイを、可溶性のPseudomonas aeruginosa PA01または170003の高分子量のPSを使用して、上記に記載したように行った。最大吸収の50%を阻害するために必要なPSの濃度を計算し(I₅₀)、そしてこの値の逆数を、相対的な結合力を示すために使用した。

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【0125】

トランスジェニックマウス中で産生されたS20は、P. aeruginosa 06ad株のO側鎖に特異的であった。ブロッキングアッセイは、コントロールのPS(精製型

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6 B p n e y m o c c o c c a l c a p s u l a r P S ; 図 2) での 10 % 未満の阻害と比較して、同じ P S との M a b の予備インキュベーション後の、固相 P s e u d o m o n a s a e r u g i n o s a 0 6 a d L P S の高分子量の P S への S 2 0 の結合の 90 % 以上の減少を明らかにした。しかし、S 2 0 は、他の P . a e r u g i n o s a 血清型由来の L P S とは交差反応しなかった。なぜなら、種々の p s e u d o m o n a s 株 (0 1 1 、 H a b s 1 6 、 1 7 0 0 0 3 、 および P A 0 1 H a l l o w a y を含む) 由来の固相 L P S O 側鎖の高分子量の P S への結合は、示すことができなかったからである (図 3) 。

【 0 1 2 6 】

高分子量の P S への S 2 0 の結合の最大吸収の 50 % を阻害した高分子量の P S の濃度を決定し、この値の逆数 ($1 / I_{50}$) を、S 2 0 の相対的な結合力を計算するために使用した。この値を、以前に開発した同じ高分子量の P S に対する防御型マウスのクローナル抗体と、そして同様の技術を使用して誘導した以前に公開された (2 、 3 3) 抗多糖の値と比較した。本発明者らは、ヒトの M a b の相対的な結合力が、マウスの M a b よりも高いことを見出した (1 . 0 対 0 . 0 3 6) 。

【 0 1 2 7 】

H 1 2 および C 3 でのブロッキングアッセイは、コントロールの P S (精製型 6 B p n e y m o c c o c c a l c a p s u l a r P S ; 図 7) での 20 % 未満の阻害と比較して、同じ P S との m A b の予備インキュベーション後に、固相 P s e u d o m o n a s a e r u g i n o s a P A 0 1 の L P S の高分子量の P S への結合において、それぞれ 90 % および 85 % 以上の減少を明らかにした。P s e u d o m o n a s a e r u g i n o s a 1 7 0 0 0 3 L P S の高分子量の P S との L N 1 H 1 0 の予備インキュベーションは、コントロールの P S での 40 % 未満の阻害と比較して、89 % まで固相 P S への m A b の結合を減少させた (図 8) 。

【 0 1 2 8 】

(実施例 4)

(抗 P s e u d o m o n a s a e r u g i n o s a L P S 抗体のオプソニン作用は補体依存性の食作用を促進する)

P . a e r u g i n o s a 0 6 a d にヒト多形核リンパ球 (P M N) による取り込みのためにオプソニン作用させるヒトモノクローナル抗体の能力を、以前に記載されているようにフローサイトメトリーによって測定した。S c h r e i b e r ら、J . I n f e c t . D i s 1 6 7 : 2 2 1 - 2 2 6 (1 9 9 3) を参照のこと。P s e u d o m o n a s a e r u g i n o s a 0 6 a d を増殖させ、熱によって死滅させ、そして F I T C 標識した。オプソニン作用を、補体供給源として無ガンマグロブリン血症患者由来の 1 % のヒト血清とともにまたはそれを伴わずに、S 2 0 で標識した細菌をインキュベートすることによって行った。細菌を、6 % のデキストランおよび 0 . 2 % のグルコースを含有する P B S 中で洗浄し、次いで 0 . 1 % のゼラチンを含有する H B S S 中に再懸濁した。多形核白血球 (P M N) を、健常な成体のボランティア由来のヒト末梢血から単離した。S c h r e i b e r ら、J . I n f e c t . D i s 1 6 7 : 2 2 1 - 2 2 6 (1 9 9 3) ; T o s i ら、J . C l i n . I n v e s t . 8 6 : 3 0 0 - 3 0 8 (1 9 9 0) を参照のこと。M N を、 10^7 個の細胞 / m l の濃度を生じるように再懸濁し、そして F M L P の細胞 1 m l あたり 10^{-6} 希釈の $10 \mu l$ とともに 30 分間活性化した (P e n i n s u l a L a b o r a t o r i e s , S a n C a r l o s , C A) 。P M N を、オプソニン作用させた各細菌サンプルに添加し、37 °C でインキュベートし、次いで、種々の遠心分離によって遊離の細菌を分離し、そして P B S 中に再懸濁した。単色フローサイトメトリー分析を、F A C S c a n および C e l l Q u e s t ソフトウェア (B e c t o n D i c k i n s o n , M o u n t a i n V i e w , C A) を利用して P M N について行い、そして食作用を、それぞれのサンプルについて 10 , 0 0 0 個の P M N の平均の蛍光の相対的な単位で示した。観察したオプソニン作用性食作用 (o p s o n o p h a g o c y t o s i s) が、細菌の死滅に関係するかどうかを決定するために、生存している P . a e

ruginosa 06 ad の 10^6 CFU を、細菌に吸収させた新鮮なヒト血清、S20、および 10^6 個の新鮮なヒト PMN と混合した、別のアッセイを使用した。サンプルを、90 分間の 37°C でのインキュベーションの開始時および終了時に採取し、細菌を希釈し、次いで細菌数を数え上げるためにプレートした。

【0129】

抗 *Pseudomonas aeruginosa* 06 ad LPS 抗体の機能的活性を示すために、S20 が、補体依存性アッセイにおいて新鮮なヒト PMN による標識された PA の取り込みについて高度にオプソニン作用性であることを示した。実際、ヒト Mab は、以前に記載された同じエピトープに対する防御型マウス Mab (D8) よりもほぼ 2 倍、オプソニン作用性であった (2.5 mg のヒト Mab が、5 mg のマウス Mab の平均の蛍光の 2 倍を生じた; 図 4)。細菌を熱によって死滅させ、その後、FITC で標識したので、抗体に対する感受性の増大および補体媒介性のオプソニン作用が、細菌表面への損傷に起因して生じ得たことを観察することができたようである。従って、本発明者らはまた、 10^6 の CFU の生存している細菌が抗体およびヒト補体とともにオプソニン作用され、次いでコロニー数が 10^6 個の新鮮な PMN への暴露後に決定される、死滅アッセイにおいて、*Pseudomonas aeruginosa* 06 ad のオプソニン作用および食作用を測定した。S20 Mab はまた、5 mg/ml の抗体によって PA cfu の 80% の減少を生じたように、このアッセイでも有効である。

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【0130】

(実施例 5)

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(好中球減少症のマウスの胎児の *Pseudomonas aeruginosa* 敗血症からの防御)

Pseudomonas aeruginosa の侵襲性感染に対するヒト Mab の防御効果を、以前に記載された好中球減少症マウスのモデルにおいて測定した。Pier ら、*Infect. Immun.* 57:174-179 (1989); Schreiber ら、*J. Immunol.* 146:188-193 (1991) を参照のこと。雌性の 6 週齢の BALB/c ByJ マウス (Jackson Laboratories, Bar Harbor, ME) を、病原体を含まない、*pseudomonas* を含まない環境下で維持した。水、敷き藁、および食物を、使用前にオートクレーブした。1、3、および 5 日目にそれぞれのマウスに 3 ml のシクロホスファミド (Cytosan (登録商標)、Bristol-Myers Squibb, Princeton, NJ) を腹腔内注射で投与することによって、好中球減少症を確立させた。5 日目に、シクロホスファミドを、0 時間で投与し、そして 2 時間後に、 $10\mu\text{g}$ の S20 または PBS コントロールを、ip 投与し、続いて 2 時間後に、 10^3 cfu の生存している *Pseudomonas aeruginosa* 06 ad の PA を投与した。マウスを、その後毎日観察し、そして死亡率を測定結果とした。

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【0131】

図 5 に示すように、PBS コントロールで処置した感染させたマウスは、*Pseudomonas aeruginosa* 感染の 1 日後に死亡し始めた。2 日後、PBS で処置した感染させたマウスの 100% が死亡した。対照的に、S20 mAb で処置したマウスの 100% が防御を示し、そして *Pseudomonas aeruginosa* 感染の 2 日後に生存していた。このことは、患者の *Pseudomonas aeruginosa* による致死力を妨げる上での S20 の防御能力を示している。

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【0132】

上記の実施態様は、例示の目的のためのみであり; 意図される本発明は、これらの特定の実施態様のいずれかによっても限定されず、そして実際、1 つ以上の実施態様の組合せを含む場合がある。

【0133】

(微生物の寄託)

ハイブリドーマ細胞株 S20、H12、C3、および LN1H10 を、ブダペスト条約の

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取り決めに従って、アメリカンタイプカルチャーコレクション (ATCC)、10801 University Blvd., Manassas, VA 20110-2209、USAに寄託し、そして以下の登録番号を与えられた：

ハイブリドーマ S20 HB
 ハイブリドーマ H12 HB
 ハイブリドーマ C3 HB
 ハイブリドーマ Ln1H10 HB _____。

【図面の簡単な説明】

【図1】

図1は、S20モノクローナル抗*Pseudomonas aeruginosa*抗体を 10
 産生するハイブリドーマによって利用されるヒト免疫グロブリン可変(V)領域、多様性(D)領域および結合(J)領域を示す。

【図2】

図2は、*Pseudomonas aeruginosa* LPSとの特異性を有するS20ヒトモノクローナル抗体結合を示す結合阻害アッセイを示す。可溶性の*Pseudomonas aeruginosa* LPSは、固相*Pseudomonas aeruginosa* LPSへのS20結合を阻害し得たが、可溶性のPneumococcal 6Bコントロール多糖類は、これを阻害できなかった。

【図3】

図3は、S20モノクローナル抗体が、*Pseudomonas aeruginosa* 20
 06ad血清型LPSを特異的に結合するが、011、Habs 16、170003およびPAOI Halloway株由来の固相LPSを結合しないことを示す。

【図4】

図4は、S20オプソニン作用が、補体依存性食作用を促進することを示す。末梢核単球(PMN)のフローサイトメトリー分析は、PMNが、補体の存在下においてのみ、FITC標識されるオプソニン作用性*Pseudomonas aeruginosa*を食作用したことを示した。

【図5】

図5は、S20モノクローナル抗体が、致死的な*Pseudomonas aeruginosa* 30
 敗血症から、好中球減少性マウスを保護したことを示す。

【図6】

図6は、ヒトモノクローナル抗体S20(IgG)の重鎖可変領域および軽鎖可変領域のDNA配列およびアミノ酸配列を示す。

【図7】

図7は、*Pseudomonas aeruginosa* LPSとの特異性を有するH12ヒトモノクローナル抗体結合およびC3ヒトモノクローナル抗体結合を示す結合阻害アッセイを示す。可溶性の*Pseudomonas aeruginosa* PA01 LPSは、固相*Pseudomonas aeruginosa* PA01 LPSへの結合を阻害し得たが、可溶性のPneumococcal 6Bコントロール多糖類は、これを阻害できなかった。 40

【図8】

図8は、*Pseudomonas aeruginosa* LPSとの特異性を有するLn1H10ヒトモノクローナル抗体結合を示す結合阻害アッセイを示す。可溶性の*Pseudomonas aeruginosa* 170003 LPSは、固相*Pseudomonas aeruginosa* 170003 LPSへの結合を阻害し得たが、可溶性のPneumococcal 6Bコントロール多糖類は、これを阻害できなかった。

【図9】

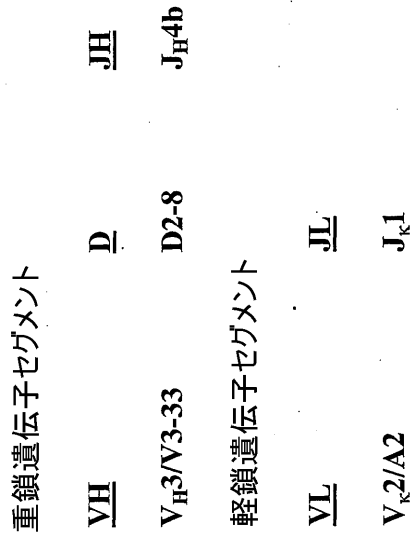
図9は、S20ヒトモノクローナル抗体の重鎖可変領域のCDR1～3の位置を示す。

【図10】

図 1 0 は、S 2 0 ヒトモノクローナル抗体の κ 軽鎖可変領域の C D R 1 ～ 3 の位置を示す。

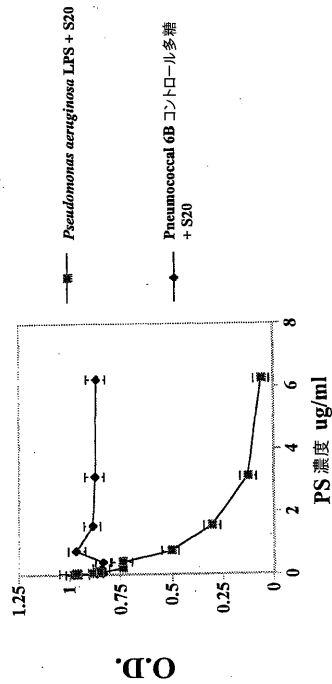
【 図 1 】

Figure 1: S20 モノクローナル抗体遺伝子用法

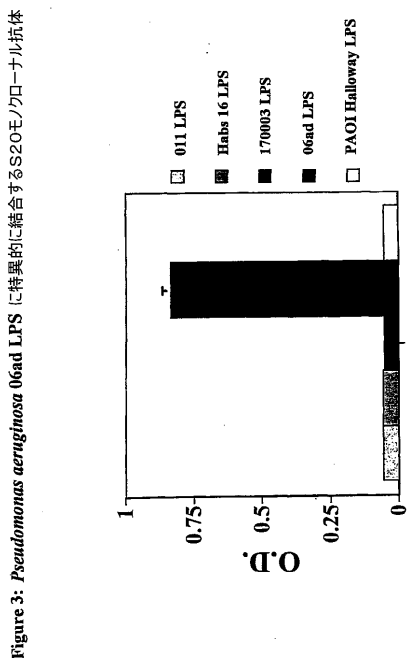


【 図 2 】

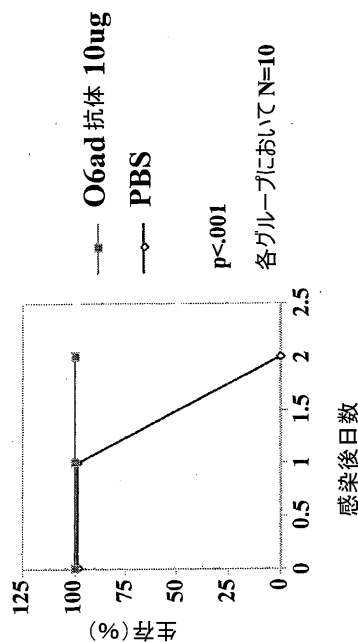
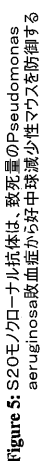
Figure 2: 固相 *Pseudomonas aeruginosa* LPS に特異的に結合するS20モノクローナル抗体



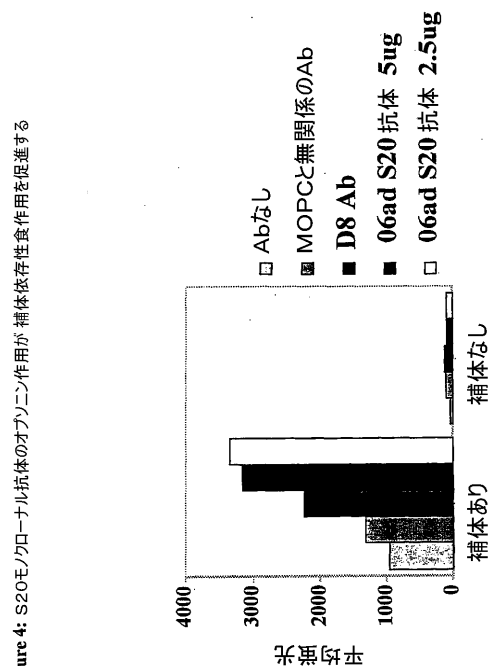
【图 3】



【图 5】



【図 4】



【图 6】

重鎖可變領域配列(配列番号1)

[illegible]

重鎖可變領域配列(配列番号2)

QVQLVESGGGVVQPGSRSLRLSCAASGFTFSYSGIDWVRQAPGKGLEWVGRINWYSGSNKYY
ADSVKGRFTISRDNKNTLYLQMNSLRAEETAVYYCARDPHYTRGFDWGKGLTVTSSA
STKGPGLPLAPCSRSTSESTAALGCLVKDYFAE

輕鎖可變領域配列(配列番号3)

GATGTTGTGATGACCAGACTCCACTCTCTCTGTCGGTCACCCCTGGACAGCGCGGCTCC
AICTCTCGCAGGTTAGTACAGAGCTCTGCATAGTGTGATGAAGTACCTATTTTTCTGG
TATTTCGACGAAGAGAGCTCCAGCTCCACAGCTCTGCTGATGTATGAAGTTTCCACCGGCTG
CTCGGAGTCGCAGATAGAGTTTCAGTGGCAGCGGTCAGGCGAGGATTTTACACTGAATAT
AGCGGGTGGAGCTGTAGATGTGTGGGTTTCTGATTCGCTAGAAATATACAGCTTCCG
TGGACTCTCCGCGACGGGACAGGTTGGAAATCAACAGCACTGTGGCTGCACACTCTGTCT
TTCATCTTCGCGCCATCTGAT

軽鎖可変領域配列(配列番号4)

DVVMTQTPLSLSVTPGQPASISCRSSQSLHSDGMTYFSWYLQKPGQPPQLLIYEVSNRF
SGVPDRFSGSGSGTDFTLTKISRVEAEDVGVYYCMQNIQLPWTFGQGTKEIKRTVAAPSV
FIPTPSD

FIGURE 6

【図 7】

PA01 IgMヒトモノクローナル抗体と純粋なPA01多糖(PS)との結合の阻害

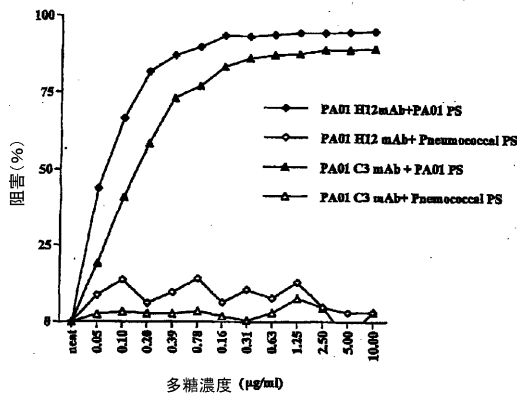


Figure 7

【図 8】

170003 IgMヒトモノクローナル抗体と純粋な170003多糖(P S)との結合の阻害

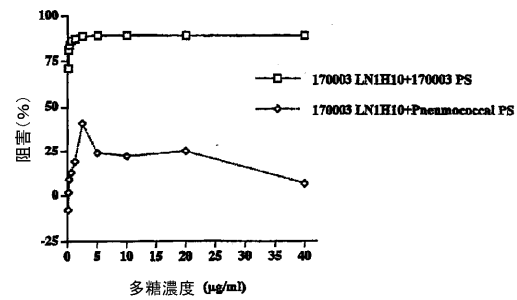


Figure 8

【図 9】

重鎖可変領域配列

フレームワーク1
CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCC
Q V Q L V E S G G G V V Q P G R S

CDR1
CTGAGACTCTCTGTGTCAGCGTCTGGATTACCTTCAGTACCTATGGCATG
L R L S C A A S G F T F S S Y G M

フレームワーク2
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGCT
H W V R Q A P G K G L E W V A A

CDR2

フレームワーク3
ATATGGTATAGTGAAGTAATAAATACTATGCAGACTCCGTAAGGGCCGA
I W Y S G S N K Y Y A D S V K G R

フレームワーク4
TTCACCATCTCCAGAGACAATCCAAGAACACGCTGTATCTGCAAATGAAC
F T I S R D N S K N T L Y L Q M N

CDR3
AGCCTGAGAGCCGAGGACACGGCTGTGTAATTACTGTGCGAGAGATCCCCAC
S L R A E D T A V Y Y C A R D P H

フレームワーク4
TATACCCGGGGTTTGGTACTACTGGGGCAAGGAAACCTGGTCAACGCTCTCC
Y T R G F D Y W G K G T L V T V S

定常
TCAGCCTCCACCAAAGGGCCATCGGTCTTCCCTGGCGCCCTGCTCCAGG
S A S T K G P I G L P L A P C S R

AGCACCTCCGAGAGCAGACGGCCCTGGGCTGCCTGGTCAAGGACTACTTC
S T S E S T A A L G C L V K D Y F

GCCGAA
A E

Figure 9

【図 10】

軽鎖可変領域配列

フレームワーク1
GATGTTGTGATGACCCAGACTCCACTCTCTCTGTCGTCACCCCTGGACAG
D V V M T Q T P L S L S V T P G Q

CDR1
CCGGCCTCCATCTCTGCAGGTCTAGTCAGAGCCTCTGCATAGTGATGGA
P A S I S C R S S Q S L L H S D G

フレームワーク2
ATGACCTATTTTCTCTGTTATTCAGAGCCAGGCCAGCCTCCACAGCTC
M T Y F S W Y L Q K P G Q P P Q L

CDR2

フレームワーク3
CTGATCTATGAAGTTTCCAACCGGTTCTCTGAGTGCCAGATAGGTTCACT
L I Y E V S N R F S G V P D R F S

GGCAGCGGCTCAGGACAGATTTCACTGAAAATCAGCCGGGTGGAG
G S G S G T D F T L K I S R V E

CDR3
GCTGAGGATGTTGGGTTTATTACTGCATGCAAAATATACAGCTTCCG
A E D V G V Y Y C M Q N I Q L P

フレームワーク4
TGCAGCTTCGGCCAAGGACCAAGGTGAAATCAACCAACTGTGGCT
W T F G Q G T K V E I K R T V A

定常
GCACCATCTGCTTCACTCTCCCGCCATCTGAT
A P S V F I F P P S D

Figure 10

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(54) Title: HUMAN ANTIBODIES AGAINST *PSEUDOMONAS AERUGINOSA* LPS DERIVED FROM TRANSGENIC
XENOMOUSE®

S20 Monoclonal Antibody Gene Usage

Heavy Chain Gene Segments

<u>VH</u>	<u>D</u>	<u>JH</u>
V _H 3/V3-33	D2-8	J _H 4b

Light Chain Gene Segments

<u>VL</u>	<u>JL</u>
V _κ 2/A2	J _κ 1

(57) Abstract: The invention described herein provides for human antibodies produced in non-human animals that specifically
bind to *Pseudomonas aeruginosa* Lipopolysaccharide (LPS). The invention further provides methods for making the antibodies in a
non-human animal, expression of the antibodies in cell lines including hybridomas and recombinant host cell systems. Also provided
are kits and pharmaceutical compositions comprising the antibodies and methods of treating or preventing pseudomonas infection
by administering to patient the pharmaceutical compositions described herein.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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HUMAN ANTIBODIES AGAINST *PSEUDOMONAS AERUGINOSA*
LPS DERIVED FROM TRANSGENIC XENOMOUSE®

TECHNICAL FIELD OF THE INVENTION

The present invention relates to compositions and
5 methods for treating or preventing *Pseudomonas*
aeruginosa infection and conditions caused by such
infection. Specifically, the present invention relates
to human antibodies that specifically bind to
Pseudomonas aeruginosa Lipopolysaccharide (LPS) and
10 encoding nucleic acid molecules thereof. The invention
further relates to methods for making the antibodies in
a non-human animal and expressing the antibodies in
cell lines including hybridomas and recombinant host
cell systems. The invention also relates to kits and
15 pharmaceutical compositions comprising the antibodies.
The invention further relates to methods of treating or
preventing *Pseudomonas* infection by administering to a
patient any of the compositions described herein.

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BACKGROUND OF THE INVENTION*Pseudomonas aeruginosa* Infections

Pseudomonas aeruginosa are Gram-negative, flagellated rod bacteria that continue to be a significant pathogen in nosocomial infections resulting from surgery, prosthesis implantation and respiratory tract procedures. *Pseudomonas aeruginosa* also is an opportunistic pathogen in the etiology of cancer, cystic fibrosis, diabetes, heart disease, otitis externa (swimmer's ear), osteomyelitis, corneal ulcers, folliculitis, mastitis, pneumonia, meningitis, urinary tract infections, endocarditis, peritonitis and other diseases found in geriatric or immunocompromised patients.

Surgical patients are often at increased risk of *Pseudomonas aeruginosa* infection by virtue of their illness (e.g., trauma, burns, inhalation injury and cancer) or treatment (e.g., disruption of natural epithelial barriers by incision or percutaneous catheterization, endotracheal intubation, cardiac and thoracic surgery, neurosurgery, and gastrointestinal surgery). Disruption of natural intestinal flora by antibiotic treatments or prophylaxis, therapeutic immunosuppression of solid organ transplant recipients, or environmental exposure to *Pseudomonas aeruginosa* can place patients at increased risk. Moreover, multi-drug-resistant strains can cause significant infections in inpatient units as well as nursing homes.

Surgical patients are affected by nosocomial pneumonia, often caused by *Pseudomonas aeruginosa*. Onset occurs after the first 72 hours of hospitalization and is characterized by fever, purulent sputum, leukocytosis and a new or changed lung

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infiltrate revealed by chest radiography. The oropharynx is colonized rapidly, which may spread into the lower respiratory tract. Incidence of nosocomial infection in surgical patients overall is approximately 5% to 8%, and is probably higher in all critically ill patients. The incidence of pneumonia reported from surgical intensive care units (ICUs) is 15% to 20%, and occasionally higher. See Barie et al. *Am. J. Surgery* 179:2S-7S (2000).

10 Cystic fibrosis (CF) patients suffer chronic colonization with a narrow but evolving spectrum of bacterial pathogens. *Pseudomonas aeruginosa* remains the major CF pathogen with a worldwide prevalence of up to 80% to 90% in CF adults. Such infections lead to 15 intermittent episodes of debilitating inflammatory exacerbations and progressive lung damage. Emerging pathogens also tend to be resistant to multiple antibiotic regimens, thus infection control plays a critical role in the quality of life and life 20 expectancy of CF patients.

The onset of chronic colonization is associated with acceleration of forced expiratory volume (FEV). The original colonizing strain transforms into a mucoid colonial form which is due to copious production of a 25 highly viscid exopolysaccharide known as alginate. The colonizing strain becomes significantly more mucinophilic and chemotactic and is associated with impaired mucociliary clearance. See Govan J. *Royal Soc. Med.* 93 Supp. 38:40-45 (2000). Moreover, the 30 *Pseudomonas aeruginosa* isolated from lungs of CF patients show changes in the LPS fatty acid acylation pattern and enhanced resistance to the bactericidal

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activity of some cationic antimicrobial peptides (CAMPs).

Alterations in *Pseudomonas aeruginosa* LPS lipid A were found in CF isolates that increased both bacterial resistance to antimicrobial peptides and the ability of LPS to elicit inflammatory mediators. CF patients have very high antibody titers to *Pseudomonas aeruginosa* LPS in both serum and sputum, which might neutralize its biological activities in vivo (e.g. proinflammatory mediator release). See Pier *Trends Microbiol.* **8**:247-251 (2000).

The leading cause of morbidity and mortality in severe burn wounds patients is infection with *Pseudomonas aeruginosa*, See Lee et al. *Vaccine* **18**:1952-1961 (2000). Burn wounds are highly exudative, creating a moist, nutrient-rich environment for bacterial colonization. Burn wounds are largely inaccessible to the patient's immune responses and vascularly-delivered antibiotics due to the severe tissue injury. Moreover, burn wounds leave the host immunocompromised with endogenously decreased levels of immunoglobulin gamma (IgG). Without treatment, burn wound infections can spread and develop into sepsis with the associated production of inflammatory cytokines, including interleukin-1 (IL-1), IL-6, and tumor necrosis factors (TNFs). Burn wound infections may also result in delayed healing, increased scarring, conversion of a partial thickness defect to a full thickness defect and increased nutritional demands.

Intravenous immunoglobulin (IVIG) has been used increasingly to treat both bacterial and viral infections and primary and secondary immunodeficiency disorders. IVIG is comprised of pooled human

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polyclonal antibodies from normal donors which are used as a substitution therapy for primary and secondary antibody deficiencies and to treat immune-mediated diseases, including autoimmune and systemic inflammatory conditions. Immunoglobulins promote the opsonization and phagocytosis of bacteria, neutralization of bacterial toxins, inhibition of microbial attachment, and the complement-induced lysis of bacteria. See Felts et al. *Burns* 25:415-423 (1999).

Direct and local delivery of protective immunoglobulins to wound and burn sites represents a rational means to overcome the lack of vascularization of burn wounds as well as biofilm barriers. Local delivery of IgG, both prophylactically and post-infection, was demonstrated to improve survival in mouse models of *Pseudomonas aeruginosa* infected burn wounds. See Felts et al. *Burns* 25:415-423 (1999).

Advances in the bioengineering of prosthetic devices has improved the lives of millions of patients. However, this progress has been tempered by implant-associated infections that often resist antibiotic treatment. Infectious organisms, including *Pseudomonas aeruginosa*, preferentially target synthetic implanted materials, eliciting serious and costly infections that frequently require removal of the colonized device.

Initial microbial adhesion is a primary determinant of biomaterial colonization because initially adhering microorganisms often progress to a mature biofilm attached to the biomaterial surface. The focus of research aimed at reducing biofilm formation on prostheses has been directed toward modifying or coating the surface of the implanted materials. Approaches utilizing surface chemistry and

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antibiotic-releasing coatings, however, have not been fully successful.

Because surgical sites are often immunocompromised, a promising approach involves the immunostimulation of the local wound site. Studies have shown that pooled polyclonal human antibodies opsonize infecting bacteria, and pooled antibodies can inhibit *Pseudomonas aeruginosa* adhesion rates and surface-growth dynamics, thus reducing biofilm formation. See Poelstra et al. *J. Biomed. Mat. Res.* 51:224-232 (2000).

Peritonitis is often caused by ulcers, appendicitis, diverticulitis, ileus, gunshot or stab wounds, disturbances during abdominal surgery, and continuous ambulatory peritoneal dialysis (CAPD). Nosocomial peritonitis, caused by exogenous pathogenic bacteria including *Pseudomonas aeruginosa*, is an especially acute problem for immunocompromised and geriatric populations.

Current treatment regimens for peritonitis focus on antibiotics, however, antibiotic resistance occurs at a significant rate and is frequently associated with clinical failure. IVIG has shown promising but inconsistent results in peritonitis, however, as with burn wounds, local (peritoneal) delivery of pooled polyclonal immunoglobulin against *Pseudomonas aeruginosa* was shown to significantly reduce infection in a mouse model. See Barekzi et al. *Antimicrob. Agents Chemotherap.* 43:1609-1615 (1999).

Treating *Pseudomonas aeruginosa* infections with antibiotic regimens has become increasingly difficult because, *inter alia*, antibiotic resistant strains have arisen. The emergence of passive antibody therapy for

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the prevention and treatment of *Pseudomonas aeruginosa* infections, though promising, has been tempered by the availability purified human antibodies, free of non-human animal antibodies, that bind specifically to

5 *Pseudomonas aeruginosa* in clinical quantities.

Non-human antibody preparations, including murine monoclonal antibodies, are not generally acceptable for human therapies because of their immunogenicity. Human polyclonal antibody preparations, although suitable for

10 human therapies, have variable titers of protective antibodies for *Pseudomonas aeruginosa* and a high cost of purifying antibodies from multiple donors.

Monoclonal antibodies theoretically can be made in unlimited quantity, at a low cost and with a desired

15 specificity. However, efficacious human monoclonal antibodies are difficult to make and require human B cells expressing appropriate antibodies to be transformed with Epstein-Barr virus. The resulting monoclonal antibody preparations would not likely be

20 appropriate for human therapeutic use. Moreover, most of the human monoclonal antibodies tested to date have been IgM which penetrate poorly into pulmonary tissue and can be associated with immune complex formation and enhanced inflammation.

25 Therefore, there is a need for purified human IgG antibodies that bind specifically to *Pseudomonas aeruginosa*, methods for its preparation and use, and pharmaceutical compositions and kits thereof.

SUMMARY OF THE INVENTION

30 The present invention provides isolated human antibodies that specifically bind to *Pseudomonas aeruginosa* Lipopolysaccharide (LPS). The invention

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further provides methods for making the antibodies in a non-human animal, expression of the antibodies in cell lines including hybridomas and recombinant host cell systems. The invention also provides kits and
5 pharmaceutical compositions comprising the antibodies. Moreover, the invention provides methods of treating or preventing pseudomonas infection by administering to a patient the pharmaceutical compositions described herein.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the human immunoglobulin variable (V), diversity (D) and joining (J) regions utilized by the hybridoma that produces the S20 monoclonal anti-*Pseudomonas aeruginosa* antibody.

15 Figure 2 depicts binding inhibition assays that show the S20 human monoclonal antibody binding with specificity to the *Pseudomonas aeruginosa* LPS. Soluble *Pseudomonas aeruginosa* LPS, but not soluble *Pneumococcal* 6B control polysaccharide, was able to
20 inhibit S20 binding to solid phase *Pseudomonas aeruginosa* LPS.

Figure 3 shows that the S20 monoclonal antibody specifically binds the *Pseudomonas aeruginosa* 06ad serotype LPS but does not bind solid phase LPS derived
25 from the 011, Habs 16, 170003 and PAOI Halloway strains.

Figure 4 shows that S20 opsonization promotes complement-dependent phagocytosis. Flow cytometry analysis of peripheral nuclear monocytes (PMNs) showed
30 that the PMNs phagocytosed FITC labeled, opsonized *Pseudomonas aeruginosa* only in the presence of complement.

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Figure 5 shows that the S20 monoclonal antibody protected neutropenic mice from fatal *Pseudomonas aeruginosa* sepsis.

Figure 6 sets forth the DNA and amino acid sequences of the heavy chain and light chain variable regions of human monoclonal antibody S20 (IgG).

Figure 7 depicts binding inhibition assays that show the H12 and C3 human monoclonal antibodies binding with specificity to the *Pseudomonas aeruginosa* LPS. Soluble *Pseudomonas aeruginosa* PA01 LPS, but not soluble *Pneumococcal* 6B control polysaccharide, was able to inhibit binding to solid phase *Pseudomonas aeruginosa* PA01 LPS.

Figure 8 depicts binding inhibition assays that show the LN1H10 human monoclonal antibody binding with specificity to the *Pseudomonas aeruginosa* LPS. Soluble *Pseudomonas aeruginosa* 170003 LPS, but not soluble *Pneumococcal* 6B control polysaccharide, was able to inhibit binding to solid phase *Pseudomonas aeruginosa* 170003 LPS.

Figure 9 indicates the location of CDRs 1-3 of the heavy chain variable region of the S20 human monoclonal antibody.

Figure 10 indicates the location of CDRs 1-3 of the kappa light chain variable region of the S20 human monoclonal antibody.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided fully human isolated antibodies or antigen-binding portions thereof that specifically binds to *Pseudomonas aeruginosa* LPS. In a preferred embodiment, the fully human antibodies are monoclonal.

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Other preferred embodiments include nucleotide sequences encoding and amino acid sequences comprising the antibodies' heavy and light chains, and in particular sequences corresponding to a contiguous heavy and light chain sequences from CDR1 through CDR3. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein. Hybridomas expressing such immunoglobulin molecules and monoclonal antibodies are also provided.

The terms herein generally have their usual meaning as understood by those of ordinary skill in the art. The following terms are intended to have the following general meanings as they are used herein:

"B lymphocytic cells or progeny thereof" refer to any cell descending from, or destined for, the B lymphocytic lineage. Examples include, but are not limited to, all B lymphocytes in the B cell developmental pathway starting from the earliest B lymphocyte stem cells through memory B cells, plasma cells, and any hybridomas created *in vitro*.

"Bispecific antibodies" are single antibodies that have affinities for two separate antigens. For example, a bispecific antibody might recognize *Pseudomonas aeruginosa* LPS using one combination of heavy and light chains and might recognize a leukocyte cell surface marker using a second combination of heavy and light chains attached to the first combination. See McCormick et al. *J. Immunol.* 158:3474-3482 (1997).

"Chimeric antibodies" are antibodies that have been altered from their original form to comprise amino acid sequences from another protein. Chimeric antibodies retain at least a portion of the original

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antibody amino acid sequence, typically the portion comprising the antigen binding region (F_{ab}). Examples of chimeric antibodies include, but are not limited to, bispecific antibodies and fusions with other non-immunoglobulin protein sequences.

"Cytokines" refer generally to signaling molecules of the immune system. Cytokines include, but are not limited to, Interleukins (IL), transforming growth factors (TGF), tumor necrosis factors (TNF), lymphotoxins (LT), interferons, granulocyte-macrophage colony stimulating factors (GM-CSF), macrophage CSF, Granulocyte CSF, and migration inhibition factors.

"Derivatize" refers to the process of attaching a non-immunoglobulin agent to the immunoglobulin molecules. Examples of derivatizing agents include, but are not limited to, toxins, complement, antibiotics, peptides, polysaccharides, lipids, organic polymers, radiolabels, and inorganic compounds.

"Expression control sequences" refer to sequences that allow for the inducible or constitutive expression of gene sequences under specific conditions or in specific cells. Examples of cellular processes that expression control sequence regulate include, but are not limited to, gene transcription, protein translation, messenger RNA splicing, immunoglobulin isotype switching, protein glycosylation, protein cleavage, protein secretion, intracellular protein localization and extracellular protein homing.

"Fusion Proteins" refer to chimeric proteins comprising amino acid sequences of two or more different proteins. Typically, fusion proteins result from *in vitro* recombinatory techniques well known in

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the art. However, fusion proteins may result from *in vivo* crossover or other recombinatory events.

"Human immunoglobulin molecules" refer to immunoglobulin proteins that are encoded by human immunoglobulin gene sequences. The immunoglobulin gene sequences may be expressed in any non-human animal.

"Human monoclonal antibodies" refer to antibodies that are members of a population of human antibodies with identical specificities. The population of human antibodies may be produced in a hybridoma or other immortalized cell line as well as in recombinant cell lines expressing the exogenous human antibody gene sequences.

"Immunocompromised patients" refer to patients whose immune responses to foreign antigens or agents is impaired either by disease (e.g. AIDS), by invasive surgery, or by drug therapies in connection with treatments for other conditions (e.g. organ transplant patients).

"Label" refers to any molecule that attaches to the claimed immunoglobulin a functional characteristic not normally associated with that immunoglobulin. Labels can be attached via chemical modification of the immunoglobulin, recognition of the label by one of the two F_{ab} regions of a bispecific immunoglobulin, affinity for a third agent (e.g. the avidin/biotin interaction), radiolabeling, or as a fusion protein expressed recombinantly. Labels can function as molecular or radioactive tags for clinical or research purposes or as agents for combating *Pseudomonas aeruginosa* infection (e.g. toxins or complement proteins). Other examples of labels can include enzymes, fluorescent molecules, magnetic labels, epitope tags (e.g. H.

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influenza hemagglutinin), antibiotics, complement proteins, and cytokines.

"Respiratory patients" refer to any patient that is either being treated for a disease of the
5 respiratory system or is receiving respiratory care, e.g. intubation or ventilation, in connection with some other medical treatment.

"Surgical patients" refer to any patient that is subject to an invasive surgical procedure, typically
10 involving puncturing or incising the dermis.

"Toxins" refer to protein or non-protein compounds that can be attached to antibodies for the purpose of killing the cells to which the antibodies have attached. Examples of toxins include, but are not
15 limited to, complement, antibiotics, peptides, polysaccharides, lipids, organic polymers, radiolabels, and inorganic compounds.

"Vectors" refer to DNA molecules that allow DNA sequences of interest to be cloned, propagated,
20 recombined, mutated, or expressed outside of their native cells. Often vectors have expression control sequences that allow for the inducible or constitutive expression of gene sequences under specific conditions or in specific cells. Examples of vectors include, but
25 are not limited to, plasmids, yeast artificial chromosomes (YACs), viruses, bacteriophages, and phagemids.

"XenoMouse[™]" refers to mice bearing homologously targeted endogenous immunoglobulin loci, rendering them
30 incapable of expressing endogenous murine immunoglobulin, but bearing substantial portions of human immunoglobulin loci. Mice of the XenoMouse[™] line are capable of somatic rearrangement of the human immunoglobulin genes, hypermutation of the human

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variable genes, and immunoglobulin isotype switching. Therefore, the mice of the XenoMouse™ line are capable of mounting effective humoral responses to antigenic challenge utilizing the human immunoglobulin gene sequences. The resulting antibodies are fully human and can be isolated from the animals themselves, from cultured cells extracted from the animals, and from hybridomas created from XenoMouse™ B lymphocytic lines or progeny thereof. Moreover, the rearranged human gene sequences encoding immunoglobulins raised against specific antigenic challenges can be isolated by recombinant means well known in the art.

Antibody Structure

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, *Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety

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for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site.

Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk *J. Mol. Biol.* **196**:901-917 (1987); Chothia et al. *Nature* **342**:878-883 (1989).

A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann *Clin. Exp. Immunol.* **79**:315-321 (1990), Kostelny et al. *J. Immunol.* **148**:1547-1553 (1992). In addition, bispecific antibodies may be formed as "diabodies" (Holliger et al. "Diabodies: small bivalent and bispecific antibody fragments" *PNAS USA* **90**:6444-6448 (1993)) or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J*

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10:3655-3659 (1991) and Traunecker et al. "Janusin: new
molecular design for bispecific reagents" *Int J Cancer*
Suppl 7:51-52 (1992)). Production of bispecific
antibodies can be a relatively labor intensive process
5 compared with production of conventional antibodies and
yields and degree of purity are generally lower for
bispecific antibodies. Bispecific antibodies do not
exist in the form of fragments having a single binding
site (e.g., Fab, Fab', and Fv).

10 Human Antibodies from Non-human Animals

Human antibodies avoid certain of the problems
associated with antibodies that possess murine or rat
variable and/or constant regions. The presence of such
murine or rat derived proteins can lead to the rapid
15 clearance of the antibodies or can lead to the
generation of an immune response against the antibody
by a patient. In order to avoid the utilization of
murine or rat derived antibodies, it has been
postulated that one can develop humanized antibodies or
20 generate fully human antibodies through the
introduction of human antibody function into a rodent
so that the rodent would produce fully human
antibodies.

The ability to clone and reconstruct
25 megabase-sized human loci in YACs and to introduce them
into the mouse germline provides a powerful approach to
elucidating the functional components of very large or
crudely mapped loci as well as generating useful models
of human disease. Furthermore, the utilization of such
30 technology for substitution of mouse loci with their
human equivalents could provide unique insights into
the expression and regulation of human gene products

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during development, their communication with other systems, and their involvement in disease induction and progression.

An important practical application of such a strategy is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated offers the opportunity to study the mechanisms underlying programmed expression and assembly of antibodies as well as their role in B-cell development. Furthermore, such a strategy could provide an ideal source for production of fully human monoclonal antibodies (Mabs) - an important milestone towards fulfilling the promise of antibody therapy in human disease. Fully human antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized Mabs and thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can be expected to provide a substantial advantage in the treatment of chronic and recurring human diseases, such as inflammation, autoimmunity, cancer and bacterial infections, which potentially require repeated antibody administrations.

One approach towards this goal was to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce a large repertoire of human antibodies in the absence of mouse antibodies. Large human Ig fragments would preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of

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immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains should yield high affinity antibodies against any antigen of interest, including human antigens.

5 Using the hybridoma technology, antigen-specific human Mabs with the desired specificity could be readily produced and selected.

This general strategy was demonstrated in connection with the generation of the first XenoMouse™ strains as published in 1994. See Green et al. *Nature Genetics* 7:13-21 (1994). The XenoMouse™ strains were engineered with yeast artificial chromosomes (YACs) containing 245 kb and 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus, respectively, which contained core variable and constant region sequences.

10 Id. The human Ig containing YACs proved to be compatible with the mouse system for both rearrangement and expression of antibodies and were capable of substituting for the inactivated mouse Ig genes. This was demonstrated by their ability to induce B-cell development, to produce an adult-like human repertoire of fully human antibodies, and to generate antigen-specific human Mabs. These results also

15 suggested that introduction of larger portions of the human Ig loci containing greater numbers of V genes, additional regulatory elements, and human Ig constant regions might recapitulate substantially the full repertoire that is characteristic of the human humoral response to infection and immunization. The work of

20 Green et al. was recently extended to the introduction of greater than approximately 80% of the human antibody repertoire through introduction of megabase sized,

25

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germline configuration YAC fragments of the human heavy chain loci and kappa light chain loci, respectively, to produce XenoMouse™ mice. See Mendez et al. *Nature Genetics* 15:146-156 (1997), Green and Jakobovits *J.*

5 *Exp. Med.* 188:483-495 (1998), and U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996, the disclosures of which are hereby incorporated by reference.

Such approach is further discussed and delineated
10 in U.S. Patent Application Serial Nos. 07/466,008, filed January 12, 1990, 07/610,515, filed November 8, 1990, 07/919,297, filed July 24, 1992, 07/922,649, filed July 30, 1992, filed 08/031,801, filed March 15, 1993, 08/112,848, filed August 27, 1993,
15 08/234,145, filed April 28, 1994, 08/376,279, filed January 20, 1995, 08/430, 938, April 27, 1995, 08/464,584, filed June 5, 1995, 08/464,582, filed June 5, 1995, 08/463,191, filed June 5, 1995, 08/462,837, filed June 5, 1995, 08/486,853, filed June 5, 1995,
20 08/486,857, filed June 5, 1995, 08/486,859, filed June 5, 1995, 08/462,513, filed June 5, 1995, 08/724,752, filed October 2, 1996, and 08/759,620, filed December 3, 1996. See also Mendez et al. *Nature Genetics* 15:146-156 (1997) and Green and Jakobovits *J.*
25 *Exp. Med.* 188:483-495 (1998). See also European Patent No. EP 0 463 151 B1, grant published June 12, 1996, International Patent Application No. WO 94/02602, published February 3, 1994, International Patent Application No. WO 96/34096, published October 31,
30 1996, and WO 98/24893, published June 11, 1998. The disclosures of each of the above-cited patents, applications, and references are hereby incorporated by reference in their entirety.

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Antibodies in accordance with the present invention are preferably prepared through the utilization of a transgenic mouse that has a substantial portion of the human antibody producing genome inserted but that is rendered deficient in the production of endogenous, murine antibodies. Such mice, then, are capable of producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed herein.

Through use of such technology, fully human monoclonal antibodies, or the antigen binding portions thereof, to *Pseudomonas aeruginosa* LPS were produced. Essentially, we immunized XenoMouse™ lines of mice with heat killed *Pseudomonas aeruginosa*, recovered spleen and lymph node cells (such as B-cells) from the mice that express *Pseudomonas aeruginosa* LPS antibodies, fused such recovered cells with nonsecreting myeloma cells to prepare immortal hybridoma cell lines, and screened hybridoma cell lines to identify those that produce antibodies specific to the antigen of interest.

As will be appreciated, antibodies in accordance with the present invention can be expressed in cell lines other than hybridoma cell lines. Sequences encoding particular antibodies can be used for transformation of a suitable host cell. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures

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known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference). The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NS/O, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. Cell lines of particular preference are selected through determining which cell lines have high expression levels and produce antibodies with constitutive *Pseudomonas aeruginosa* LPS binding properties.

Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, enhanced expression can be realized by the coamplification expression system utilizing dihydrofolate reductase (DHFR) or the glutamine synthetase gene expression system (the GS system). See, e.g. Kaufman and Sharp *J. Mol. Biol.*

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159:601-621 (1982); European Patent Nos. 0 216 846, 0 256 055, and 0 323 997; and European Patent Application No. 89303964.4.

Antibodies of the invention can also be produced
5 through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. In connection with the transgenic production in mammals, antibodies can be
10 produced in, and recovered from, the milk of goats, cows, or other mammals. See, e.g., U.S. Patent Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that was
15 expressed in a non-human animal and specifically binds to *Pseudomonas aeruginosa* LPS. In a preferred embodiment, the isolated human antibody or antigen-binding portion thereof is a monoclonal antibody.

The invention further contemplates the isolated
20 human antibody or antigen-binding portion thereof that is opsonic for *Pseudomonas aeruginosa* cells. In a preferred embodiment, the isolated human antibody or antigen-binding portion thereof facilitates phagocytosis of the *Pseudomonas aeruginosa* cells.

25 The invention also contemplates that the isolated human antibody or antigen-binding portion thereof enhances the immune response to *Pseudomonas aeruginosa*. In a preferred embodiment, the isolated human antibody or antigen-binding portion thereof facilitates the
30 killing of *Pseudomonas aeruginosa* cells. In a more preferred embodiment, the isolated human antibody or antigen-binding portion thereof facilitates the killing

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of *Pseudomonas aeruginosa* cells by delivering an agent that is lethal to the *Pseudomonas aeruginosa* cells.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that
5 specifically binds to *Pseudomonas aeruginosa* LPS, wherein the antibody or antigen-binding portion thereof inhibits *Pseudomonas aeruginosa* infection.

The invention also contemplates an isolated human antibody or antigen-binding portion thereof that
10 specifically binds to *Pseudomonas aeruginosa* LPS, wherein the antibody or antigen-binding portion thereof binds to *Pseudomonas aeruginosa* LPS with a dissociation constant (K_d) of 5×10^{-7} M or less, preferably 5×10^{-7} M to 1×10^{-7} M. In a more preferred embodiment, the
15 antibody or antigen-binding portion thereof binds to *Pseudomonas aeruginosa* LPS with a K_d of 1×10^{-7} M to 5×10^{-8} M. In a more preferred embodiment, the antibody or antigen-binding portion thereof binds to *Pseudomonas aeruginosa* LPS with a K_d of 5×10^{-8} M to 1×10^{-8} M.

The invention contemplates an isolated human
20 antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and has a half-life *in vivo* of one hour or more. In a preferred embodiment, the antibody or antigen-binding
25 portion thereof has a half-life *in vivo* of between one hour and thirty days. In a more preferred embodiment, the antibody or antigen-binding portion thereof has a half-life *in vivo* of between sixteen and thirty days. In another more preferred embodiment, the antibody or
30 antigen-binding portion thereof has a half-life *in vivo* of between one hour and fifteen days.

The isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas*

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aeruginosa LPS of the invention may be immunoglobulin G (IgG), IgM, IgE, IgA and IgD. In a preferred embodiment, the IgG may be an IgG1, IgG2, IgG3 or IgG4 subtype.

5 The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and is labeled. In a preferred embodiment, the label is a radiolabel, an enzyme label, a fluorescent label, a
10 toxin, a magnetic agent, a second antibody, an affinity label, an epitope tag, an antibiotic, a complement protein or a cytokine.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that
15 specifically binds to *Pseudomonas aeruginosa* LPS and comprises a kappa light chain and framework sequences thereof. In a preferred embodiment, the framework sequences of the kappa light chain are encoded by a human Vk2/A2 gene. In a preferred embodiment, the
20 kappa light chain comprises between seven and fifteen changes from a kappa light chain encoded by a germline Vk2/A2 gene. In a more preferred embodiment, the kappa light chain comprises no more than six amino acid changes from a kappa light chain encoded by a germline
25 Vk2/A2 gene. In a more preferred embodiment, the kappa light chain comprises no more than three amino acid changes from a kappa light chain encoded by a germline Vk2/A2 gene.

The invention contemplates an isolated human
30 antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and comprises a kappa light chain having the amino acid sequence of SEQ ID NO: 4. The invention further

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contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and comprises a kappa light chain that is encoded by the nucleic acid sequence of SEQ ID NO: 3. The invention also contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and comprises a lambda light chain.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, comprising a heavy chain composed of variable (V), diversity (D), and Joining (J) regions and composed of framework sequences thereof. In a preferred embodiment, the variable region of the heavy chain is encoded by a human V_H3/V3-33 gene. In another preferred embodiment, the diversity region of the heavy chain is encoded by a human D2-8 gene. In another preferred embodiment, the joining region of the heavy chain is encoded by a human J_H4b gene. In a more preferred embodiment, the variable region comprises between seven and fifteen amino acid changes from a variable region encoded by a germline V_H3/V3-33 gene. In a more preferred embodiment, the heavy chain comprises no more than six amino acid changes from a variable region encoded by a germline V_H3/V3-33 gene. In a more preferred embodiment, the heavy chain comprises no more than three amino acid changes from a variable region encoded by a germline V_H3/V3-33 gene.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and

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comprises a heavy chain having the amino acid sequence of SEQ ID NO: 2.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that
5 specifically binds to *Pseudomonas aeruginosa* LPS and is encoded by the nucleic acid sequence of SEQ ID NO: 1,.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and
10 comprises an antigen binding domain chosen from the list consisting of an Fab fragment, an F(ab')₂ fragment and an F_v fragment.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that
15 specifically binds to *Pseudomonas aeruginosa* LPS and the antibody is a single chain antibody.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and
20 the antibody is a chimeric antibody. In a preferred embodiment, the chimeric antibody comprises framework regions and CDR regions from different human antibodies. In a more preferred embodiment, the chimeric antibody is bispecific. In a more preferred
25 embodiment, the chimeric antibody is bispecific for *Pseudomonas aeruginosa* LPS and a label selected from the list consisting of a radiolabeled molecule, an enzymatic label, a fluorescent label, a toxin, a magnetic agent, a second antibody, an affinity label,
30 an epitope tag, an antibiotic, a complement protein and a cytokine.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that

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specifically binds to *Pseudomonas aeruginosa* LPS and the antibody or portion thereof is derivatized. In a preferred embodiment, the antibody or portion thereof is derivatized with polyethylene glycol, at least one
5 methyl or ethyl group or at least one carbohydrate moiety.

The invention contemplates a pharmaceutical composition comprising the an isolated human antibody or antigen-binding portion thereof that specifically
10 binds to *Pseudomonas aeruginosa* LPS and a pharmaceutically acceptable carrier. The invention further contemplates a kit comprising the antibody or antigen-binding portion thereof, a pharmaceutically acceptable carrier therefor, and a container. In a
15 preferred embodiment, the kit further comprising instructions for use.

The invention contemplates a method for treating or preventing *Pseudomonas aeruginosa* infection, comprising the step of administering a pharmaceutical
20 composition to a patient at risk of being infected with, or currently infected with, *Pseudomonas aeruginosa*.

In a preferred embodiment, the human antibody is a monoclonal antibody. In another preferred embodiment,
25 the pharmaceutical composition is administered via an injection, transmucosal, oral, inhalation, ocular, rectal, long acting implantation, liposomes, emulsion, cream, topical or sustained release means. In another preferred embodiment, the antibody is a fusion with a
30 second protein. In a more preferred embodiment the second protein is chosen from the list consisting of a toxic peptide moiety, a complement protein, a radiolabeled protein, a cytokine or an antibiotic

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protein. In another preferred embodiment, the antibody is labeled with a radiolabel, a toxin, a complement protein, a cytokine or an antibiotic. In another preferred embodiment, the patient is a burn patient, a surgical patient, a prosthesis recipient, a respiratory patient, a cancer patient, a cystic fibrosis patient or an immunocompromised patient. In another preferred embodiment, the pharmaceutical composition further comprises toxins, complement proteins, radiolabeled proteins, cytokines, antibiotics, or any combination thereof.

The invention contemplates an isolated cell line that produces a human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS. In a preferred embodiment, the cell line is a hybridoma.

The invention contemplates a method of producing an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, comprising:

- a) culturing a non-human cell capable of producing the antibody under conditions in which the antibody is produced;
- b) isolating the antibody from the cell culture.

In a preferred embodiment, the method of producing an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS utilizes a hybridoma. In another preferred embodiment, the method utilizes a cell that is transformed with isolated nucleic acid molecules encoding the human antibody or antigen-binding portion thereof and the cell is chosen from the list consisting

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of a bacterial cell, a yeast cell, an insect cell, an amphibian cell and a mammalian cell. In a more preferred embodiment, the mammalian cell is selected from the list consisting of a human cell, a mouse cell, 5 a rat cell, a dog cell, a monkey cell, a goat cell, a pig cell, a bovine cell and a hamster cell. In a more preferred embodiment, the mammalian cell is selected from the list consisting of a HeLa cell, a NIH 3T3 cell, a CHO cell, a BHK cell, a VERO cell, a CV-1 cell, 10 a NS/O cell and a COS cell.

The invention contemplates a method for making an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, comprising:

- 15 a) immunizing a non-human animal having incorporated a human immunoglobulin locus therein with a *Pseudomonas aeruginosa* antigenic composition;
- b) allowing the non-human animal to mount a humoral response to the antigenic composition; and
- 20 c) isolating the human antibody from the non-human animal.

The invention contemplates a nucleic acid molecule isolated from a non-human animal that encodes a human antibody heavy chain or the antigen-binding portion 25 thereof that specifically binds to *Pseudomonas aeruginosa* LPS. In a preferred embodiment, the nucleic acid molecule is isolated from a hybridoma that produces the human antibody.

The invention contemplates an isolated nucleic 30 acid molecule, or a fragment thereof, encoding a human antibody heavy chain or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS having the nucleotide sequence of SEQ ID: 1. In a

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preferred embodiment, the isolated nucleic acid molecule comprises the sequence encoding between one to three of the CDR regions of the human antibody.

The invention contemplates a vector comprising a
5 nucleic acid molecule, or fragment thereof, encoding a human antibody heavy chain or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa*. In a preferred embodiment, the vector further comprises expression control sequences operably
10 linked to the nucleic acid.

The invention contemplates a nucleic acid molecule isolated from a non-human animal that encodes a human antibody light chain or the antigen-binding portion thereof that specifically binds to *Pseudomonas*
15 *aeruginosa* LPS. In a preferred embodiment, the nucleic acid molecule is isolated from a hybridoma that produces the human antibody.

The invention contemplates an isolated nucleic acid molecule, or a fragment thereof, encoding a human
20 antibody light chain or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS having the nucleotide sequence of SEQ ID: 3. In a preferred embodiment, the isolated nucleic acid molecule comprises the sequence encoding between one to
25 three of the CDR regions of the human antibody.

The invention contemplates a vector comprising a nucleic acid molecule, or fragment thereof, encoding a human antibody light chain or antigen-binding portion thereof that specifically binds to *Pseudomonas*
30 *aeruginosa*. In a preferred embodiment, the vector further comprises an expression control sequence operably linked to the nucleic acid.

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The invention contemplates an isolated host cell comprising

5 a) a nucleic acid molecule that was isolated from a non-human animal and encodes a light chain or the antigen-binding portion thereof of a human antibody that specifically binds to *Pseudomonas aeruginosa* LPS; or

b) a vector comprising the nucleic acid molecule.

10 The invention contemplates an isolated host cell comprising:

a) a nucleic acid molecule that was isolated from a non-human animal and encodes a heavy chain or the antigen-binding portion thereof of a human antibody that specifically binds to *Pseudomonas*

15 *aeruginosa* LPS; or

b) a vector comprising the nucleic acid molecule.

20 The invention contemplates an isolated host cell comprising:

a) a nucleic acid molecule that was isolated from a non-human animal and encodes a heavy chain or the antigen-binding portion thereof and an isolated nucleic acid molecule that encodes a light chain or the antigen-binding portion thereof of a human antibody that specifically binds to *Pseudomonas*

25 *aeruginosa* LPS; or

b) a vector or vectors comprising the nucleic acid molecules.

30 In a preferred embodiment, the isolated host cells described above are chosen from the list consisting of hybridoma cells, bacterial cells, yeast cells, insect cells, amphibian cells and mammalian cells. In a more

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preferred embodiment, the mammalian cells are selected from the list consisting of human cells, mouse cells, rat cells, dog cells, monkey cells, goat cells, pig cells, bovine cells and hamster cells. In a more

5 preferred embodiment, the mammalian cells are selected from the list consisting of HeLa cells, NIH 3T3 cells, CHO cells, BHK cells, VERO cells, CV-1 cells, NS/0 cells and COS cells.

The invention contemplates a method of

10 recombinantly producing the heavy chain or the antigen-binding portion thereof, the light chain or the antigen-binding portion thereof, or both the light chain and heavy chain or antigen-binding portions thereof, of a human antibody that was identified from a

15 non-human animal and specifically binds to *Pseudomonas aeruginosa* LPS, comprising the step of cultivating the host cells described above under conditions in which the nucleic acid molecules are expressed.

The invention contemplates an isolated human

20 antibody heavy chain or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, encoded by any of the nucleic acid molecules encoding the heavy chain described above, or isolated from any of the host cells described above. In a preferred

25 embodiment, the isolated human antibody heavy chain or antigen-binding portion thereof comprises between one to ten amino acid substitutions that increase the serum half-life of the antibody.

The invention contemplates an isolated human

30 antibody light chain or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, encoded by any of the nucleic acid molecules encoding the heavy chain described above; or isolated from any

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of the host cells described above. In a preferred embodiment, the isolated human antibody light chain or antigen-binding portion thereof comprises between one to ten amino acid substitutions that increase the serum half-life of the antibody.

The invention contemplates a non-human transgenic animal comprising any of the nucleic acid molecules described above. In a preferred embodiment, the non-human transgenic animal expresses the nucleic acid molecule or molecules. In a more preferred embodiment, the non-human transgenic animal comprises an isolated nucleic acid molecule that encodes a heavy chain or the antigen-binding portion thereof and an isolated nucleic acid molecule that encodes a light chain or the antigen-binding portion thereof of a human antibody that specifically binds to *Pseudomonas aeruginosa* LPS, and the non-human animal expresses both nucleic acid molecules. In a more preferred embodiment, the non-human animal is selected from the list consisting of a mouse, a rat, a hamster, a cow, a sheep, a primate, a horse and a pig. In a more preferred embodiment, a human antibody resulting from expression of the isolated nucleic acid molecules or portions thereof is expressed on the surface of cells derived from the animal's B lymphocytic cells or progeny thereof. In another preferred embodiment, the human antibody resulting from expression of the isolated nucleic acid molecules or a portion thereof is secreted into the lymph, blood, milk, saliva, or ascites of the animal.

The invention contemplates a fusion protein comprising the an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and a second polypeptide sequence. In a preferred embodiment, the second

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polypeptide sequence is chosen from the list consisting of an epitope tag, an affinity tag, a toxic polypeptide, an antibiotic, an enzyme, a second antibody sequence, a complement protein, and a cytokine.

5 The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, wherein the heavy chain isotype of the antibody is mu, gamma, delta, epsilon or alpha.

10 The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, wherein the antibody or antigen-binding portion thereof is produced by a process comprising the steps of:

15 a) immunizing a non-human animal comprising a human immunoglobulin locus with an antigen selected from the group consisting of an isolated *Pseudomonas aeruginosa* LPS preparation, a virile *Pseudomonas aeruginosa* cell preparation, an attenuated *Pseudomonas aeruginosa* cell preparation, and a killed *Pseudomonas aeruginosa* cell preparation;

20 b) allowing the non-human animal to mount an immune response to the antigen; and

25 c) isolating the antibody from the non-human animal.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS wherein the *Pseudomonas aeruginosa* LPS is derived from a *Pseudomonas aeruginosa* strain chosen from the list consisting of 06ad, 011, Habs16, 170003 and PA01 Halloway.

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The invention contemplates an isolated human antibody or antigen-binding portion thereof isolated from an animal or cell that was free of contaminating human biomaterials. In a preferred embodiment, the
5 biomaterials are viruses, enzymes, hormones, cytokines, receptors, receptor ligands, immunoglobulins, complement, nuclear proteins, and cytoplasmic signaling proteins. In a more preferred embodiment, the viruses are Epstein-Barr virus or retroviruses.

10 Pharmaceutical compositions may be manufactured by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance
15 with the present invention thus may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used
20 pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's
25 solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. For ocular administration, suspensions in
30 an appropriate saline solution are used as is well known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in

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the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained as a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in

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admixture with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or
5 suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

10 For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are
15 conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other
20 suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin, for use in an inhaler or insufflator, may be formulated containing a powder mix of the
25 compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may
30 be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and

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may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, such as sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

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A pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase.

5 The cosolvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists

10 of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without

15 destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied;

20 other biocompatible polymers may replace polyethylene glycol, e.g., polyvinyl pyrrolidone; and other sugars or polysaccharides may be substituted for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed.

25 Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually with a greater toxicity.

30 Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those

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skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days.

Depending on the chemical nature and the
5 biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients.

10 Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

The isolated human antibody or antigen-binding
15 portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS of the invention may be provided as salts with pharmaceutically compatible counterions. Pharmaceutically compatible salts may be formed with many acids, including but not limited to hydrochloric,
20 sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms.

A component of the kits of the present invention
25 comprise instructions for utilizing the compositions of the present invention for prevention or treatment of *Pseudomonas aeruginosa* infections. Applicant has, for the first time, disclosed herein a method of preventing or treating *Pseudomonas aeruginosa* infections with an
30 isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS. The printed instructions on the kit

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enable one of skill in the art to utilize the kit for practicing the methods of the present invention.

EXAMPLE 1

5 Generation of Mice and Hybridomas That Produce Fully Human Antibodies to *Pseudomonas aeruginosa* LPS
A. *Pseudomonas aeruginosa* serotype 06ad

Pseudomonas aeruginosa serotype 06ad was used for mouse immunizations, mouse protection assays and opsonic assays. Bacteria for mouse challenge assays were fresh plated onto pseudosel agar (BBL, Becton Dickinson, Sparks, MD), then were incubated at 37° C, and one cfu was inoculated into LB broth and was incubated at 37°C in a shaking water bath to a concentration of 5×10^8 cfu/ml. Bacteria were
10 centrifuged at 10,000 rpm for 5 minutes, resuspended and washed in chilled phosphate buffered saline (PBS). Bacteria for immunizations were grown as above and heat-killed at 60°C for one hour and stored at 4°C until use. Labeled bacteria used in the flow cytometry
15 opsonic assay were grown and heat-killed as above. However, these bacteria were resuspended in Alkaline Conjugation Buffer (ACB: a 1:3 solution of .5M Na_2CO_3 and .5M NaHCO_3 , pH 9.5) to give a concentration of 10^9 /ml. An equal volume of ACB with .06% Fluorescein
20 Isothiocyanate Isomer I (FITC, Amresco, Solon, OH) was added and incubated for 20 hours at room temperature with gentle shaking. Bacteria were washed in veronal buffered saline and then were resuspended in PBS at 10^9 /ml, and stored at -80° C.
25 The high molecular weight polysaccharide portion of the LPS O-specific side chains from *Pseudomonas aeruginosa* strains 06ad, 011, Habs16, 170003, and PA01 Halloway LPS (high MW PS) were made as described, and were lyophilized for storage. See Hatano et al.
30

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Infect. Immun. 62:3608-3616 (1994). These high MW PS were used to coat 96-well plates for enzyme-linked immunosorbent assays (ELISA) as described below. The 06ad high MW PS was also used in blocking and avidity assays described below.

Mice that were transgenic for human heavy and light Ig were bred and maintained by Abgenix Inc., Fremont, CA. The strain of Xenomouse™ used was Xma2a-3, which is an Ig-inactivated mouse reconstituted with a YAC containing cointegrated human heavy and light chain transgenes. Mice were housed in micro-isolator cages in a pathogen-free facility after shipping, and food and water were autoclaved prior to use. Mice were immunized with 10^7 heat-killed *Pseudomonas aeruginosa* 06ad PA twice per week intraperitoneally (ip) (10^7 bacteria in PBS) and/or in the foot pad (10^7 bacteria and RIBI adjuvant, Sigma, St. Louis, MO), and their sera screened for anti-*Pseudomonas aeruginosa* 06ad LPS antibodies by ELISA described below.

Hybridomas were generated by fusing spleen and/or lymph node cells from immunized, seropositive Xenomouse™ animals with the nonsecreting sp2/0 myeloma cell line, as described previously. See Mendez et al. Nat. Gen. 15:146-156 (1997); Schreiber et al. J. Immunol. 146:188-193 (1991). Supernatants from hybridomas were screened for production of human anti-*Pseudomonas aeruginosa* 06ad LPS using the ELISA procedure described below, and hybridomas found to be secreting IgG anti-LPS antibodies were then cloned three times by limiting dilution. One IgG2-secreting clone (S20) was chosen based on initial measurements of strength of binding to solid phase *Pseudomonas aeruginosa* 06ad PS.

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**B. *Pseudomonas aeruginosa* serotypes
PA01 and 170003**

Pseudomonas aeruginosa serotypes PA01 and 170003

were used for mouse immunizations. Bacteria were fresh
5 plated onto pseudosel agar (BBL, Becton Dickinson,
Sparks, MD), then were incubated at 37° C, and one cfu
was inoculated into LB broth and was incubated at 37°C
in a shaking water bath to a concentration of 5X10⁸
cfu/ml. Bacteria were centrifuged at 10,000 rpm for 5
10 minutes, resuspended and washed in chilled phosphate
buffered saline (PBS). Bacteria for immunizations were
grown as above and heat-killed at 60°C for one hour and
stored at 4°C until use.

The high molecular weight polysaccharide portion
15 of the LPS O-specific side chains from *Pseudomonas*
aeruginosa strains 06ad, 011, Habs16, 170003, and PA01
Halloway LPS (high MW PS) were made as described, and
were lyophilized for storage. See Hatano et al.
Infect. Immun. 62:3608-3616 (1994). These high MW PS
20 were used to coat 96-well plates for enzyme-linked
immunosorbent assays (ELISA) as described below. The
PA01 and 170003 high MW PS also were used in blocking
assays described below.

Mice that were transgenic for human heavy and
25 light Ig were bred and maintained by Abgenix Inc.,
Fremont, CA. The strain of Xenomouse™ used was Xma2a-3,
which is an Ig-inactivated mouse reconstituted with a
YAC containing cointegrated human heavy and light chain
transgenes. Mice were housed in micro-isolator cages
30 in a pathogen-free facility after shipping, and food
and water were autoclaved prior to use. Mice were
immunized with 10⁷ heat-killed *Pseudomonas aeruginosa*
PA01 or 170003 twice per week intraperitoneally (ip) (10⁷
bacteria in PBS) and/or in the foot pad (10⁷ bacteria

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- and RIBI adjuvant, Sigma, St. Louis, MO), and their sera screened for anti-*Pseudomonas aeruginosa* PA01 or 170003 LPS antibodies, respectively, by ELISA described below.
- 5 Hybridomas were generated by fusing spleen and/or lymph node cells from immunized, seropositive Xenomouse™ animals with the nonsecreting sp2/0 myeloma cell line, as described previously. See Mendez et al. *Nat. Gen.* 15:146-156 (1997); Schreiber et al. *J. Immunol.* 146:188-193 (1991). Supernatants from
- 10 hybridomas were screened for production of human anti-*Pseudomonas aeruginosa* LPS using the ELISA procedure described below, and hybridomas found to be secreting anti-LPS antibodies were then cloned three times by
- 15 limiting dilution. Two IgM-secreting clones (H12 and C3) were chosen based on initial measurements of strength of binding to solid phase *Pseudomonas aeruginosa* PA01 PS. These were derived from fusion of splenic cells as described above. One IgM-secreting
- 20 clone (LN1H10) derived from fusion of lymph node cells was chosen based on initial measurements of strength of binding to solid phase *Pseudomonas aeruginosa* 170003 PS.

EXAMPLE 2

- 25 Characterization and Usage of Variable Region Genes From Transgenic Mouse-derived Anti-LPS Antibody
- Dideoxy DNA sequencing was performed as previously described to determine the sequence of the variable region of the human monoclonal antibodies (30, 33).
- 30 Total RNA was isolated from hybridoma cells from nine

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different clones using TRIZOL reagent (Gibco BRL) and converted into random primed cDNA for use as a template in PCR. Human heavy chain and light chain variable regions were amplified using degenerate leader peptide primers and constant region primers provided in the Human Ig-Primer Set (Novagen, Madison, WI). The PCR products were analyzed on a Tris-acetate-EDTA agarose gel. The positive PCR reactions were chloroform isoamyl alcohol (24:1) extracted and cloned into the EcoRI site of pT7Blue (Novagen). The clones were sequenced based on the dideoxy method with Sequenase V2.0 DNA sequencing kit (USB, Cleveland, OH). Gene usage analysis was performed using the Vbase database (Tomlinson et al., MRC Centre for Protein Engineering, Cambridge, UK:

Variable region genes from hybridomas obtained from fusion of spleen cells from PA-immunized transgenic mice with the non-secreting SP2/0 cell line were cloned and sequenced in order to determine variable region gene usage (Figure 1). The protective IgG2 anti-LPS monoclonal antibody chosen for further study that was made in the human Ig transgenic mice, utilized genes from the V_H3 gene family, similar to the restricted V_H gene usage found after immunization of humans with a variety of bacterial polysaccharides. Nine other Mab made from fusions with spleen cells from pseudomonas immunized transgenic mice yielded antibodies that also used the V_H3 gene family for the heavy chain gene elements as determined by DNA sequencing. More specifically, DNA sequence analysis showed that the V_H3/V3-33 and JH4 genes were used in the protective anti-LPS Mab. Similarly, light chain gene segments used were Vk2/A2 and Jk1, as is commonly used in humans after PS vaccine immunization. In summary,

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gene usage for anti-LPS antibodies after PA immunization in these transgenic mice appeared to be remarkably similar to that observed in humans after PS immunization.

5

EXAMPLE 3Detection of Anti-*Pseudomonas aeruginosa* LPS antibodies

Enzyme-linked immunosorbent assay (ELISA) was used to detect antibodies to the *Pseudomonas aeruginosa* 06ad LPS in sera of immunized mice and in hybridoma supernatants as we have previously described (34). Briefly, 96-well microtiter polystyrene plates (NUNC, Denmark) were coated with 2ug/ml of *Pseudomonas aeruginosa* 06ad high MW PS overnight at 4°C, washed, and
15 blocked with 200ul/well of 1% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO) in PBS and .05 % Tween 20 (Amresco, Solon, OH). Plates were washed and incubated over night with serial dilutions of S20 or sera in 1% BSA in PBS. Plates were washed, and bound
20 antibodies were detected by adding isotype specific alkaline phosphatase-conjugated mouse-anti-human polyclonal antibodies (Southern Biotechnology Associates, Birmingham, AL). Plates were developed with 100ul/well of p-nitrophenyl phosphate (PNPP, Sigma-
25 Aldrich) chromogenic substrate in DEA buffer. Optical densities were measured at 415nm with a microplate reader (Biorad, Hercules, CA).
Blocking assays to determine the specificity of S20 were performed in an identical fashion as above except
30 that soluble *Pseudomonas aeruginosa* 06ad high MW PS or

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control PS of different concentrations was added to the S20 prior to addition to PS-coated 96-well ELISA plates. Relative avidity of the Mab was calculated as described. See Chung et al. *Infect. Immun.* 63:4219-4223 (1995); Schreiber et al. *J. Infect. Dis* 167:221-226 (1993). Antibodies were added to the wells of a high MW PS-coated ELISA plate followed by serial dilutions of *Pseudomonas aeruginosa* 06ad high MW PS or equal volumes of PBS (negative control). The blocking assays to determine the binding specificity of the H12, C3 and LN1H10 monoclonal antibodies were conducted as described above, using soluble *Pseudomonas aeruginosa* PA01 or 170003 high MW PS. The concentration of PS required to inhibit 50% of the maximum absorbance was calculated (I_{50}) and the inverse of this value was used to represent relative avidity.

The S20 produced in the transgenic mouse was specific for the O-side chain of *P. aeruginosa* strain 06ad. Blocking assays revealed over 90% reduction in binding of S20 to solid phase *Pseudomonas aeruginosa* 06ad LPS high MW PS after preincubation of the Mab with the same PS, compared to less than 10% inhibition with the control PS (purified type 6B pneumococcal capsular PS; Figure 2). S20, however, did not cross-react with LPS from other *P. aeruginosa* serotypes since no binding could be demonstrated to solid phase LPS O-side chain high MW PS from a variety of pseudomonas strains including 011, Habs 16, 170003, and PA01 Halloway (Figure 3).

The concentration of high MW PS that inhibited 50% of the maximum absorbance of S20 binding to high MW PS was determined so that the inverse of this value ($1/I_{50}$) was used to calculate relative avidity of S20. This

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value was compared to a previously developed protective mouse monoclonal antibody directed to the same high MW PS and to previously published anti-polysaccharide values derived by using similar techniques (2, 33). We found that the relative avidity of the human Mab was higher than the mouse Mab (1.0 vs. .036).

Blocking assays with H12 and C3 revealed over 90% and 85% reduction, respectively, in binding to solid phase *Pseudomonas aeruginosa* PA01 LPS high MW PS after preincubation of the mAbs with the same PS, compared to less than 20% inhibition with the control PS (purified type 6B pneumococcal capsular PS; Figure 7). Preincubation of LNIH10 with *Pseudomonas aeruginosa* 170003 LPS high MW PS reduced binding of the mAb to solid phase PS by 89% compared to less than 40% inhibition with the control PS (Figure 8).

EXAMPLE 4

Anti-*Pseudomonas aeruginosa* LPS Antibody Opsonization Promotes Complement-Dependent Phagocytosis

The ability of the human monoclonal antibody to opsonize *P. aeruginosa* 06ad for uptake by human polymorphonuclear leukocytes (PMN) was measured via flow cytometry as previously described. See Schreiber et al. *J. Infect. Dis* 167:221-226 (1993). *Pseudomonas aeruginosa* 06ad was grown, heat-killed and FITC labeled. Opsonization was carried out by incubating the labeled bacteria with S20 with or without 1% human serum from an agammaglobulinemic patient as the complement source. Bacteria were washed in PBS containing 6% dextran and .2% glucose, and then were

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resuspended in HBSS with .1% gelatin.
Polymorphonuclear leukocytes (PMN) were isolated from peripheral human blood from healthy, adult volunteers. See Schreiber et al. *J. Infect. Dis* 167:221-226 (1993);

5 Tosi et al. *J. Clin. Invest.* 86:300-308 (1990). MN were resuspended to achieve a concentration of 10^7 cells/ml, and activated for 30 minutes with 10ul of a 10^{-6} dilution per ml of cells of FMLP (Peninsula Laboratories, San Carlos, CA). PMNs were added to each

10 opsonized bacteria sample, incubated at 37°C , and then were separated from free bacteria by differential centrifugation and resuspended in PBS. Single color flow cytometry analysis was performed on PMN utilizing a FACScan and CellQuest software (Becton Dickinson,

15 MountainView, CA), and phagocytosis was expressed in relative units of mean fluorescence of 10,000 PMN for each sample. To demonstrate that the observed opsonophagocytosis was associated with bacterial killing, an alternative assay was used in which 10^6 CFU

20 of live *P. aeruginosa* 06ad was mixed with fresh human serum absorbed with the bacteria, S20 and 10^6 fresh human PMN. Samples were obtained at the beginning and end of a 90 minute 37° incubation, bacteria were diluted and then plated for bacterial enumeration.

25 To show functional activity of the Anti-*Pseudomonas aeruginosa* 06ad LPS antibody, S20 was shown to be highly opsonic for uptake of labeled PA by fresh human PMN in a complement-dependent assay. In fact, the human Mab was almost two-fold more opsonic than a

30 previously described protective mouse Mab (D8) against the same epitope (2.5 mg of human Mab yielded twice the mean fluorescence as 5 mg of mouse Mab; Figure 4). Since the bacteria were heat killed prior to labeling

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with FITC it seemed possible that increased susceptibility to antibody and complement-mediated opsonization could have occurred due to damage to the bacterial surface. Thus, we also measured opsonization and phagocytosis of *Pseudomonas aeruginosa* 06ad in a killing assay in which 10^6 CFU of live bacteria are opsonized with antibody and human complement and then colony counts determined after exposure to 10^6 fresh PMN. The S20 Mab was also effective in this assay so that 5mg/ml antibody resulted in 80% reduction in the PA cfu.

EXAMPLE 5

Protection of neutropenic mice from fatal *Pseudomonas aeruginosa* sepsis

The protective efficacy of the human Mab against invasive infection with *Pseudomonas aeruginosa* was measured in the neutropenic mouse model, described previously. See Pier et al. *Infect. Immun.* 57:174-179 (1989); Schreiber et al. *J. Immunol.* 146:188-193 (1991). Female, six week-old BALB/c ByJ mice (Jackson Laboratories, Bar Harbor, ME) were maintained in a pathogen-free, pseudomonas-free environment in which water, bedding, and food were autoclaved prior to use. Neutropenia was established by administering 3mg of cyclophosphamide (Cytoxan[®], Bristol-Myers Squibb, Princeton, NJ) intra-peritoneally to each mouse on days 1, 3, and 5. On day 5, the cyclophosphamide was administered at time 0 hours, and 2 hours later 10ug of S20 or PBS control was administered ip, followed by 10^3 cfu of live *Pseudomonas aeruginosa* 06ad PA two hours later. Mice were observed daily thereafter and

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mortality was the outcome measured.

As illustrated in Figure 5, infected mice treated with the PBS control began dying one day after *Pseudomonas aeruginosa* infection. After two days, 100% of the infected mice treated with PBS had died. In contrast, 100% of the mice treated with the S20 mAb showed protection and were alive two days after *Pseudomonas aeruginosa* infection, demonstrating the protective potential of S20 in preventing *Pseudomonas aeruginosa*-related fatalities in patients.

The embodiments listed above are for illustrative purposes only; the invention as contemplated is not limited to any of these particular embodiments and in fact may encompass a combination of one or more of the embodiments.

BIOLOGICAL DEPOSITS

Hybridoma cell lines S20, H12, C3 and LN1H10 were deposited in accordance with the provisions of the Budapest Treaty at the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209, USA, and were assigned the following accession numbers:

Hybridoma S20	HB _____
Hybridoma H12	HB _____
Hybridoma C3	HB _____
Hybridoma Ln1H10	HB _____

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We claim:

1. An isolated human antibody or antigen-binding portion thereof that was expressed in a non-human animal and specifically binds to *Pseudomonas aeruginosa* LPS.
5
2. The isolated human antibody or antigen-binding portion thereof according to claim 1, wherein said human antibody is a monoclonal antibody.
3. The isolated human antibody or antigen-binding portion thereof according to claim 1 or
10 claim 2, wherein the antibody or portion thereof is opsonic for *Pseudomonas aeruginosa* cells.
4. The isolated human antibody or antigen-binding portion thereof according to claim 3 wherein
15 said antibody or portion thereof facilitates phagocytosis of said *Pseudomonas aeruginosa* cells.
5. The isolated human antibody or antigen-binding portion thereof according to claim 1 or
claim 2, wherein the antibody or portion thereof
20 enhances the immune response to *Pseudomonas aeruginosa*.
6. The isolated human antibody or antigen-binding portion thereof according to claim 1 or
claim 2, wherein the antibody or portion thereof
facilitates the killing of *Pseudomonas aeruginosa*
25 cells.
7. The isolated human antibody or antigen-

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binding portion thereof according to claim 6, wherein said facilitating the killing of *Pseudomonas aeruginosa* cells comprises delivery of an agent lethal to said *Pseudomonas aeruginosa* cells.

5 8. The isolated human antibody or antigen-binding portion thereof according to claim 6, wherein said facilitating the killing of *Pseudomonas aeruginosa* cells comprises enhancing the immune response to *Pseudomonas aeruginosa*.

10 9. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody or antigen-binding portion thereof inhibits *Pseudomonas aeruginosa* infection.

15 10. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody or antigen-binding portion thereof binds to *Pseudomonas aeruginosa* LPS with a K_d of 5×10^{-7} M to 1×10^{-7} M.

20 11. The isolated human antibody or antigen-binding portion thereof according to claim 10, wherein said antibody or antigen-binding portion thereof binds to *Pseudomonas aeruginosa* LPS with a K_d of 1×10^{-7} to 5×10^{-8} M.

25 12. The isolated human antibody or antigen-binding portion thereof according to claim 10, wherein said antibody or antigen-binding portion thereof binds to *Pseudomonas aeruginosa* LPS with a K_d of 5×10^{-8} M to 1×10^{-9} M.

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13. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2 wherein the *Pseudomonas aeruginosa* LPS is derived from a *Pseudomonas aeruginosa* strain chosen from the list
5 consisting of 06ad, 011, Habs16, 170003 and PA01 Halloway.

14. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody or antigen-binding portion
10 thereof has a half-life *in vivo* of between one hour and thirty days

15. The isolated human antibody or antigen-binding portion thereof according to claim 14, wherein said antibody or antigen-binding portion thereof has a
15 half-life *in vivo* of between sixteen and thirty days.

16. The isolated human antibody or antigen-binding portion thereof according to claim 14, wherein said antibody or antigen-binding portion thereof has a half-life *in vivo* of between one hour and fifteen days.

20 17. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody is or is derived from an immunoglobulin molecule having a heavy chain isotype chosen from the list consisting of immunoglobulin G
25 (IgG), IgM, IgE, IgA and IgD.

18. The isolated human antibody according to claim 17, wherein said IgG is a subtype selected from the list consisting of IgG1, IgG2, IgG3 and IgG4.

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19. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody or portion thereof is labeled.

20. The isolated human antibody or antigen-binding portion thereof according to claim 19, wherein said label is selected from the list consisting of a radiolabel, an enzyme label, a fluorescent label, a toxin, a magnetic agent, a second antibody, an affinity label, an epitope tag, an antibiotic, a complement protein and a cytokine.

21. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, comprising a kappa light chain and framework sequences thereof.

22. The isolated human antibody or antigen-binding portion thereof according to claim 21, wherein said framework sequences of said kappa light chain are encoded by a human Vx2/A2 gene.

23. The isolated human antibody or antigen-binding portion thereof according to claim 21, wherein said kappa light chain comprises between seven and fifteen amino acid changes from a kappa light chain encoded by a germline Vx2/A2 gene.

24. The isolated human antibody or antigen-binding portion thereof according to claim 21, wherein said kappa light chain comprises no more than six amino acid changes from a kappa light chain encoded by a germline Vx2/A2 gene.

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25. The isolated human antibody or antigen-binding portion thereof according to claim 24, wherein said kappa light chain comprises no more than three amino acid changes from a kappa light chain encoded by a germline Vk2/A2 gene.

26. The isolated human antibody or antigen-binding portion thereof according to claim 21, wherein said kappa light chain comprises the amino acid sequence of SEQ ID NO: 4.

27. The isolated human antibody or antigen-binding portion thereof according to claim 21, wherein said light chain is encoded by the nucleic acid sequence of SEQ ID NO: 3.

28. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, comprising a lambda light chain.

29. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, comprising a heavy chain composed of variable (V), diversity (D), and Joining (J) regions and composed of framework sequences thereof.

30. The isolated human antibody or antigen-binding portion thereof according to claim 29, wherein said variable region of said heavy chain is encoded by a human V_H3/V3-33 gene.

31. The isolated human antibody or antigen-binding portion thereof according to claim 29, wherein said diversity region of said heavy chain is

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encoded by a human D2-8 gene.

32. The isolated human antibody or
antigen-binding portion thereof according to claim 29,
wherein said joining region of said heavy chain is
5 encoded by a human J_H4b gene.

33. The isolated human antibody or
antigen-binding portion thereof according to claim 29,
wherein said variable region comprises between seven
and fifteen amino acid changes from a variable region
10 encoded by a germline V_H3/V3-33 gene.

34. The isolated human antibody or
antigen-binding portion thereof according to claim 29,
wherein said heavy chain comprises no more than six
amino acid changes from a variable region encoded by a
15 germline V_H3/V3-33 gene.

35. The isolated human antibody or
antigen-binding portion thereof according to claim 29,
wherein said heavy chain comprises no more than three
amino acid changes from a variable region encoded by a
20 germline V_H3/V3-33 gene.

36. The isolated human antibody or
antigen-binding portion thereof according to claim 1 or
claim 2, wherein said heavy chain comprises the amino
acid sequence of SEQ ID NO: 2.

25 37. The isolated human antibody or
antigen-binding portion thereof according to claim 1 or
claim 2 wherein said heavy chain is encoded by the
nucleic acid sequence of SEQ ID NO: 1.

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38. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2 comprising an antigen binding domain chosen from the list consisting of an Fab fragment, an F(ab')₂ fragment
5 and an F_v fragment.

39. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody is a single chain antibody.

40. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody is a chimeric antibody.

41. The chimeric antibody according to claim 40, comprising framework regions and CDR regions from different human antibodies.

15 42. The chimeric antibody according to claim 40, wherein the chimeric antibody is bispecific.

43. The chimeric antibody according to claim 42, wherein the chimeric antibody is bispecific for *Pseudomonas aeruginosa* LPS and a label selected from
20 the list consisting of a radiolabeled molecule, an enzymatic label, a fluorescent label, a toxin, a magnetic agent, a second antibody, an affinity label, an epitope tag, an antibiotic, a complement protein and a cytokine.

25 44. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody or portion thereof is derivatized.

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45. The isolated human antibody or antigen-binding portion thereof according to claim 44, wherein said antibody or portion thereof is derivatized with polyethylene glycol, at least one methyl or ethyl group
5 or at least one carbohydrate moiety.

46. A pharmaceutical composition comprising the antibody or antigen-binding portion thereof according to claim 1 or claim 2 and a pharmaceutically acceptable carrier.

10 47. A kit comprising the antibody or antigen-binding portion thereof according to claim 1 or claim 2, a pharmaceutically acceptable carrier therefor, and a container.

48. The kit according to claim 47, further
15 comprising instructions for use.

49. A method for treating or preventing *Pseudomonas aeruginosa* infection, comprising the step of administering an isolated human antibody according to claim 1 to a patient at risk of being infected with,
20 or currently infected with, *Pseudomonas aeruginosa*.

50. The method according to claim 49 wherein said human antibody is a monoclonal antibody.

51. The method according to claim 49, wherein said administering is performed via an
25 injection, transmucosal, oral, inhalation, ocular, rectal, long acting implantation, liposomes, emulsion, cream, topical or sustained release means.

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52. The method according to claim 49,
wherein said antibody is a fusion with a second
protein.

53. The method according to claim 49,
5 wherein said antibody is labeled with a radiolabel, a
toxin, a complement protein, a cytokine or an
antibiotic.

54. The method according to claim 52,
wherein said second protein is chosen from the list
10 consisting of a toxic peptide moiety, a complement
protein, a radiolabeled protein, a cytokine or an
antibiotic protein.

55. The method according to claim 49 wherein
said patient is a burn patient, a surgical patient, a
15 prosthesis recipient, a respiratory patient, a cancer
patient, a cystic fibrosis patient or an
immunocompromised patient.

56. The method according to claim 49,
wherein said pharmaceutical composition further
20 comprises toxins, complement proteins, radiolabeled
proteins, cytokines, antibiotics, or any combination
thereof.

57. An isolated cell line that produces the
antibody according to claim 1 or claim 2.

25 58. The cell line according to claim 57
wherein said cell line is a hybridoma.

59. A method of producing an isolated human

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antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, comprising:

- a) culturing a non-human cell capable of producing said antibody under conditions in which the antibody is produced;
- b) isolating said antibody from said cell culture.

60. The method according to claim 59, wherein said cell is a hybridoma.

61. The method according to claim 59, wherein said cell is transformed with isolated nucleic acid molecules encoding said human antibody or antigen-binding portion thereof and said cell is chosen from the list consisting of a bacterial cell, a yeast cell, an insect cell, an amphibian cell and a mammalian cell.

62. The method according to claim 61, wherein said mammalian cell is selected from the list consisting of a human cell, a mouse cell, a rat cell, a dog cell, a monkey cell, a goat cell, a pig cell, a bovine cell and a hamster cell.

63. The method according to claim 61, wherein said mammalian cell is selected from the list consisting of a HeLa cell, a NIH 3T3 cell, a CHO cell, a BHK cell, a VERO cell, a CV-1 cell, a NS/O cell and a COS cell.

64. A method for making an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS,

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comprising:

- a) immunizing a non-human animal having incorporated a human immunoglobulin locus therein with a *Pseudomonas aeruginosa* antigenic composition;
- b) allowing said non-human animal to mount a humoral response to said antigenic composition; and
- c) isolating said human antibody from said non-human animal.

65. A nucleic acid molecule isolated from a non-human animal that encodes a human antibody heavy chain or the antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS.

66. The isolated nucleic acid molecule according to claim 65, wherein said nucleic acid molecule is isolated from a hybridoma that produces said human antibody.

67. An isolated nucleic acid molecule, or a fragment thereof, encoding a human antibody heavy chain or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS having the nucleotide sequence of SEQ ID: _.

68. The isolated nucleic acid molecule of claim 67 wherein said fragment comprises the sequence encoding between one to three of the CDR regions of said human antibody.

69. A vector comprising the nucleic acid

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molecule, or fragment thereof, according to any one of claims 65-68.

70. The vector according to claim 69,
wherein said vector further comprises expression
5 control sequences operably linked to said nucleic acid.

71. A nucleic acid molecule isolated from a
non-human animal that encodes a human antibody light
chain or the antigen-binding portion thereof that
specifically binds to *Pseudomonas aeruginosa* LPS.

10 72. The isolated nucleic acid molecule
according to claim 71, wherein said nucleic acid
molecule is isolated from a hybridoma that produces
said human antibody.

73. An isolated nucleic acid molecule, or a
15 fragment thereof, encoding a human antibody light chain
or antigen-binding portion thereof that specifically
binds to *Pseudomonas aeruginosa* LPS having the
nucleotide sequence of SEQ ID: 3.

74. The isolated nucleic acid molecule of
20 claim 71 wherein said fragment comprises the sequence
encoding between one to three of the CDR regions of
said human antibody.

75. A vector comprising the nucleic acid
molecule according to any one of claims 71-74.

25 76. The vector according to claim 75,
wherein said vector further comprises an expression
control sequence operably linked to said nucleic acid.

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77. An isolated host cell comprising
a) a nucleic acid molecule that was
isolated from a non-human animal and encodes a light
chain or the antigen-binding portion thereof of a human
5 antibody that specifically binds to *Pseudomonas*
aeruginosa LPS; or
b) a vector comprising said nucleic
acid molecule.
78. An isolated host cell comprising:
10 a) a nucleic acid molecule that was
isolated from a non-human animal and encodes a heavy
chain or the antigen-binding portion thereof of a human
antibody that specifically binds to *Pseudomonas*
aeruginosa LPS; or
15 b) a vector comprising said nucleic
acid molecule.
79. An isolated host cell comprising:
a) an nucleic acid molecule that was
isolated from a non-human animal and encodes a heavy
20 chain or the antigen-binding portion thereof and an
isolated nucleic acid molecule that encodes a light
chain or the antigen-binding portion thereof of a human
antibody that specifically binds to *Pseudomonas*
aeruginosa LPS or
25 b) a vector or vectors comprising said
nucleic acid molecules.
80. The isolated host cell of any one of
claims 77-79 wherein said cells are chosen from the
list consisting of hybridoma cells, bacterial cells,
30 yeast cells, insect cells, amphibian cells and

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mammalian cells.

81. The host cell according to claim 80, wherein said mammalian cell is selected from the list consisting of a human cell, a mouse cell, a rat cell, a
5 dog cell, a monkey cell, a goat cell, a pig cell, a bovine cell and a hamster cell.

82. The method according to claim 80, wherein said mammalian cells are selected from the list consisting of HeLa cells, NIH 3T3 cells, CHO cells, BHK
10 cells, VERO cells, CV-1 cells, NS/O cells and COS cells.

83. A method of recombinantly producing the heavy chain or the antigen-binding portion thereof, the light chain or the antigen-binding portion thereof, or
15 both the light chain and heavy chain or antigen-binding portions thereof, of a human antibody that was identified from a non-human animal and specifically binds to *Pseudomonas aeruginosa* LPS, comprising the step of cultivating the host cell according to any one
20 of claims 77-81 under conditions in which the nucleic acid molecules are expressed.

84. An isolated heavy chain or antigen-binding portion thereof obtained from the antibody according to claim 1 or claim 2, encoded by the nucleic
25 acid molecule according to claim 67, or isolated from the host cell according to claim 78.

85. An isolated light chain or antigen-binding portion thereof obtained from the antibody according to claim 1 or claim 2, encoded by the nucleic

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acid molecule according to claim 73, or isolated from the host cell according to claim 77.

86. A non-human transgenic animal comprising the nucleic acid molecule according to claim 67,
5 wherein said non-human transgenic animal expresses said nucleic acid molecule.

87. A non-human transgenic animal comprising the nucleic acid molecule according to claim 73,
wherein said non-human transgenic animal expresses said
10 nucleic acid molecule.

88. A non-human transgenic animal comprising an isolated nucleic acid molecule that encodes a heavy chain or the antigen-binding portion thereof and an isolated nucleic acid molecule that encodes a light
15 chain or the antigen-binding portion thereof of a human antibody that specifically binds to *Pseudomonas aeruginosa* LPS, wherein said animal expresses said nucleic acid molecules.

89. The non-human transgenic animal
20 according to any one of claims 86-88, wherein said animal is selected from the list consisting of a mouse, a rat, a hamster, a cow, a sheep, a primate, a horse and a pig.

90. The non-human transgenic animal
25 according to any one of claims 86-88, wherein a human antibody resulting from expression of said isolated nucleic acid molecules or portions thereof is expressed on the surface of cells derived from said animal's B lymphocytic cells or progeny thereof.

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91. The non-human transgenic animal according to any one of claims 86-88, wherein a human antibody resulting from expression of said isolated nucleic acid molecules or a portion thereof is secreted
5 into the lymph, blood, milk, saliva, or ascites of said animal.

92. A fusion protein comprising the isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2 and a second
10 polypeptide sequence.

93. The fusion protein of claim 92, wherein said second polypeptide sequence is chosen from the list consisting of an epitope tag, an affinity tag, a toxic polypeptide, an antibiotic, an enzyme, a second
15 antibody sequence, a complement protein, and a cytokine.

94. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein the heavy chain isotype of said antibody is
20 mu, gamma, delta, epsilon or alpha.

95. The isolated heavy chain or antigen-binding portion thereof according to claim 84 comprising between one to ten amino acid substitutions that increase the serum half-life of said antibody.

25 96. The isolated light chain or antigen-binding portion thereof according to claim 85 comprising between one to ten amino acid substitutions that increase the serum half-life of said antibody.

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97. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein the antibody or portion thereof is produced by a process comprising the steps of:

5 a) immunizing a non-human animal comprising a human immunoglobulin locus with an antigen selected from the group consisting of an isolated *Pseudomonas aeruginosa* LPS preparation, a virile *Pseudomonas aeruginosa* cell preparation, an attenuated

10 *Pseudomonas aeruginosa* cell preparation, and a killed *Pseudomonas aeruginosa* cell preparation;

 b) allowing said non-human animal to mount an immune response to said antigen; and

 c) isolating said antibody from said

15 non-human animal.

98. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody or portion thereof is isolated from an animal or cell that is free of contaminating

20 human biomaterials.

99. The isolated human antibody or antigen-binding portion thereof according to claim 99 wherein said biomaterials are chosen from the list consisting of viruses, enzymes, hormones, cytokines, receptors,

25 receptor ligands, immunoglobulins, complement, nuclear proteins, and cytoplasmic signaling proteins.

100. The isolated human antibody or antigen-binding portion thereof according to claim 100 wherein said viruses are Epstein-Barr virus or retroviruses.

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101. A hybridoma cell line that produces the S20 mAb, said hybridoma cell line having American Type Culture Collection Accession Number ____.

102. A monoclonal antibody produced by the
5 hybridoma cell line according to claim 101.

103. An isolated human antibody or an antigen-binding portion thereof, that specifically binds *Pseudomonas aeruginosa* LPS O-specific side chain.

104. The antibody antigen-binding portion
10 according to claim 103, wherein the *Pseudomonas aeruginosa* strain is selected from the group consisting of: 06ad, 011, Habs16, 17003 an PA01 Holloway.

105. The antibody antigen-binding portion according to claim 103 or 104, which is monoclonal.

15 106. The antibody or antigen-binding portion according to any one of claim 103-105, having a relative binding avidity of about 1.0.

107. The antibody or antigen-binding portion according to claim 104, that specifically binds the LPS
20 O-specific side chain of one strain and does not bind to the LPS O-specific side chain of the other four strains.

108. A human monoclonal antibody or antigen-binding portion thereof that specifically binds
25 *Pseudomonas aeruginosa* strain PA01 LPS O-specific side chain.

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109. The human monoclonal antibody or antigen-binding portion according to claim 108, which is an IgM antibody.

110. The human monoclonal antibody or
5 antigen-binding portion according to claim 108, that is produced by the hybridoma cell line having ATCC accession # _____ or ATCC accession # _____.

111. A human monoclonal antibody or antigen-binding portion thereof that specifically binds
10 *Pseudomonas aeruginosa* strain 170003 LPS O-specific side chain.

112. The human monoclonal antibody or antigen-binding portion according to claim 111, which is an IgM antibody.

15 113. The human monoclonal antibody or antigen-binding portion according to claim 111, that is produced by the hybridoma cell line having ATCC accession # _____.

114. A human monoclonal antibody or an
20 antigen-binding portion thereof, that inhibits the binding of an antibody according to any one of claim 108-113.

115. An antibody or antigen-binding portion thereof that specifically binds *Pseudomonas aeruginosa*
25 LPS O-specific side chain, wherein said antibody has comprises a heavy chain that includes one or more of CDR1, CDR2 and CDR3 shown in Figure 9.

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116. The antibody or antigen-binding portion according to claim 115, wherein said heavy chain comprises the CDR3 shown in Figure 9.

117. The antibody or antigen-binding portion
5 according to claim 115 comprising the amino acid sequence shown in SEQ ID NO: 2.

118. An antibody or antigen-binding portion thereof that specifically binds *Pseudomonas aeruginosa* LPS O-specific side chain, wherein said antibody has
10 comprises a light chain that includes one or more of CDR1, CDR2 and CDR3 shown in Figure 10.

119. The antibody or antigen-binding portion according to claim 118, wherein said heavy chain comprises the CDR3 shown in Figure 10.

120. The antibody or antigen-binding portion
15 according to claim 118 comprising the amino acid sequence shown in SEQ ID NO: 4.

121. A passive vaccine for preventing or inhibiting *Pseudomonas aeruginosa* infection comprising
20 one or more human monoclonal antibodies or an antigen-binding portion thereof, selected from the group consisting of the antibody or portion according to claim 1, claim 102, claim 103, and claims 115-120.

122. The passive vaccine according to claim
25 121 comprising two or more human monoclonal antibodies or an antigen-binding portion thereof, wherein said human monoclonal antibodies or portions specifically

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bind different strains of *Pseudomonas aeruginosa*.

123. A method for detecting the presence of
Pseudomonas aeruginosa in a biological sample
comprising the steps of contacting said sample with an
5 antibody or antigen-binding portion thereof according
to any one of claims 1, 102, 103 or 115-120.

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Figure 1: S20 Monoclonal Antibody Gene Usage

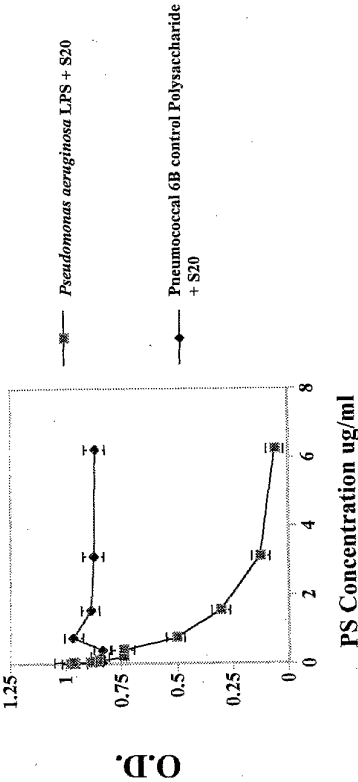
Heavy Chain Gene Segments		
<u>V_H</u>	<u>D</u>	<u>J_H</u>
V _H 3/V3-33	D2-8	J _H 4b
Light Chain Gene Segments		
<u>V_L</u>	<u>J_L</u>	
V _κ 2/A2	J _κ 1	

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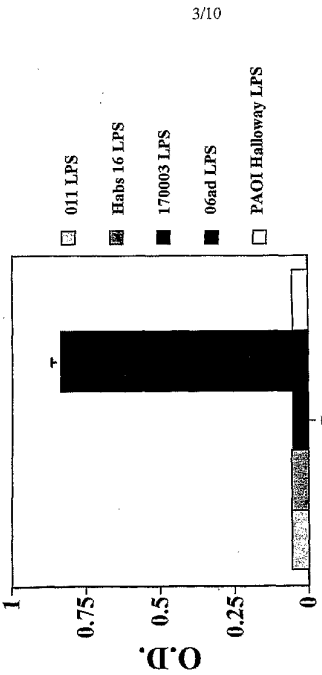
Figure 2: S20 Monoclonal Antibody Specifically Binds Solid Phase *Pseudomonas aeruginosa* LPS



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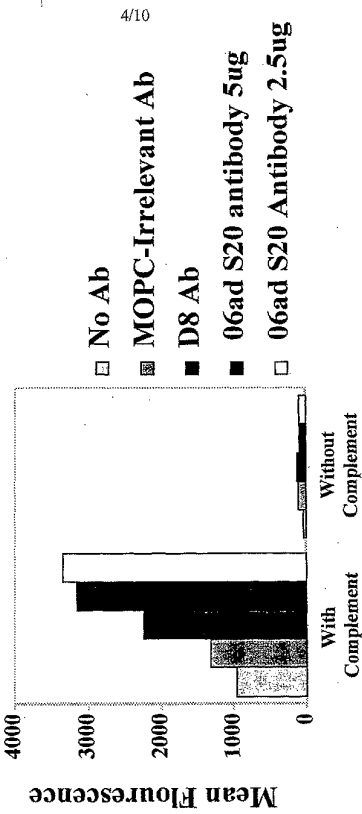
Figure 3: S20 Monoclonal Antibody Specifically Binds *Pseudomonas aeruginosa* 06ad LPS



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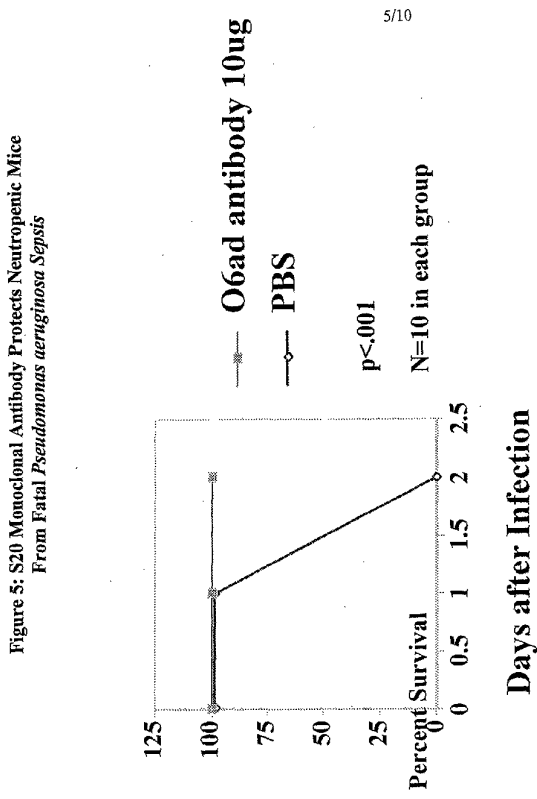
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Figure 4: S20 Monoclonal Antibody Opsonization Promotes Complement-Dependent Phagocytosis



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Heavy Chain Variable Region Sequence (SEQ ID:1)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTC
TCCTGTGCAGCGCTGGATTACCTTCAGTAGCTATGGCATCGACTGGGTCCGCCAGGCT
CCAGGCAAGGGGCTGGAGTGGGTCGGACGTATATGGTATAGTGGAGTAATAAATACTAT
GCAGACTCCGTGAAGGGCCGATTACCATCTCCAGAGACAATCCAAGAACACGCTGTAT
CTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATCCC
CACTATACCCGGGTTTGTACTTGGGGCAAGGGAACCTGGTCACCGTCTCCTCAGCC
TCCACCAAAGGGCCCATCGGTCTTCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGC
ACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCGCCGAA

Heavy Chain Variable Region Sequence (SEQ ID:2)

QVQLVESGGGVVQPRSLRLSCAASGFTFSSYGIDWVRQAPGKGLEWVGRWISGSNKYY
ADSVKGRFTISRDNKNTLYLQMNSLRADTAVYYCARDPHYTRGFDYWGKGLTVTVSSA
STKGPIGLPLAPCSRSTSESTAALGCLVKDYFAE

Light Chain Variable Region Sequence (SEQ ID:3)

GATGTTGTGATGACCCAGACTCCACTCTCTCTGTCCTCCGTACCCCTGGACAGCCGGCCTCC
ATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCATAGTGATGGAAAGACCTATTTTCTTGG
TATTTGCAGAGCCAGGCCAGCCTCCACAGCTCCTGATCTATGAAGTTTCCAACCGGTTT
TCTGGAGTGCCAGATAGGTTTCACTGGCAGCGGGTCAGGGACAGATTTCACTGAAATC
AGCCGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAAATATACAGCTTCG
TGGACGTTCCGCCAAGGACCAAGGTGGAAATCAAACGAACGTGGCTGCACCATCTGTG
TTCATCTTCCGCCATCTGAT

Light Chain Variable Region Sequence (SEQ ID:4)

DVVMQTPLSLVTPGQPASISCRSSQSLHSDGMTYFSWYLQKPGQPPQLLIYEVSNR
SGVPRDRFGSGGTDFTLKISRVEAEDVGVYYCMQNIQLPWTFGQGTKEIKRTVAAPSV
FIAPPSSD

FIGURE 6

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Inhibition of binding of PA01 IgM human monoclonal antibody with pure PA01 polysaccharide (PS)

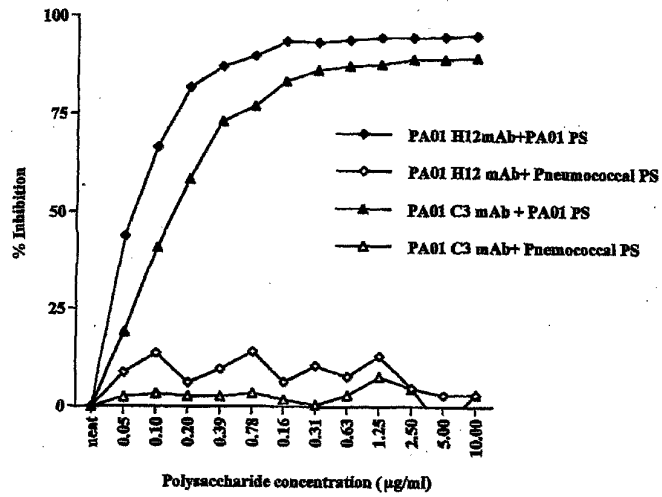


Figure 7

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Inhibition of binding of 170003 IgM human monoclonal antibody with pure 170003 polysaccharide (PS)

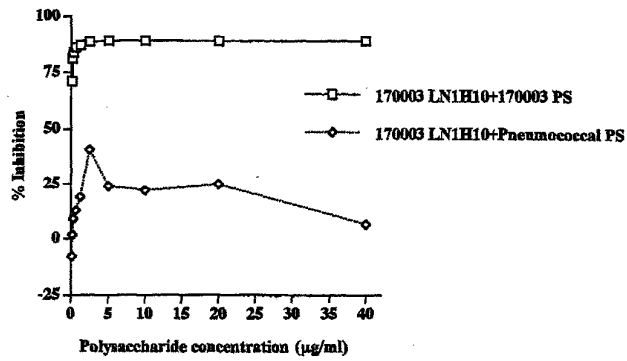


Figure 8

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Heavy Chain Variable Region Sequence**Framework 1**

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCC
 Q V Q L V E S G G G V V Q P G R S

CDR1

CTGAGACTCTCTGTGCAGCGTCTGGATTACCTTCAGTAGCTATGGCATG
 L R L S C A A S G F T F S S Y G M

Framework 2

CAGTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGCT
 H W V R Q A P G K G L E W V A A

CDR2

ATATGGTATAGTGGAACTAATAAATACTATGCAGACTCCGTGAAGGGCCGA
 I W Y S G S N K Y Y A D S V K G R

Framework 3

TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAAC
 F T I S R D N S K N T L Y L Q M N

CDR3

AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATCCCCAC
 S L R A E D T A V Y Y C A R D P H

Framework 4

TATACCCGGGGTTTGACTACTGGGGCAAGGGAACCCCTGGTCACCGTCTCC
 Y T R G F D Y W G K G T L V T V S

Constant

TCAGCCTCCACCAAAGGGCCCATCGGTCTTCCCTGGCGCCCTGCTCCAGG
 S A S T K G P I G L P L A P C S R

AGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC
 S T S E S T A A L G C L V K D Y F

GCCGAA
 A E

Figure 9

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Light Chain Variable Region Sequence

Framework 1
GATGTTGTGATGACCCAGACTCCACTCTCTCTGTCGGTCACCCCTGGACAG
D V V M T Q T P L S L S V T P G Q

CDR 1
CCGGCTCCATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCATAGTGATGGA
P A S I S C R S S Q S L L H S D G

Framework 2
ATGACCTATTTTCTTGGTATTTGCAGAAGCCAGGCCAGCCTCCACAGCTC
M T Y F S W Y L Q K P G Q P P Q L

CDR 2 Framework 3
CTGATCTATGAAGTTTCCAACCGGTTCTCTGGAGTGCCAGATAGGTTCACT
L I Y E V S N R F S G V P D R F S

GGCAGCGGGTCAGGGACAGATTTCACTGAAAATCAGCCGGTGGAG
G S G S G T D F T L K I S R V E

CDR 3
GCTGAGGATGTTGGGGTTTATTACTGCATGCAAAATATACAGCTTCCG
A E D V G V Y Y C M Q N I Q L P

Framework 4 Constant
TGGACGTTTCGGCCAAGGACCAAGGTGAAATCAAACGAACTGTGGCT
W T F G Q G T K V E I K R T V A

GCACCATCTGTCTTCATCTTCCCGCCATCTGAT
A P S V F I F P P S D

Figure 10

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[Continued on next page]

(54) Title: HUMAN ANTIBODIES AGAINST *PSEUDOMONAS AERUGINOSA* LPS DERIVED FROM TRANSGENIC XLINOMOUSE[®]

S20 Monoclonal Antibody Gene Usage

Heavy Chain Gene Segments

VHDJHV_H3/V3-33

D2-8

J_H4b

Light Chain Gene Segments

VLJLV_K2/A2J_K1

(57) Abstract: The invention described herein provides for human antibodies produced in non-human animals that specifically bind to *Pseudomonas aeruginosa* lipopolysaccharide (LPS). The invention further provides methods for making the antibodies in a non-human animal, expression of the antibodies in cell lines including hybridomas and recombinant host cell systems. Also provided are kits and pharmaceutical compositions comprising the antibodies and methods of treating or preventing pseudomonas infection by administering to patient the pharmaceutical compositions described herein.

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with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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15/85, A01K 67/027, C07K 19/00, G01N 33/577, 33/569Road, Gates Mills, OH 44040 (US). KAMBOJ, Kulwant,
Kauer [IN/US]; 906 Longridge Drive, Seven Hills, OH
44131 (US).

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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, PH, PL, PT, RO, RU, SD, SE, SG, SI,
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patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,

[Continued on next page]

(54) Title: HUMAN ANTIBODIES AGAINST *PSEUDOMONAS AERUGINOSA* LPS DERIVED FROM TRANSGENIC
XENOMOUSE®

S20 Monoclonal Antibody Gene Usage

Heavy Chain Gene Segments

<u>VH</u>	<u>D</u>	<u>JH</u>
V _H 3/V3-33	D2-8	J _H 4b

Light Chain Gene Segments

<u>VL</u>	<u>JL</u>
V _κ 2/A2	J _κ 1

(57) Abstract: The invention described herein provides for human antibodies produced in non-human animals that specifically bind to *Pseudomonas aeruginosa* Lipopolysaccharide (LPS). The invention further provides methods for making the antibodies in a non-human animal, expression of the antibodies in cell lines including hybridomas and recombinant host cell systems. Also provided are kits and pharmaceutical compositions comprising the antibodies and methods of treating or preventing pseudomonas infection by administering to patient the pharmaceutical compositions described herein.

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HUMAN ANTIBODIES AGAINST *PSEUDOMONAS AERUGINOSA*
LPS DERIVED FROM TRANSGENIC XENOMOUSE[®]

TECHNICAL FIELD OF THE INVENTION

The present invention relates to compositions and
5 methods for treating or preventing *Pseudomonas*
aeruginosa infection and conditions caused by such
infection. Specifically, the present invention relates
to human antibodies that specifically bind to
Pseudomonas aeruginosa Lipopolysaccharide (LPS) and
10 encoding nucleic acid molecules thereof. The invention
further relates to methods for making the antibodies in
a non-human animal and expressing the antibodies in
cell lines including hybridomas and recombinant host
cell systems. The invention also relates to kits and
15 pharmaceutical compositions comprising the antibodies.
The invention further relates to methods of treating or
preventing *Pseudomonas* infection by administering to a
patient any of the compositions described herein.

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BACKGROUND OF THE INVENTION*Pseudomonas aeruginosa* Infections

Pseudomonas aeruginosa are Gram-negative, flagellated rod bacteria that continue to be a significant pathogen in nosocomial infections resulting from surgery, prosthesis implantation and respiratory tract procedures. *Pseudomonas aeruginosa* also is an opportunistic pathogen in the etiology of cancer, cystic fibrosis, diabetes, heart disease, otitis externa (swimmer's ear), osteomyelitis, corneal ulcers, folliculitis, mastitis, pneumonia, meningitis, urinary tract infections, endocarditis, peritonitis and other diseases found in geriatric or immunocompromised patients.

Surgical patients are often at increased risk of *Pseudomonas aeruginosa* infection by virtue of their illness (e.g., trauma, burns, inhalation injury and cancer) or treatment (e.g., disruption of natural epithelial barriers by incision or percutaneous catheterization, endotracheal intubation, cardiac and thoracic surgery, neurosurgery, and gastrointestinal surgery). Disruption of natural intestinal flora by antibiotic treatments or prophylaxis, therapeutic immunosuppression of solid organ transplant recipients, or environmental exposure to *Pseudomonas aeruginosa* can place patients at increased risk. Moreover, multi-drug-resistant strains can cause significant infections in inpatient units as well as nursing homes.

Surgical patients are affected by nosocomial pneumonia, often caused by *Pseudomonas aeruginosa*. Onset occurs after the first 72 hours of hospitalization and is characterized by fever, purulent sputum, leukocytosis and a new or changed lung

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infiltrate revealed by chest radiography. The oropharynx is colonized rapidly, which may spread into the lower respiratory tract. Incidence of nosocomial infection in surgical patients overall is approximately 5% to 8%, and is probably higher in all critically ill patients. The incidence of pneumonia reported from surgical intensive care units (ICUs) is 15% to 20%, and occasionally higher. See Barie et al. *Am. J. Surgery* 179:2S-7S (2000).

10 Cystic fibrosis (CF) patients suffer chronic colonization with a narrow but evolving spectrum of bacterial pathogens *Pseudomonas aeruginosa* remains the major CF pathogen with a worldwide prevalence of up to 80% to 90% in CF adults. Such infections lead to 15 intermittent episodes of debilitating inflammatory exacerbations and progressive lung damage. Emerging pathogens also tend to be resistant to multiple antibiotic regimens, thus infection control plays a critical role in the quality of life and life 20 expectancy of CF patients.

The onset of chronic colonization is associated with acceleration of forced expiratory volume (FEV). The original colonizing strain transforms into a mucoid colonial form which is due to copious production of a 25 highly viscid exopolysaccharide known as alginate. The colonizing strain becomes significantly more mucinophilic and chemotactic and is associated with impaired mucociliary clearance. See Govan J. *Royal Soc. Med.* 93 Supp. 38:40-45 (2000). Moreover, the 30 *Pseudomonas aeruginosa* isolated from lungs of CF patients show changes in the LPS fatty acid acylation pattern and enhanced resistance to the bactericidal

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activity of some cationic antimicrobial peptides (CAMPs).

Alterations in *Pseudomonas aeruginosa* LPS lipid A were found in CF isolates that increased both bacterial resistance to antimicrobial peptides and the ability of LPS to elicit inflammatory mediators. CF patients have very high antibody titers to *Pseudomonas aeruginosa* LPS in both serum and sputum, which might neutralize its biological activities in vivo (e.g. proinflammatory mediator release). See Pier Trends Microbiol. 8:247-251 (2000).

The leading cause of morbidity and mortality in severe burn wounds patients is infection with *Pseudomonas aeruginosa*, See Lee et al. Vaccine 18:1952-1961 (2000). Burn wounds are highly exudative, creating a moist, nutrient-rich environment for bacterial colonization. Burn wounds are largely inaccessible to the patient's immune responses and vascularly-delivered antibiotics due to the severe tissue injury. Moreover, burn wounds leave the host immunocompromised with endogenously decreased levels of immunoglobulin gamma (IgG). Without treatment, burn wound infections can spread and develop into sepsis with the associated production of inflammatory cytokines, including interleukin-1 (IL-1), IL-6, and tumor necrosis factors (TNFs). Burn wound infections may also result in delayed healing, increased scarring, conversion of a partial thickness defect to a full thickness defect and increased nutritional demands.

Intravenous immunoglobulin (IVIG) has been used increasingly to treat both bacterial and viral infections and primary and secondary immunodeficiency disorders. IVIG is comprised of pooled human

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polyclonal antibodies from normal donors which are used as a substitution therapy for primary and secondary antibody deficiencies and to treat immune-mediated diseases, including autoimmune and systemic inflammatory conditions. Immunoglobulins promote the opsonization and phagocytosis of bacteria, neutralization of bacterial toxins, inhibition of microbial attachment, and the complement-induced lysis of bacteria. See Felts et al. *Burns* 25:415-423 (1999).

10 Direct and local delivery of protective immunoglobulins to wound and burn sites represents a rational means to overcome the lack of vascularization of burn wounds as well as biofilm barriers. Local delivery of IgG, both prophylactically and post-infection, was demonstrated to improve survival in mouse models of *Pseudomonas aeruginosa* infected burn wounds. See Felts et al. *Burns* 25:415-423 (1999).

Advances in the bioengineering of prosthetic devices has improved the lives of millions of patients. However, this progress has been tempered by implant-associated infections that often resist antibiotic treatment. Infectious organisms, including *Pseudomonas aeruginosa*, preferentially target synthetic implanted materials, eliciting serious and costly infections that frequently require removal of the colonized device.

Initial microbial adhesion is a primary determinant of biomaterial colonization because initially adhering microorganisms often progress to a mature biofilm attached to the biomaterial surface. The focus of research aimed at reducing biofilm formation on prostheses has been directed toward modifying or coating the surface of the implanted materials. Approaches utilizing surface chemistry and

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antibiotic-releasing coatings, however, have not been fully successful.

Because surgical sites are often immunocompromised, a promising approach involves the immunostimulation of the local wound site. Studies have shown that pooled polyclonal human antibodies opsonize infecting bacteria, and pooled antibodies can inhibit *Pseudomonas aeruginosa* adhesion rates and surface-growth dynamics, thus reducing biofilm formation. See Poelstra et al. *J. Biomed. Mat. Res.* 51:224-232 (2000).

Peritonitis is often caused by ulcers, appendicitis, diverticulitis, ileus, gunshot or stab wounds, disturbances during abdominal surgery, and continuous ambulatory peritoneal dialysis (CAPD). Nosocomial peritonitis, caused by exogenous pathogenic bacteria including *Pseudomonas aeruginosa*, is an especially acute problem for immunocompromised and geriatric populations.

Current treatment regimens for peritonitis focus on antibiotics, however, antibiotic resistance occurs at a significant rate and is frequently associated with clinical failure. IVIG has shown promising but inconsistent results in peritonitis, however, as with burn wounds, local (peritoneal) delivery of pooled polyclonal immunoglobulin against *Pseudomonas aeruginosa* was shown to significantly reduce infection in a mouse model. See Barekzi et al. *Antimicrob. Agents Chemotherap.* 43:1609-1615 (1999).

Treating *Pseudomonas aeruginosa* infections with antibiotic regimens has become increasingly difficult because, *inter alia*, antibiotic resistant strains have arisen. The emergence of passive antibody therapy for

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the prevention and treatment of *Pseudomonas aeruginosa* infections, though promising, has been tempered by the availability purified human antibodies, free of non-human animal antibodies, that bind specifically to

5 *Pseudomonas aeruginosa* in clinical quantities.

Non-human antibody preparations, including murine monoclonal antibodies, are not generally acceptable for human therapies because of their immunogenicity. Human polyclonal antibody preparations, although suitable for

10 human therapies, have variable titers of protective antibodies for *Pseudomonas aeruginosa* and a high cost of purifying antibodies from multiple donors.

Monoclonal antibodies theoretically can be made in unlimited quantity, at a low cost and with a desired

15 specificity. However, efficacious human monoclonal antibodies are difficult to make and require human B cells expressing appropriate antibodies to be transformed with Epstein-Barr virus. The resulting monoclonal antibody preparations would not likely be

20 appropriate for human therapeutic use. Moreover, most of the human monoclonal antibodies tested to date have been IgM which penetrate poorly into pulmonary tissue and can be associated with immune complex formation and enhanced inflammation.

25 Therefore, there is a need for purified human IgG antibodies that bind specifically to *Pseudomonas aeruginosa*, methods for its preparation and use, and pharmaceutical compositions and kits thereof.

SUMMARY OF THE INVENTION

30 The present invention provides isolated human antibodies that specifically bind to *Pseudomonas aeruginosa* Lipopolysaccharide (LPS). The invention

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further provides methods for making the antibodies in a non-human animal, expression of the antibodies in cell lines including hybridomas and recombinant host cell systems. The invention also provides kits and pharmaceutical compositions comprising the antibodies. Moreover, the invention provides methods of treating or preventing pseudomonas infection by administering to a patient the pharmaceutical compositions described herein.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the human immunoglobulin variable (V), diversity (D) and joining (J) regions utilized by the hybridoma that produces the S20 monoclonal anti-*Pseudomonas aeruginosa* antibody.

15 Figure 2 depicts binding inhibition assays that show the S20 human monoclonal antibody binding with specificity to the *Pseudomonas aeruginosa* LPS. Soluble *Pseudomonas aeruginosa* LPS, but not soluble *Pneumococcal* 6B control polysaccharide, was able to
20 inhibit S20 binding to solid phase *Pseudomonas aeruginosa* LPS.

Figure 3 shows that the S20 monoclonal antibody specifically binds the *Pseudomonas aeruginosa* 06ad serotype LPS but does not bind solid phase LPS derived
25 from the 011, Habs 16, 170003 and PAOI Halloway strains.

Figure 4 shows that S20 opsonization promotes complement-dependent phagocytosis. Flow cytometry analysis of peripheral nuclear monocytes (PMNs) showed
30 that the PMNs phagocytosed FITC labeled, opsonized *Pseudomonas aeruginosa* only in the presence of complement.

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Figure 5 shows that the S20 monoclonal antibody protected neutropenic mice from fatal *Pseudomonas aeruginosa* sepsis.

Figure 6 sets forth the DNA and amino acid sequences of the heavy chain and light chain variable regions of human monoclonal antibody S20 (IgG).

Figure 7 depicts binding inhibition assays that show the H12 and C3 human monoclonal antibodies binding with specificity to the *Pseudomonas aeruginosa* LPS. Soluble *Pseudomonas aeruginosa* PA01 LPS, but not soluble *Pneumococcal* 6B control polysaccharide, was able to inhibit binding to solid phase *Pseudomonas aeruginosa* PA01 LPS.

Figure 8 depicts binding inhibition assays that show the LN1H10 human monoclonal antibody binding with specificity to the *Pseudomonas aeruginosa* LPS. Soluble *Pseudomonas aeruginosa* 170003 LPS, but not soluble *Pneumococcal* 6B control polysaccharide, was able to inhibit binding to solid phase *Pseudomonas aeruginosa* 170003 LPS.

Figure 9 indicates the location of CDRs 1-3 of the heavy chain variable region of the S20 human monoclonal antibody.

Figure 10 indicates the location of CDRs 1-3 of the kappa light chain variable region of the S20 human monoclonal antibody.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided fully human isolated antibodies or antigen-binding portions thereof that specifically binds to *Pseudomonas aeruginosa* LPS. In a preferred embodiment, the fully human antibodies are monoclonal.

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Other preferred embodiments include nucleotide sequences encoding and amino acid sequences comprising the antibodies' heavy and light chains, and in particular sequences corresponding to a contiguous heavy and light chain sequences from CDR1 through CDR3. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein. Hybridomas expressing such immunoglobulin molecules and monoclonal antibodies are also provided.

The terms herein generally have their usual meaning as understood by those of ordinary skill in the art. The following terms are intended to have the following general meanings as they are used herein:

"B lymphocytic cells or progeny thereof" refer to any cell descending from, or destined for, the B lymphocytic lineage. Examples include, but are not limited to, all B lymphocytes in the B cell developmental pathway starting from the earliest B lymphocyte stem cells through memory B cells, plasma cells, and any hybridomas created *in vitro*.

"Bispecific antibodies" are single antibodies that have affinities for two separate antigens. For example, a bispecific antibody might recognize *Pseudomonas aeruginosa* LPS using one combination of heavy and light chains and might recognize a leukocyte cell surface marker using a second combination of heavy and light chains attached to the first combination. See McCormick et al. *J. Immunol.* **158**:3474-3482 (1997).

"Chimeric antibodies" are antibodies that have been altered from their original form to comprise amino acid sequences from another protein. Chimeric antibodies retain at least a portion of the original

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antibody amino acid sequence, typically the portion comprising the antigen binding region (F_{ab}). Examples of chimeric antibodies include, but are not limited to, bispecific antibodies and fusions with other non-immunoglobulin protein sequences.

"Cytokines" refer generally to signaling molecules of the immune system. Cytokines include, but are not limited to, Interleukins (IL), transforming growth factors (TGF), tumor necrosis factors (TNF), lymphotoxins (LT), interferons, granulocyte-macrophage colony stimulating factors (GM-CSF), macrophage CSF, Granulocyte CSF, and migration inhibition factors.

"Derivatize" refers to the process of attaching a non-immunoglobulin agent to the immunoglobulin molecules. Examples of derivatizing agents include, but are not limited to, toxins, complement, antibiotics, peptides, polysaccharides, lipids, organic polymers, radiolabels, and inorganic compounds.

"Expression control sequences" refer to sequences that allow for the inducible or constitutive expression of gene sequences under specific conditions or in specific cells. Examples of cellular processes that expression control sequence regulate include, but are not limited to, gene transcription, protein translation, messenger RNA splicing, immunoglobulin isotype switching, protein glycosylation, protein cleavage, protein secretion, intracellular protein localization and extracellular protein homing.

"Fusion Proteins" refer to chimeric proteins comprising amino acid sequences of two or more different proteins. Typically, fusion proteins result from *in vitro* recombinatory techniques well known in

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the art. However, fusion proteins may result from *in vivo* crossover or other recombinatory events.

"Human immunoglobulin molecules" refer to immunoglobulin proteins that are encoded by human immunoglobulin gene sequences. The immunoglobulin gene sequences may be expressed in any non-human animal.

"Human monoclonal antibodies" refer to antibodies that are members of a population of human antibodies with identical specificities. The population of human antibodies may be produced in a hybridoma or other immortalized cell line as well as in recombinant cell lines expressing the exogenous human antibody gene sequences.

"Immunocompromised patients" refer to patients whose immune responses to foreign antigens or agents is impaired either by disease (e.g. AIDS), by invasive surgery, or by drug therapies in connection with treatments for other conditions (e.g. organ transplant patients).

"Label" refers to any molecule that attaches to the claimed immunoglobulin a functional characteristic not normally associated with that immunoglobulin. Labels can be attached via chemical modification of the immunoglobulin, recognition of the label by one of the two F_{ab} regions of a bispecific immunoglobulin, affinity for a third agent (e.g. the avidin/biotin interaction), radiolabeling, or as a fusion protein expressed recombinantly. Labels can function as molecular or radioactive tags for clinical or research purposes or as agents for combating *Pseudomonas aeruginosa* infection (e.g. toxins or complement proteins). Other examples of labels can include enzymes, fluorescent molecules, magnetic labels, epitope tags (e.g. H.

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influenza hemagglutinin), antibiotics, complement proteins, and cytokines.

"Respiratory patients" refer to any patient that is either being treated for a disease of the respiratory system or is receiving respiratory care, e.g. intubation or ventilation, in connection with some other medical treatment.

"Surgical patients" refer to any patient that is subject to an invasive surgical procedure, typically involving puncturing or incising the dermis.

"Toxins" refer to protein or non-protein compounds that can be attached to antibodies for the purpose of killing the cells to which the antibodies have attached. Examples of toxins include, but are not limited to, complement, antibiotics, peptides, polysaccharides, lipids, organic polymers, radiolabels, and inorganic compounds.

"Vectors" refer to DNA molecules that allow DNA sequences of interest to be cloned, propagated, recombined, mutated, or expressed outside of their native cells. Often vectors have expression control sequences that allow for the inducible or constitutive expression of gene sequences under specific conditions or in specific cells. Examples of vectors include, but are not limited to, plasmids, yeast artificial chromosomes (YACs), viruses, bacteriophages, and phagemids.

"XenoMouse[™]" refers to mice bearing homologously targeted endogenous immunoglobulin loci, rendering them incapable of expressing endogenous murine immunoglobulin, but bearing substantial portions of human immunoglobulin loci. Mice of the XenoMouse[™] line are capable of somatic rearrangement of the human immunoglobulin genes, hypermutation of the human

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variable genes, and immunoglobulin isotype switching. Therefore, the mice of the XenoMouse™ line are capable of mounting effective humoral responses to antigenic challenge utilizing the human immunoglobulin gene sequences. The resulting antibodies are fully human and can be isolated from the animals themselves, from cultured cells extracted from the animals, and from hybridomas created from XenoMouse™ B lymphocytic lines or progeny thereof. Moreover, the rearranged human gene sequences encoding immunoglobulins raised against specific antigenic challenges can be isolated by recombinant means well known in the art.

Antibody Structure

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, *Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety

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for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site.

Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific

5 antibodies, the two binding sites are the same.

The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs

10 from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to
15 each domain is in accordance with the definitions of Kabat *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk *J. Mol. Biol.* **196**:901-917 (1987); Chothia et al. *Nature* **342**:878-883 (1989).

20 A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or
25 linking of Fab' fragments. See, e.g., Songsivilai & Lachmann *Clin. Exp. Immunol.* **79**:315-321 (1990), Kostelny et al. *J. Immunol.* **148**:1547-1553 (1992). In addition, bispecific antibodies may be formed as "diabodies" (Holliger et al. "Diabodies": small
30 bivalent and bispecific antibody fragments" *PNAS USA* **90**:6444-6448 (1993)) or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J*

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10:3655-3659 (1991) and Traunecker et al. "Janusin: new
molecular design for bispecific reagents" *Int J Cancer*
Suppl 7:51-52 (1992)). Production of bispecific
antibodies can be a relatively labor intensive process
5 compared with production of conventional antibodies and
yields and degree of purity are generally lower for
bispecific antibodies. Bispecific antibodies do not
exist in the form of fragments having a single binding
site (e.g., Fab, Fab', and Fv).

10 Human Antibodies from Non-human Animals

Human antibodies avoid certain of the problems
associated with antibodies that possess murine or rat
variable and/or constant regions. The presence of such
murine or rat derived proteins can lead to the rapid
15 clearance of the antibodies or can lead to the
generation of an immune response against the antibody
by a patient. In order to avoid the utilization of
murine or rat derived antibodies, it has been
postulated that one can develop humanized antibodies or
20 generate fully human antibodies through the
introduction of human antibody function into a rodent
so that the rodent would produce fully human
antibodies.

The ability to clone and reconstruct
25 megabase-sized human loci in YACs and to introduce them
into the mouse germline provides a powerful approach to
elucidating the functional components of very large or
crudely mapped loci as well as generating useful models
of human disease. Furthermore, the utilization of such
30 technology for substitution of mouse loci with their
human equivalents could provide unique insights into
the expression and regulation of human gene products

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during development, their communication with other systems, and their involvement in disease induction and progression.

An important practical application of such a strategy is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated offers the opportunity to study the mechanisms underlying programmed expression and assembly of antibodies as well as their role in B-cell development. Furthermore, such a strategy could provide an ideal source for production of fully human monoclonal antibodies (Mabs) - an important milestone towards fulfilling the promise of antibody therapy in human disease. Fully human antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized Mabs and thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can be expected to provide a substantial advantage in the treatment of chronic and recurring human diseases, such as inflammation, autoimmunity, cancer and bacterial infections, which potentially require repeated antibody administrations.

One approach towards this goal was to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce a large repertoire of human antibodies in the absence of mouse antibodies. Large human Ig fragments would preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of

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immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains should yield high affinity antibodies against any antigen of interest, including human antigens.

- 5 Using the hybridoma technology, antigen-specific human Mabs with the desired specificity could be readily produced and selected.

This general strategy was demonstrated in connection with the generation of the first XenoMouse™ strains as published in 1994. See Green et al. *Nature Genetics* 7:13-21 (1994). The XenoMouse™ strains were engineered with yeast artificial chromosomes (YACs) containing 245 kb and 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus, respectively, which contained core variable and constant region sequences. *Id.* The human Ig containing YACs proved to be compatible with the mouse system for both rearrangement and expression of antibodies and were capable of substituting for the inactivated mouse Ig genes. This was demonstrated by their ability to induce B-cell development, to produce an adult-like human repertoire of fully human antibodies, and to generate antigen-specific human Mabs. These results also suggested that introduction of larger portions of the human Ig loci containing greater numbers of V genes, additional regulatory elements, and human Ig constant regions might recapitulate substantially the full repertoire that is characteristic of the human humoral response to infection and immunization. The work of Green et al. was recently extended to the introduction of greater than approximately 80% of the human antibody repertoire through introduction of megabase sized,

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germline configuration YAC fragments of the human heavy chain loci and kappa light chain loci, respectively, to produce XenoMouse™ mice. See Mendez et al. *Nature Genetics* 15:146-156 (1997), Green and Jakobovits *J.*

- 5 *Exp. Med.* 188:483-495 (1998), and U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996, the disclosures of which are hereby incorporated by reference.

- Such approach is further discussed and delineated
- 10 in U.S. Patent Application Serial Nos. 07/466,008, filed January 12, 1990, 07/610,515, filed November 8, 1990, 07/919,297, filed July 24, 1992, 07/922,649, filed July 30, 1992, filed 08/031,801, filed March 15, 1993, 08/112,848, filed August 27, 1993,
- 15 08/234,145, filed April 28, 1994, 08/376,279, filed January 20, 1995, 08/430, 938, April 27, 1995, 08/464,584, filed June 5, 1995, 08/464,582, filed June 5, 1995, 08/463,191, filed June 5, 1995, 08/462,837, filed June 5, 1995, 08/486,853, filed June 5, 1995,
- 20 08/486,857, filed June 5, 1995, 08/486,859, filed June 5, 1995, 08/462,513, filed June 5, 1995, 08/724,752, filed October 2, 1996, and 08/759,620, filed December 3, 1996. See also Mendez et al. *Nature Genetics* 15:146-156 (1997) and Green and Jakobovits *J.*
- 25 *Exp. Med.* 188:483-495 (1998). See also European Patent No. EP 0 463 151 B1, grant published June 12, 1996, International Patent Application No. WO 94/02602, published February 3, 1994, International Patent Application No. WO 96/34096, published October 31,
- 30 1996, and WO 98/24893, published June 11, 1998. The disclosures of each of the above-cited patents, applications, and references are hereby incorporated by reference in their entirety.

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Antibodies in accordance with the present invention are preferably prepared through the utilization of a transgenic mouse that has a substantial portion of the human antibody producing genome inserted but that is rendered deficient in the production of endogenous, murine antibodies. Such mice, then, are capable of producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed herein.

Through use of such technology, fully human monoclonal antibodies, or the antigen binding portions thereof, to *Pseudomonas aeruginosa* LPS were produced. Essentially, we immunized XenoMouse™ lines of mice with heat killed *Pseudomonas aeruginosa*, recovered spleen and lymph node cells (such as B-cells) from the mice that express *Pseudomonas aeruginosa* LPS antibodies, fused such recovered cells with nonsecreting myeloma cells to prepare immortal hybridoma cell lines, and screened hybridoma cell lines to identify those that produce antibodies specific to the antigen of interest.

As will be appreciated, antibodies in accordance with the present invention can be expressed in cell lines other than hybridoma cell lines. Sequences encoding particular antibodies can be used for transformation of a suitable host cell. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures

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known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference). The transformation procedure used depends upon the host
5 to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion,
10 electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are well known in the art and include many
15 immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NS/O, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma
20 cells (e.g., Hep G2), and a number of other cell lines. Cell lines of particular preference are selected through determining which cell lines have high expression levels and produce antibodies with constitutive *Pseudomonas aeruginosa* LPS binding
25 properties.

Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, enhanced expression can be
30 realized by the coamplification expression system utilizing dihydrofolate reductase (DHFR) or the glutamine synthetase gene expression system (the GS system). See, e.g. Kaufman and Sharp *J. Mol. Biol.*

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159:601-621 (1982); European Patent Nos. 0 216 846, 0 256 055, and 0 323 997; and European Patent Application No. 89303964.4.

Antibodies of the invention can also be produced
5 through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. In connection with the transgenic production in mammals, antibodies can be
10 produced in, and recovered from, the milk of goats, cows, or other mammals. See, e.g., U.S. Patent Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that was
15 expressed in a non-human animal and specifically binds to *Pseudomonas aeruginosa* LPS. In a preferred embodiment, the isolated human antibody or antigen-binding portion thereof is a monoclonal antibody.

The invention further contemplates the isolated
20 human antibody or antigen-binding portion thereof that is opsonic for *Pseudomonas aeruginosa* cells. In a preferred embodiment, the isolated human antibody or antigen-binding portion thereof facilitates phagocytosis of the *Pseudomonas aeruginosa* cells.

25 The invention also contemplates that the isolated human antibody or antigen-binding portion thereof enhances the immune response to *Pseudomonas aeruginosa*. In a preferred embodiment, the isolated human antibody or antigen-binding portion thereof facilitates the
30 killing of *Pseudomonas aeruginosa* cells. In a more preferred embodiment, the isolated human antibody or antigen-binding portion thereof facilitates the killing

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of *Pseudomonas aeruginosa* cells by delivering an agent that is lethal to the *Pseudomonas aeruginosa* cells.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that
5 specifically binds to *Pseudomonas aeruginosa* LPS, wherein the antibody or antigen-binding portion thereof inhibits *Pseudomonas aeruginosa* infection.

The invention also contemplates an isolated human antibody or antigen-binding portion thereof that
10 specifically binds to *Pseudomonas aeruginosa* LPS, wherein the antibody or antigen-binding portion thereof binds to *Pseudomonas aeruginosa* LPS with a dissociation constant (K_d) of 5×10^{-7} M or less, preferably 5×10^{-7} M to 1×10^{-7} M. In a more preferred embodiment, the
15 antibody or antigen-binding portion thereof binds to *Pseudomonas aeruginosa* LPS with a K_d of 1×10^{-7} M to 5×10^{-8} M. In a more preferred embodiment, the antibody or antigen-binding portion thereof binds to *Pseudomonas aeruginosa* LPS with a K_d of 5×10^{-8} M to 1×10^{-8} M.

20 The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and has a half-life *in vivo* of one hour or more. In a preferred embodiment, the antibody or antigen-binding
25 portion thereof has a half-life *in vivo* of between one hour and thirty days. In a more preferred embodiment, the antibody or antigen-binding portion thereof has a half-life *in vivo* of between sixteen and thirty days. In another more preferred embodiment, the antibody or
30 antigen-binding portion thereof has a half-life *in vivo* of between one hour and fifteen days.

The isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas*

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aeruginosa LPS of the invention may be immunoglobulin G (IgG), IgM, IgE, IgA and IgD. In a preferred embodiment, the IgG may be an IgG1, IgG2, IgG3 or IgG4 subtype.

5 The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and is labeled. In a preferred embodiment, the label is a radiolabel, an enzyme label, a fluorescent label, a
10 toxin, a magnetic agent, a second antibody, an affinity label, an epitope tag, an antibiotic, a complement protein or a cytokine.

 The invention contemplates an isolated human antibody or antigen-binding portion thereof that
15 specifically binds to *Pseudomonas aeruginosa* LPS and comprises a kappa light chain and framework sequences thereof. In a preferred embodiment, the framework sequences of the kappa light chain are encoded by a human Vk2/A2 gene. In a preferred embodiment, the
20 kappa light chain comprises between seven and fifteen changes from a kappa light chain encoded by a germline Vk2/A2 gene. In a more preferred embodiment, the kappa light chain comprises no more than six amino acid changes from a kappa light chain encoded by a germline
25 Vk2/A2 gene. In a more preferred embodiment, the kappa light chain comprises no more than three amino acid changes from a kappa light chain encoded by a germline Vk2/A2 gene.

 The invention contemplates an isolated human
30 antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and comprises a kappa light chain having the amino acid sequence of SEQ ID NO: 4. The invention further

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contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and comprises a kappa light chain that is encoded by the nucleic acid sequence of SEQ ID NO: 3. The invention also contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and comprises a lambda light chain.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, comprising a heavy chain composed of variable (V), diversity (D), and Joining (J) regions and composed of framework sequences thereof. In a preferred embodiment, the variable region of the heavy chain is encoded by a human V_H3/V3-33 gene. In another preferred embodiment, the diversity region of the heavy chain is encoded by a human D2-8 gene. In another preferred embodiment, the joining region of the heavy chain is encoded by a human J_H4b gene. In a more preferred embodiment, the variable region comprises between seven and fifteen amino acid changes from a variable region encoded by a germline V_H3/V3-33 gene. In a more preferred embodiment, the heavy chain comprises no more than six amino acid changes from a variable region encoded by a germline V_H3/V3-33 gene. In a more preferred embodiment, the heavy chain comprises no more than three amino acid changes from a variable region encoded by a germline V_H3/V3-33 gene.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and

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comprises a heavy chain having the amino acid sequence of SEQ ID NO: 2.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that
5 specifically binds to *Pseudomonas aeruginosa* LPS and is encoded by the nucleic acid sequence of SEQ ID NO: 1,.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and
10 comprises an antigen binding domain chosen from the list consisting of an Fab fragment, an F(ab')₂ fragment and an F_v fragment.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that
15 specifically binds to *Pseudomonas aeruginosa* LPS and the antibody is a single chain antibody.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and
20 the antibody is a chimeric antibody. In a preferred embodiment, the chimeric antibody comprises framework regions and CDR regions from different human antibodies. In a more preferred embodiment, the chimeric antibody is bispecific. In a more preferred
25 embodiment, the chimeric antibody is bispecific for *Pseudomonas aeruginosa* LPS and a label selected from the list consisting of a radiolabeled molecule, an enzymatic label, a fluorescent label, a toxin, a magnetic agent, a second antibody, an affinity label,
30 an epitope tag, an antibiotic, a complement protein and a cytokine.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that

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specifically binds to *Pseudomonas aeruginosa* LPS and the antibody or portion thereof is derivatized. In a preferred embodiment, the antibody or portion thereof is derivatized with polyethylene glycol, at least one
5 methyl or ethyl group or at least one carbohydrate moiety.

The invention contemplates a pharmaceutical composition comprising the an isolated human antibody or antigen-binding portion thereof that specifically
10 binds to *Pseudomonas aeruginosa* LPS and a pharmaceutically acceptable carrier. The invention further contemplates a kit comprising the antibody or antigen-binding portion thereof, a pharmaceutically acceptable carrier therefor, and a container. In a
15 preferred embodiment, the kit further comprising instructions for use.

The invention contemplates a method for treating or preventing *Pseudomonas aeruginosa* infection, comprising the step of administering a pharmaceutical
20 composition to a patient at risk of being infected with, or currently infected with, *Pseudomonas aeruginosa*.

In a preferred embodiment, the human antibody is a monoclonal antibody. In another preferred embodiment,
25 the pharmaceutical composition is administered via an injection, transmucosal, oral, inhalation, ocular, rectal, long acting implantation, liposomes, emulsion, cream, topical or sustained release means. In another preferred embodiment, the antibody is a fusion with a
30 second protein. In a more preferred embodiment the second protein is chosen from the list consisting of a toxic peptide moiety, a complement protein, a radiolabeled protein, a cytokine or an antibiotic

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protein. In another preferred embodiment, the antibody is labeled with a radiolabel, a toxin, a complement protein, a cytokine or an antibiotic. In another preferred embodiment, the patient is a burn patient, a surgical patient, a prosthesis recipient, a respiratory patient, a cancer patient, a cystic fibrosis patient or an immunocompromised patient. In another preferred embodiment, the pharmaceutical composition further comprises toxins, complement proteins, radiolabeled proteins, cytokines, antibiotics, or any combination thereof.

The invention contemplates an isolated cell line that produces a human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS. In a preferred embodiment, the cell line is a hybridoma.

The invention contemplates a method of producing an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, comprising:

- a) culturing a non-human cell capable of producing the antibody under conditions in which the antibody is produced;
- b) isolating the antibody from the cell culture.

In a preferred embodiment, the method of producing an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS utilizes a hybridoma. In another preferred embodiment, the method utilizes a cell that is transformed with isolated nucleic acid molecules encoding the human antibody or antigen-binding portion thereof and the cell is chosen from the list consisting

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of a bacterial cell, a yeast cell, an insect cell, an amphibian cell and a mammalian cell. In a more preferred embodiment, the mammalian cell is selected from the list consisting of a human cell, a mouse cell, 5 a rat cell, a dog cell, a monkey cell, a goat cell, a pig cell, a bovine cell and a hamster cell. In a more preferred embodiment, the mammalian cell is selected from the list consisting of a HeLa cell, a NIH 3T3 cell, a CHO cell, a BHK cell, a VERO cell, a CV-1 cell, 10 a NS/O cell and a COS cell.

The invention contemplates a method for making an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, comprising:

- 15 a) immunizing a non-human animal having incorporated a human immunoglobulin locus therein with a *Pseudomonas aeruginosa* antigenic composition;
- b) allowing the non-human animal to mount a humoral response to the antigenic composition; and
- 20 c) isolating the human antibody from the non-human animal.

The invention contemplates a nucleic acid molecule isolated from a non-human animal that encodes a human antibody heavy chain or the antigen-binding portion 25 thereof that specifically binds to *Pseudomonas aeruginosa* LPS. In a preferred embodiment, the nucleic acid molecule is isolated from a hybridoma that produces the human antibody.

The invention contemplates an isolated nucleic acid molecule, or a fragment thereof, encoding a human 30 antibody heavy chain or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS having the nucleotide sequence of SEQ ID: 1. In a

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preferred embodiment, the isolated nucleic acid molecule comprises the sequence encoding between one to three of the CDR regions of the human antibody.

The invention contemplates a vector comprising a
5 nucleic acid molecule, or fragment thereof, encoding a human antibody heavy chain or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa*. In a preferred embodiment, the vector further comprises expression control sequences operably
10 linked to the nucleic acid.

The invention contemplates a nucleic acid molecule isolated from a non-human animal that encodes a human antibody light chain or the antigen-binding portion thereof that specifically binds to *Pseudomonas*
15 *aeruginosa* LPS. In a preferred embodiment, the nucleic acid molecule is isolated from a hybridoma that produces the human antibody.

The invention contemplates an isolated nucleic acid molecule, or a fragment thereof, encoding a human
20 antibody light chain or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS having the nucleotide sequence of SEQ ID: 3. In a preferred embodiment, the isolated nucleic acid molecule comprises the sequence encoding between one to
25 three of the CDR regions of the human antibody.

The invention contemplates a vector comprising a nucleic acid molecule, or fragment thereof, encoding a human antibody light chain or antigen-binding portion thereof that specifically binds to *Pseudomonas*
30 *aeruginosa*. In a preferred embodiment, the vector further comprises an expression control sequence operably linked to the nucleic acid.

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The invention contemplates an isolated host cell comprising

- a) a nucleic acid molecule that was isolated from a non-human animal and encodes a light chain or the antigen-binding portion thereof of a human antibody that specifically binds to *Pseudomonas aeruginosa* LPS; or

b) a vector comprising the nucleic acid molecule.

10 The invention contemplates an isolated host cell comprising:

- a) a nucleic acid molecule that was isolated from a non-human animal and encodes a heavy chain or the antigen-binding portion thereof of a human antibody that specifically binds to *Pseudomonas aeruginosa* LPS; or

b) a vector comprising the nucleic acid molecule.

20 The invention contemplates an isolated host cell comprising:

- a) a nucleic acid molecule that was isolated from a non-human animal and encodes a heavy chain or the antigen-binding portion thereof and an isolated nucleic acid molecule that encodes a light chain or the antigen-binding portion thereof of a human antibody that specifically binds to *Pseudomonas aeruginosa* LPS; or

b) a vector or vectors comprising the nucleic acid molecules.

30 In a preferred embodiment, the isolated host cells described above are chosen from the list consisting of hybridoma cells, bacterial cells, yeast cells, insect cells, amphibian cells and mammalian cells. In a more

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preferred embodiment, the mammalian cells are selected from the list consisting of human cells, mouse cells, rat cells, dog cells, monkey cells, goat cells, pig cells, bovine cells and hamster cells. In a more
5 preferred embodiment, the mammalian cells are selected from the list consisting of HeLa cells, NIH 3T3 cells, CHO cells, BHK cells, VERO cells, CV-1 cells, NS/O cells and COS cells.

The invention contemplates a method of
10 recombinantly producing the heavy chain or the antigen-binding portion thereof, the light chain or the antigen-binding portion thereof, or both the light chain and heavy chain or antigen-binding portions thereof, of a human antibody that was identified from a
15 non-human animal and specifically binds to *Pseudomonas aeruginosa* LPS, comprising the step of cultivating the host cells described above under conditions in which the nucleic acid molecules are expressed.

The invention contemplates an isolated human
20 antibody heavy chain or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, encoded by any of the nucleic acid molecules encoding the heavy chain described above, or isolated from any of the host cells described above. In a preferred
25 embodiment, the isolated human antibody heavy chain or antigen-binding portion thereof comprises between one to ten amino acid substitutions that increase the serum half-life of the antibody.

The invention contemplates an isolated human
30 antibody light chain or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, encoded by any of the nucleic acid molecules encoding the heavy chain described above, or isolated from any

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of the host cells described above. In a preferred embodiment, the isolated human antibody light chain or antigen-binding portion thereof comprises between one to ten amino acid substitutions that increase the serum half-life of the antibody.

The invention contemplates a non-human transgenic animal comprising any of the nucleic acid molecules described above. In a preferred embodiment, the non-human transgenic animal expresses the nucleic acid molecule or molecules. In a more preferred embodiment, the non-human transgenic animal comprises an isolated nucleic acid molecule that encodes a heavy chain or the antigen-binding portion thereof and an isolated nucleic acid molecule that encodes a light chain or the antigen-binding portion thereof of a human antibody that specifically binds to *Pseudomonas aeruginosa* LPS, and the non-human animal expresses both nucleic acid molecules. In a more preferred embodiment, the non-human animal is selected from the list consisting of a mouse, a rat, a hamster, a cow, a sheep, a primate, a horse and a pig. In a more preferred embodiment, a human antibody resulting from expression of the isolated nucleic acid molecules or portions thereof is expressed on the surface of cells derived from the animal's B lymphocytic cells or progeny thereof. In another preferred embodiment, the human antibody resulting from expression of the isolated nucleic acid molecules or a portion thereof is secreted into the lymph, blood, milk, saliva, or ascites of the animal.

The invention contemplates a fusion protein comprising the an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS and a second polypeptide sequence. In a preferred embodiment, the second

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polypeptide sequence is chosen from the list consisting of an epitope tag, an affinity tag, a toxic polypeptide, an antibiotic, an enzyme, a second antibody sequence, a complement protein, and a
5 cytokine.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, wherein the heavy chain isotype of the antibody is mu,
10 gamma, delta, epsilon or alpha.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, wherein the antibody or antigen-binding portion thereof
15 is produced by a process comprising the steps of:

a) immunizing a non-human animal comprising a human immunoglobulin locus with an antigen selected from the group consisting of an isolated *Pseudomonas aeruginosa* LPS preparation, a virile *Pseudomonas*
20 *aeruginosa* cell preparation, an attenuated *Pseudomonas aeruginosa* cell preparation, and a killed *Pseudomonas aeruginosa* cell preparation;

b) allowing the non-human animal to mount an immune response to the antigen; and

25 c) isolating the antibody from the non-human animal.

The invention contemplates an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS
30 wherein the *Pseudomonas aeruginosa* LPS is derived from a *Pseudomonas aeruginosa* strain chosen from the list consisting of 06ad, 011, Habs16, 170003 and PA01 Hallaway.

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The invention contemplates an isolated human antibody or antigen-binding portion thereof isolated from an animal or cell that was free of contaminating human biomaterials. In a preferred embodiment, the biomaterials are viruses, enzymes, hormones, cytokines, receptors, receptor ligands, immunoglobulins, complement, nuclear proteins, and cytoplasmic signaling proteins. In a more preferred embodiment, the viruses are Epstein-Barr virus or retroviruses.

Pharmaceutical compositions may be manufactured by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. For ocular administration, suspensions in an appropriate saline solution are used as is well known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in

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the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained as a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in

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admixture with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or
5 suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

10 For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are
15 conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other
20 suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin, for use in an inhaler or insufflator, may be formulated containing a powder mix of the
25 compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may
30 be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and

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may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, such as sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

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A pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase.

5 The cosolvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists

10 of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without

15 destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied;

20 other biocompatible polymers may replace polystyrene glycol, e.g., polyvinyl pyrrolidone; and other sugars or polysaccharides may be substituted for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed.

25 Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually with a greater toxicity.

30 Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those

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skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days.

Depending on the chemical nature and the
5 biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients.
10 Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

The isolated human antibody or antigen-binding
15 portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS of the invention may be provided as salts with pharmaceutically compatible counterions. Pharmaceutically compatible salts may be formed with many acids, including but not limited to hydrochloric,
20 sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms.

A component of the kits of the present invention
25 comprise instructions for utilizing the compositions of the present invention for prevention or treatment of *Pseudomonas aeruginosa* infections. Applicant has, for the first time, disclosed herein a method of preventing or treating *Pseudomonas aeruginosa* infections with an
30 isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS. The printed instructions on the kit

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enable one of skill in the art to utilize the kit for practicing the methods of the present invention.

EXAMPLE 1

5 Fully Human Antibodies to *Pseudomonas aeruginosa* LPS
A. *Pseudomonas aeruginosa* serotype 06ad

Pseudomonas aeruginosa serotype 06ad was used for mouse immunizations, mouse protection assays and opsonic assays. Bacteria for mouse challenge assays were fresh plated onto pseudosel agar (BBL, Becton Dickinson, Sparks, MD), then were incubated at 37° C, and one cfu was inoculated into LB broth and was incubated at 37°C in a shaking water bath to a concentration of 5×10^8 cfu/ml. Bacteria were centrifuged at 10,000 rpm for 5 minutes, resuspended and washed in chilled phosphate buffered saline (PBS). Bacteria for immunizations were grown as above and heat-killed at 60°C for one hour and stored at 4°C until use. Labeled bacteria used in the flow cytometry opsonic assay were grown and heat-killed as above. However, these bacteria were resuspended in Alkaline Conjugation Buffer (ACB: a 1:3 solution of .5M Na_2CO_3 and .5M NaHCO_3 , pH 9.5) to give a concentration of 10^9 /ml. An equal volume of ACB with .06% Fluorescein Isothiocyanate Isomer I (FITC, Amresco, Solon, OH) was added and incubated for 20 hours at room temperature with gentle shaking. Bacteria were washed in veronal buffered saline and then were resuspended in PBS at 10^9 /ml, and stored at -80° C.

30 The high molecular weight polysaccharide portion of the LPS O-specific side chains from *Pseudomonas aeruginosa* strains 06ad, 011, Habs16, 170003, and PA01 Halloway LPS (high MW PS) were made as described, and were lyophilized for storage. See Hatano et al.

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Infect. Immun. 62:3608-3616 (1994). These high MW PS were used to coat 96-well plates for enzyme-linked immunosorbent assays (ELISA) as described below. The 06ad high MW PS was also used in blocking and avidity assays described below.

Mice that were transgenic for human heavy and light Ig were bred and maintained by Abgenix Inc., Fremont, CA. The strain of Xenomouse™ used was Xma2a-3, which is an Ig-inactivated mouse reconstituted with a YAC containing cointegrated human heavy and light chain transgenes. Mice were housed in micro-isolator cages in a pathogen-free facility after shipping, and food and water were autoclaved prior to use. Mice were immunized with 10⁷ heat-killed *Pseudomonas aeruginosa* 06ad PA twice per week intraperitoneally (ip) (10⁷ bacteria in PBS) and/or in the foot pad (10⁷ bacteria and RIBI adjuvant, Sigma, St. Louis, MO), and their sera screened for anti-*Pseudomonas aeruginosa* 06ad LPS antibodies by ELISA described below.

Hybridomas were generated by fusing spleen and/or lymph node cells from immunized, seropositive Xenomouse™ animals with the nonsecreting sp2/0 myeloma cell line, as described previously. See Mendez et al. *Nat. Gen.* 15:146-156 (1997); Schreiber et al. *J. Immunol.* 146:188-193 (1991). Supernatants from hybridomas were screened for production of human anti-*Pseudomonas aeruginosa* 06ad LPS using the ELISA procedure described below, and hybridomas found to be secreting IgG anti-LPS antibodies were then cloned three times by limiting dilution. One IgG2-secreting clone (S20) was chosen based on initial measurements of strength of binding to solid phase *Pseudomonas aeruginosa* 06ad PS.

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**B. *Pseudomonas aeruginosa* serotypes
PA01 and 170003**

Pseudomonas aeruginosa serotypes PA01 and 170003

were used for mouse immunizations. Bacteria were fresh
5 plated onto pseudosel agar (BBL, Becton Dickinson,
Sparks, MD), then were incubated at 37° C, and one cfu
was inoculated into LB broth and was incubated at 37°C
in a shaking water bath to a concentration of 5×10^8
cfu/ml. Bacteria were centrifuged at 10,000 rpm for 5
10 minutes, resuspended and washed in chilled phosphate
buffered saline (PBS). Bacteria for immunizations were
grown as above and heat-killed at 60°C for one hour and
stored at 4°C until use.

The high molecular weight polysaccharide portion
15 of the LPS O-specific side chains from *Pseudomonas*
aeruginosa strains 06ad, 011, Habs16, 170003, and PA01
Halloway LPS (high MW PS) were made as described, and
were lyophilized for storage. See Hatano et al.
Infect. Immun. 62:3608-3616 (1994). These high MW PS
20 were used to coat 96-well plates for enzyme-linked
immunosorbent assays (ELISA) as described below. The
PA01 and 170003 high MW PS also were used in blocking
assays described below.

Mice that were transgenic for human heavy and
25 light Ig were bred and maintained by Abgenix Inc.,
Fremont, CA. The strain of Xenomouse™ used was Xma2a-3,
which is an Ig-inactivated mouse reconstituted with a
YAC containing cointegrated human heavy and light chain
transgenes. Mice were housed in micro-isolator cages
30 in a pathogen-free facility after shipping, and food
and water were autoclaved prior to use. Mice were
immunized with 10^7 heat-killed *Pseudomonas aeruginosa*
PA01 or 170003 twice per week intraperitoneally (ip) (10^7
bacteria in PBS) and/or in the foot pad (10^7 bacteria

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and RIBI adjuvant, Sigma, St. Louis, MO), and their sera screened for anti-*Pseudomonas aeruginosa* PA01 or 170003 LPS antibodies, respectively, by ELISA described below.

- 5 Hybridomas were generated by fusing spleen and/or lymph node cells from immunized, seropositive Xenomouse™ animals with the nonsecreting sp2/0 myeloma cell line, as described previously. See Mendez et al. *Nat. Gen.* 15:146-156 (1997); Schreiber et al. *J. Immunol.* 146:188-193 (1991). Supernatants from hybridomas were screened for production of human anti-*Pseudomonas aeruginosa* LPS using the ELISA procedure described below, and hybridomas found to be secreting anti-LPS antibodies were then cloned three times by limiting dilution. Two IgM-secreting clones (H12 and C3) were chosen based on initial measurements of strength of binding to solid phase *Pseudomonas aeruginosa* PA01 PS. These were derived from fusion of splenic cells as described above. One IgM-secreting clone (LN1H10) derived from fusion of lymph node cells was chosen based on initial measurements of strength of binding to solid phase *Pseudomonas aeruginosa* 170003 PS.

EXAMPLE 2

25 Characterization and Usage of Variable Region Genes From Transgenic Mouse-derived Anti-LPS Antibody

Dideoxy DNA sequencing was performed as previously described to determine the sequence of the variable region of the human monoclonal antibodies (30, 33).

- 30 Total RNA was isolated from hybridoma cells from nine

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different clones using TRIZOL reagent (Gibco BRL) and converted into random primed cDNA for use as a template in PCR. Human heavy chain and light chain variable regions were amplified using degenerate leader peptide primers and constant region primers provided in the Human Ig-Primer Set (Novagen, Madison, WI). The PCR products were analyzed on a Tris-acetate-EDTA agarose gel. The positive PCR reactions were chloroform isoamyl alcohol (24:1) extracted and cloned into the EcoRI site of pT7Blue (Novagen). The clones were sequenced based on the dideoxy method with Sequenase V2.0 DNA sequencing kit (USB, Cleveland, OH). Gene usage analysis was performed using the Vbase database (Tomlinson et al., MRC Centre for Protein Engineering, Cambridge, UK:

Variable region genes from hybridomas obtained from fusion of spleen cells from PA-immunized transgenic mice with the non-secreting SP2/0 cell line were cloned and sequenced in order to determine variable region gene usage (Figure 1). The protective IgG2 anti-LPS monoclonal antibody chosen for further study that was made in the human Ig transgenic mice, utilized genes from the V_H3 gene family, similar to the restricted V_H gene usage found after immunization of humans with a variety of bacterial polysaccharides. Nine other Mab made from fusions with spleen cells from pseudomonas immunized transgenic mice yielded antibodies that also used the V_H3 gene family for the heavy chain gene elements as determined by DNA sequencing. More specifically, DNA sequence analysis showed that the V_H3/V3-33 and JH4 genes were used in the protective anti-LPS Mab. Similarly, light chain gene segments used were Vk2/A2 and Jk1, as is commonly used in humans after PS vaccine immunization. In summary,

gene usage for anti-LPS antibodies after PA immunization in these transgenic mice appeared to be remarkably similar to that observed in humans after PS immunization.

Detection of Anti-Pseudomonas aeruginosa LPS antibodies

Enzyme-linked immunosorbent assay (ELISA) was used to detect antibodies to the *Pseudomonas aeruginosa* 06ad LPS in sera of immunized mice and in hybridoma supernatants as we have previously described (34). Briefly, 96-well microtiter polystyrene plates (NUNC, Denmark) were coated with 2ug/ml of *Pseudomonas aeruginosa* 06ad high MW PS overnight at 4°C, washed, and blocked with 200ul/well of 1% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO) in PBS and .05 % Tween 20 (Amresco, Solon, OH). Plates were washed and incubated over night with serial dilutions of S20 or sera in 1% BSA in PBS. Plates were washed, and bound antibodies were detected by adding isotype specific alkaline phosphatase-conjugated mouse-anti-human polyclonal antibodies (Southern Biotechnology Associates, Birmingham, AL). Plates were developed with 100ul/well of p-nitrophenyl phosphate (PNPP, Sigma-Aldrich) chromogenic substrate in DEA buffer. Optical densities were measured at 415nm with a microplate reader (Biorad, Hercules, CA). Blocking assays to determine the specificity of S20 were performed in an identical fashion as above except that soluble *Pseudomonas aeruginosa* 06ad high MW PS or

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control PS of different concentrations was added to the S20 prior to addition to PS-coated 96-well ELISA plates. Relative avidity of the Mab was calculated as described. See Chung et al. *Infect. Immun.* **63**:4219-4223 (1995); Schreiber et al. *J. Infect. Dis* **167**:221-226 (1993). Antibodies were added to the wells of a high MW PS-coated ELISA plate followed by serial dilutions of *Pseudomonas aeruginosa* 06ad high MW PS or equal volumes of PBS (negative control). The blocking assays to determine the binding specificity of the H12, C3 and LN1H10 monoclonal antibodies were conducted as described above, using soluble *Pseudomonas aeruginosa* PA01 or 170003 high MW PS. The concentration of PS required to inhibit 50% of the maximum absorbance was calculated (I_{50}) and the inverse of this value was used to represent relative avidity.

The S20 produced in the transgenic mouse was specific for the O-side chain of *P. aeruginosa* strain 06ad. Blocking assays revealed over 90% reduction in binding of S20 to solid phase *Pseudomonas aeruginosa* 06ad LPS high MW PS after preincubation of the Mab with the same PS, compared to less than 10% inhibition with the control PS (purified type 6B pneumococcal capsular PS; Figure 2). S20, however, did not cross-react with LPS from other *P. aeruginosa* serotypes since no binding could be demonstrated to solid phase LPS O-side chain high MW PS from a variety of pseudomonas strains including 011, Habs 16, 170003, and PA01 Halloway (Figure 3).

The concentration of high MW PS that inhibited 50% of the maximum absorbance of S20 binding to high MW PS was determined so that the inverse of this value ($1/I_{50}$) was used to calculate relative avidity of S20. This

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value was compared to a previously developed protective mouse monoclonal antibody directed to the same high MW PS and to previously published anti-polysaccharide values derived by using similar techniques (2, 33). We found that the relative avidity of the human Mab was higher than the mouse Mab (1.0 vs. .036).

Blocking assays with H12 and C3 revealed over 90% and 85% reduction, respectively, in binding to solid phase *Pseudomonas aeruginosa* PA01 LPS high MW PS after preincubation of the mAbs with the same PS, compared to less than 20% inhibition with the control PS (purified type 6B pneumococcal capsular PS; Figure 7). Preincubation of LN1H10 with *Pseudomonas aeruginosa* 170003 LPS high MW PS reduced binding of the mAb to solid phase PS by 89% compared to less than 40% inhibition with the control PS (Figure 8).

EXAMPLE 4

Anti-*Pseudomonas aeruginosa* LPS Antibody Opsonization Promotes Complement-Dependent Phagocytosis

The ability of the human monoclonal antibody to opsonize *P. aeruginosa* 06ad for uptake by human polymorphonuclear leukocytes (PMN) was measured via flow cytometry as previously described. See Schreiber et al. *J. Infect. Dis* 167:221-226 (1993). *Pseudomonas aeruginosa* 06ad was grown, heat-killed and FITC labeled. Opsonization was carried out by incubating the labeled bacteria with S20 with or without 1% human serum from an agammaglobulinemic patient as the complement source. Bacteria were washed in PBS containing 6% dextran and .2% glucose, and then were

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resuspended in HBSS with .1% gelatin.

Polymorphonuclear leukocytes (PMN) were isolated from peripheral human blood from healthy, adult volunteers. See Schreiber et al. *J. Infect. Dis* **167**:221-226 (1993);

- 5 Tosi et al. *J. Clin. Invest.* **86**:300-308 (1990). MN were resuspended to achieve a concentration of 10^7 cells/mL, and activated for 30 minutes with 10 μ l of a 10^{-6} dilution per ml of cells of FMLP (Peninsula Laboratories, San Carlos, CA). PMNs were added to each
10 opsonized bacteria sample, incubated at 37°C, and then were separated from free bacteria by differential centrifugation and resuspended in PBS. Single color flow cytometry analysis was performed on PMN utilizing a FACScan and CellQuest software (Becton Dickinson,
15 MountainView, CA), and phagocytosis was expressed in relative units of mean fluorescence of 10,000 PMN for each sample. To demonstrate that the observed opsonophagocytosis was associated with bacterial killing, an alternative assay was used in which 10^6 CFU
20 of live *P. aeruginosa* 06ad was mixed with fresh human serum absorbed with the bacteria, S20 and 10^6 fresh human PMN. Samples were obtained at the beginning and end of a 90 minute 37° incubation, bacteria were diluted and then plated for bacterial enumeration.

- 25 To show functional activity of the Anti-*Pseudomonas aeruginosa* 06ad LPS antibody, S20 was shown to be highly opsonic for uptake of labeled PA by fresh human PMN in a complement-dependent assay. In fact, the human Mab was almost two-fold more opsonic than a
30 previously described protective mouse Mab (D8) against the same epitope (2.5 mg of human Mab yielded twice the mean fluorescence as 5 mg of mouse Mab; Figure 4). Since the bacteria were heat killed prior to labeling

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with FITC it seemed possible that increased susceptibility to antibody and complement-mediated opsonization could have occurred due to damage to the bacterial surface. Thus, we also measured opsonization and phagocytosis of *Pseudomonas aeruginosa* 06ad in a killing assay in which 10^6 CFU of live bacteria are opsonized with antibody and human complement and then colony counts determined after exposure to 10^6 fresh PMN. The S20 Mab was also effective in this assay so that 5mg/ml antibody resulted in 80% reduction in the PA cfu.

EXAMPLE 5

Protection of neutropenic mice from fatal *Pseudomonas aeruginosa* sepsis

The protective efficacy of the human Mab against invasive infection with *Pseudomonas aeruginosa* was measured in the neutropenic mouse model, described previously. See Pier et al. *Infect. Immun.* **57**:174-179 (1989); Schreiber et al. *J. Immunol.* **146**:188-193 (1991). Female, six week-old BALB/c ByJ mice (Jackson Laboratories, Bar Harbor, ME) were maintained in a pathogen-free, pseudomonas-free environment in which water, bedding, and food were autoclaved prior to use. Neutropenia was established by administering 3mg of cyclophosphamide (Cytoxan[®], Bristol-Myers Squibb, Princeton, NJ) intra-peritoneally to each mouse on days 1, 3, and 5. On day 5, the cyclophosphamide was administered at time 0 hours, and 2 hours later 10ug of S20 or PBS control was administered ip, followed by 10^3 cfu of live *Pseudomonas aeruginosa* 06ad PA two hours later. Mice were observed daily thereafter and

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mortality was the outcome measured.

As illustrated in Figure 5, infected mice treated with the PBS control began dying one day after *Pseudomonas aeruginosa* infection. After two days, 100% of the infected mice treated with PBS had died. In contrast, 100% of the mice treated with the S20 mAb showed protection and were alive two days after *Pseudomonas aeruginosa* infection, demonstrating the protective potential of S20 in preventing *Pseudomonas aeruginosa*-related fatalities in patients.

The embodiments listed above are for illustrative purposes only; the invention as contemplated is not limited to any of these particular embodiments and in fact may encompass a combination of one or more of the embodiments.

BIOLOGICAL DEPOSITS

Hybridoma cell lines S20, H12, C3 and LnlH10 were deposited in accordance with the provisions of the Budapest Treaty at the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209, USA, and were assigned the following accession numbers:

	Hybridoma S20	HB _____
	Hybridoma H12	HB _____
25	Hybridoma C3	HB _____
	Hybridoma LnlH10	HB _____.

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We claim:

1. An isolated human antibody or antigen-binding portion thereof that was expressed in a non-human animal and specifically binds to *Pseudomonas aeruginosa* LPS.
5
2. The isolated human antibody or antigen-binding portion thereof according to claim 1, wherein said human antibody is a monoclonal antibody.
3. The isolated human antibody or antigen-binding portion thereof according to claim 1 or
10 claim 2, wherein the antibody or portion thereof is opsonic for *Pseudomonas aeruginosa* cells.
4. The isolated human antibody or antigen-binding portion thereof according to claim 3 wherein
15 said antibody or portion thereof facilitates phagocytosis of said *Pseudomonas aeruginosa* cells.
5. The isolated human antibody or antigen-binding portion thereof according to claim 1 or
claim 2, wherein the antibody or portion thereof
20 enhances the immune response to *Pseudomonas aeruginosa*.
6. The isolated human antibody or antigen-binding portion thereof according to claim 1 or
claim 2, wherein the antibody or portion thereof
facilitates the killing of *Pseudomonas aeruginosa*
25 cells.
7. The isolated human antibody or antigen-

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binding portion thereof according to claim 6, wherein said facilitating the killing of *Pseudomonas aeruginosa* cells comprises delivery of an agent lethal to said *Pseudomonas aeruginosa* cells.

5 8. The isolated human antibody or antigen-binding portion thereof according to claim 6, wherein said facilitating the killing of *Pseudomonas aeruginosa* cells comprises enhancing the immune response to *Pseudomonas aeruginosa*.

10 9. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody or antigen-binding portion thereof inhibits *Pseudomonas aeruginosa* infection.

15 10. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody or antigen-binding portion thereof binds to *Pseudomonas aeruginosa* LPS with a K_d of 5×10^{-7} M to 1×10^{-7} M.

20 11. The isolated human antibody or antigen-binding portion thereof according to claim 10, wherein said antibody or antigen-binding portion thereof binds to *Pseudomonas aeruginosa* LPS with a K_d of 1×10^{-7} to 5×10^{-8} M.

25 12. The isolated human antibody or antigen-binding portion thereof according to claim 10, wherein said antibody or antigen-binding portion thereof binds to *Pseudomonas aeruginosa* LPS with a K_d of 5×10^{-8} M to 1×10^{-8} M.

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13. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2 wherein the *Pseudomonas aeruginosa* LPS is derived from a *Pseudomonas aeruginosa* strain chosen from the list
5 consisting of 06ad, 011, Habs16, 170003 and PA01 Halloway.

14. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody or antigen-binding portion
10 thereof has a half-life *in vivo* of between one hour and thirty days

15. The isolated human antibody or antigen-binding portion thereof according to claim 14, wherein said antibody or antigen-binding portion thereof has a
15 half-life *in vivo* of between sixteen and thirty days.

16. The isolated human antibody or antigen-binding portion thereof according to claim 14, wherein said antibody or antigen-binding portion thereof has a half-life *in vivo* of between one hour and fifteen days.

20 17. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody is or is derived from an immunoglobulin molecule having a heavy chain isotype chosen from the list consisting of immunoglobulin G
25 (IgG), IgM, IgE, IgA and IgD.

18. The isolated human antibody according to claim 17, wherein said IgG is a subtype selected from the list consisting of IgG1, IgG2, IgG3 and IgG4.

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19. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody or portion thereof is labeled.

20. The isolated human antibody or antigen-binding portion thereof according to claim 19, wherein said label is selected from the list consisting of a radiolabel, an enzyme label, a fluorescent label, a toxin, a magnetic agent, a second antibody, an affinity label, an epitope tag, an antibiotic, a complement protein and a cytokine.

21. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, comprising a kappa light chain and framework sequences thereof.

22. The isolated human antibody or antigen-binding portion thereof according to claim 21, wherein said framework sequences of said kappa light chain are encoded by a human Vx2/A2 gene.

23. The isolated human antibody or antigen-binding portion thereof according to claim 21, wherein said kappa light chain comprises between seven and fifteen amino acid changes from a kappa light chain encoded by a germline Vx2/A2 gene.

24. The isolated human antibody or antigen-binding portion thereof according to claim 21, wherein said kappa light chain comprises no more than six amino acid changes from a kappa light chain encoded by a germline Vx2/A2 gene.

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25. The isolated human antibody or antigen-binding portion thereof according to claim 24, wherein said kappa light chain comprises no more than three amino acid changes from a kappa light chain encoded by a germline Vk2/A2 gene.

26. The isolated human antibody or antigen-binding portion thereof according to claim 21, wherein said kappa light chain comprises the amino acid sequence of SEQ ID NO: 4.

10 27. The isolated human antibody or antigen-binding portion thereof according to claim 21, wherein said light chain is encoded by the nucleic acid sequence of SEQ ID NO: 3.

15 28. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, comprising a lambda light chain.

29. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, comprising a heavy chain composed of variable (V), diversity (D), and Joining (J) regions and composed of framework sequences thereof.

30. The isolated human antibody or antigen-binding portion thereof according to claim 29, wherein said variable region of said heavy chain is encoded by a human V_H3/V3-33 gene.

31. The isolated human antibody or antigen-binding portion thereof according to claim 29, wherein said diversity region of said heavy chain is

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encoded by a human D2-8 gene.

32. The isolated human antibody or antigen-binding portion thereof according to claim 29, wherein said joining region of said heavy chain is
5 encoded by a human J_H4b gene.

33. The isolated human antibody or antigen-binding portion thereof according to claim 29, wherein said variable region comprises between seven and fifteen amino acid changes from a variable region
10 encoded by a germline V_H3/V3-33 gene.

34. The isolated human antibody or antigen-binding portion thereof according to claim 29, wherein said heavy chain comprises no more than six amino acid changes from a variable region encoded by a
15 germline V_H3/V3-33 gene.

35. The isolated human antibody or antigen-binding portion thereof according to claim 29, wherein said heavy chain comprises no more than three amino acid changes from a variable region encoded by a
20 germline V_H3/V3-33 gene.

36. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said heavy chain comprises the amino acid sequence of SEQ ID NO: 2.

25 37. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2 wherein said heavy chain is encoded by the nucleic acid sequence of SEQ ID NO: 1.

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38. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2 comprising an antigen binding domain chosen from the list consisting of an Fab fragment, an F(ab')₂ fragment
5 and an F_v fragment.

39. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody is a single chain antibody.

40. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody is a chimeric antibody.
10

41. The chimeric antibody according to claim 40, comprising framework regions and CDR regions from different human antibodies.

42. The chimeric antibody according to claim 40, wherein the chimeric antibody is bispecific.
15

43. The chimeric antibody according to claim 42, wherein the chimeric antibody is bispecific for *Pseudomonas aeruginosa* LPS and a label selected from the list consisting of a radiolabeled molecule, an enzymatic label, a fluorescent label, a toxin, a magnetic agent, a second antibody, an affinity label, an epitope tag, an antibiotic, a complement protein and a cytokine.
20

44. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody or portion thereof is derivatized.
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45. The isolated human antibody or antigen-binding portion thereof according to claim 44, wherein said antibody or portion thereof is derivatized with polyethylene glycol, at least one methyl or ethyl group
5 or at least one carbohydrate moiety.

46. A pharmaceutical composition comprising the antibody or antigen-binding portion thereof according to claim 1 or claim 2 and a pharmaceutically acceptable carrier.

10 47. A kit comprising the antibody or antigen-binding portion thereof according to claim 1 or claim 2, a pharmaceutically acceptable carrier therefor, and a container.

48. The kit according to claim 47, further
15 comprising instructions for use.

49. A method for treating or preventing *Pseudomonas aeruginosa* infection, comprising the step of administering an isolated human antibody according to claim 1 to a patient at risk of being infected with,
20 or currently infected with, *Pseudomonas aeruginosa*.

50. The method according to claim 49 wherein said human antibody is a monoclonal antibody.

51. The method according to claim 49, wherein said administering is performed via an
25 injection, transmucosal, oral, inhalation, ocular, rectal, long acting implantation, liposomes, emulsion, cream, topical or sustained release means.

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52. The method according to claim 49,
wherein said antibody is a fusion with a second
protein.

53. The method according to claim 49,
5 wherein said antibody is labeled with a radiolabel, a
toxin, a complement protein, a cytokine or an
antibiotic.

54. The method according to claim 52,
wherein said second protein is chosen from the list
10 consisting of a toxic peptide moiety, a complement
protein, a radiolabeled protein, a cytokine or an
antibiotic protein.

55. The method according to claim 49 wherein
said patient is a burn patient, a surgical patient, a
15 prosthesis recipient, a respiratory patient, a cancer
patient, a cystic fibrosis patient or an
immunocompromised patient.

56. The method according to claim 49,
wherein said pharmaceutical composition further
20 comprises toxins, complement proteins, radiolabeled
proteins, cytokines, antibiotics, or any combination
thereof.

57. An isolated cell line that produces the
antibody according to claim 1 or claim 2.

25 58. The cell line according to claim 57
wherein said cell line is a hybridoma.

59. A method of producing an isolated human

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antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS, comprising:

- a) culturing a non-human cell capable of producing said antibody under conditions in which the antibody is produced;
- b) isolating said antibody from said cell culture.

60. The method according to claim 59, wherein said cell is a hybridoma.

61. The method according to claim 59, wherein said cell is transformed with isolated nucleic acid molecules encoding said human antibody or antigen-binding portion thereof and said cell is chosen from the list consisting of a bacterial cell, a yeast cell, an insect cell, an amphibian cell and a mammalian cell.

62. The method according to claim 61, wherein said mammalian cell is selected from the list consisting of a human cell, a mouse cell, a rat cell, a dog cell, a monkey cell, a goat cell, a pig cell, a bovine cell and a hamster cell.

63. The method according to claim 61, wherein said mammalian cell is selected from the list consisting of a HeLa cell, a NIH 3T3 cell, a CHO cell, a BHK cell, a VERO cell, a CV-1 cell, a NS/O cell and a COS cell.

64. A method for making an isolated human antibody or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS,

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comprising:

- a) immunizing a non-human animal having incorporated a human immunoglobulin locus therein with a *Pseudomonas aeruginosa* antigenic composition;
- b) allowing said non-human animal to mount a humoral response to said antigenic composition; and
- c) isolating said human antibody from said non-human animal.

65. A nucleic acid molecule isolated from a non-human animal that encodes a human antibody heavy chain or the antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS.

66. The isolated nucleic acid molecule according to claim 65, wherein said nucleic acid molecule is isolated from a hybridoma that produces said human antibody.

67. An isolated nucleic acid molecule, or a fragment thereof, encoding a human antibody heavy chain or antigen-binding portion thereof that specifically binds to *Pseudomonas aeruginosa* LPS having the nucleotide sequence of SEQ ID: _.

68. The isolated nucleic acid molecule of claim 67 wherein said fragment comprises the sequence encoding between one to three of the CDR regions of said human antibody.

69. A vector comprising the nucleic acid

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molecule, or fragment thereof, according to any one of claims 65-68.

70. The vector according to claim 69,
wherein said vector further comprises expression
5 control sequences operably linked to said nucleic acid.

71. A nucleic acid molecule isolated from a
non-human animal that encodes a human antibody light
chain or the antigen-binding portion thereof that
specifically binds to *Pseudomonas aeruginosa* LPS.

10 72. The isolated nucleic acid molecule
according to claim 71, wherein said nucleic acid
molecule is isolated from a hybridoma that produces
said human antibody.

73. An isolated nucleic acid molecule, or a
15 fragment thereof, encoding a human antibody light chain
or antigen-binding portion thereof that specifically
binds to *Pseudomonas aeruginosa* LPS having the
nucleotide sequence of SEQ ID: 3.

74. The isolated nucleic acid molecule of
20 claim 71 wherein said fragment comprises the sequence
encoding between one to three of the CDR regions of
said human antibody.

75. A vector comprising the nucleic acid
molecule according to any one of claims 71-74.

25 76. The vector according to claim 75,
wherein said vector further comprises an expression
control sequence operably linked to said nucleic acid.

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77. An isolated host cell comprising
a) a nucleic acid molecule that was
isolated from a non-human animal and encodes a light
chain or the antigen-binding portion thereof of a human
5 antibody that specifically binds to *Pseudomonas*
aeruginosa LPS; or
b) a vector comprising said nucleic
acid molecule.

78. An isolated host cell comprising:
10 a) a nucleic acid molecule that was
isolated from a non-human animal and encodes a heavy
chain or the antigen-binding portion thereof of a human
antibody that specifically binds to *Pseudomonas*
aeruginosa LPS; or
15 b) a vector comprising said nucleic
acid molecule.

79. An isolated host cell comprising:
a) an nucleic acid molecule that was
isolated from a non-human animal and encodes a heavy
20 chain or the antigen-binding portion thereof and an
isolated nucleic acid molecule that encodes a light
chain or the antigen-binding portion thereof of a human
antibody that specifically binds to *Pseudomonas*
aeruginosa LPS or
25 b) a vector or vectors comprising said
nucleic acid molecules.

80. The isolated host cell of any one of
claims 77-79 wherein said cells are chosen from the
list consisting of hybridoma cells, bacterial cells,
30 yeast cells, insect cells, amphibian cells and

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mammalian cells.

81. The host cell according to claim 80,
wherein said mammalian cell is selected from the list
consisting of a human cell, a mouse cell, a rat cell, a
5 dog cell, a monkey cell, a goat cell, a pig cell, a
bovine cell and a hamster cell.

82. The method according to claim 80,
wherein said mammalian cells are selected from the list
consisting of HeLa cells, NIH 3T3 cells, CHO cells, BHK
10 cells, VERO cells, CV-1 cells, NS/O cells and COS
cells.

83. A method of recombinantly producing the
heavy chain or the antigen-binding portion thereof, the
light chain or the antigen-binding portion thereof, or
15 both the light chain and heavy chain or antigen-binding
portions thereof, of a human antibody that was
identified from a non-human animal and specifically
binds to *Pseudomonas aeruginosa* LPS, comprising the
step of cultivating the host cell according to any one
20 of claims 77-81 under conditions in which the nucleic
acid molecules are expressed.

84. An isolated heavy chain or antigen-
binding portion thereof obtained from the antibody
according to claim 1 or claim 2, encoded by the nucleic
25 acid molecule according to claim 67, or isolated from
the host cell according to claim 78.

85. An isolated light chain or antigen-
binding portion thereof obtained from the antibody
according to claim 1 or claim 2, encoded by the nucleic

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acid molecule according to claim 73, or isolated from the host cell according to claim 77.

86. A non-human transgenic animal comprising the nucleic acid molecule according to claim 67,
5 wherein said non-human transgenic animal expresses said nucleic acid molecule.

87. A non-human transgenic animal comprising the nucleic acid molecule according to claim 73,
wherein said non-human transgenic animal expresses said
10 nucleic acid molecule.

88. A non-human transgenic animal comprising an isolated nucleic acid molecule that encodes a heavy chain or the antigen-binding portion thereof and an isolated nucleic acid molecule that encodes a light
15 chain or the antigen-binding portion thereof of a human antibody that specifically binds to *Pseudomonas aeruginosa* LPS, wherein said animal expresses said nucleic acid molecules.

89. The non-human transgenic animal
20 according to any one of claims 86-88, wherein said animal is selected from the list consisting of a mouse, a rat, a hamster, a cow, a sheep, a primate, a horse and a pig.

90. The non-human transgenic animal
25 according to any one of claims 86-88, wherein a human antibody resulting from expression of said isolated nucleic acid molecules or portions thereof is expressed on the surface of cells derived from said animal's B lymphocytic cells or progeny thereof.

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91. The non-human transgenic animal according to any one of claims 86-88, wherein a human antibody resulting from expression of said isolated nucleic acid molecules or a portion thereof is secreted
5 into the lymph, blood, milk, saliva, or ascites of said animal.

92. A fusion protein comprising the isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2 and a second
10 polypeptide sequence.

93. The fusion protein of claim 92, wherein said second polypeptide sequence is chosen from the list consisting of an epitope tag, an affinity tag, a toxic polypeptide, an antibiotic, an enzyme, a second
15 antibody sequence, a complement protein, and a cytokine.

94. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein the heavy chain isotype of said antibody is
20 mu, gamma, delta, epsilon or alpha.

95. The isolated heavy chain or antigen-binding portion thereof according to claim 84 comprising between one to ten amino acid substitutions that increase the serum half-life of said antibody.

25 96. The isolated light chain or antigen-binding portion thereof according to claim 85 comprising between one to ten amino acid substitutions that increase the serum half-life of said antibody.

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97. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein the antibody or portion thereof is produced by a process comprising the steps of:

- 5 a) immunizing a non-human animal comprising a human immunoglobulin locus with an antigen selected from the group consisting of an isolated *Pseudomonas aeruginosa* LPS preparation, a virile *Pseudomonas aeruginosa* cell preparation, an attenuated *Pseudomonas aeruginosa* cell preparation, and a killed *Pseudomonas aeruginosa* cell preparation;
- 10 b) allowing said non-human animal to mount an immune response to said antigen; and
- c) isolating said antibody from said
- 15 non-human animal.

98. The isolated human antibody or antigen-binding portion thereof according to claim 1 or claim 2, wherein said antibody or portion thereof is isolated from an animal or cell that is free of contaminating

20 human biomaterials.

99. The isolated human antibody or antigen-binding portion thereof according to claim 99 wherein said biomaterials are chosen from the list consisting of viruses, enzymes, hormones, cytokines, receptors,

25 receptor ligands, immunoglobulins, complement, nuclear proteins, and cytoplasmic signaling proteins.

100. The isolated human antibody or antigen-binding portion thereof according to claim 100 wherein said viruses are Epstein-Barr virus or retroviruses.

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101. A hybridoma cell line that produces the S20 mAb, said hybridoma cell line having American Type Culture Collection Accession Number ____.

102. A monoclonal antibody produced by the hybridoma cell line according to claim 101.

103. An isolated human antibody or an antigen-binding portion thereof, that specifically binds *Pseudomonas aeruginosa* LPS O-specific side chain.

104. The antibody antigen-binding portion according to claim 103, wherein the *Pseudomonas aeruginosa* strain is selected from the group consisting of: 06ad, 011, Habs16, 17003 and PA01 Hallaway.

105. The antibody antigen-binding portion according to claim 103 or 104, which is monoclonal.

106. The antibody or antigen-binding portion according to any one of claim 103-105, having a relative binding avidity of about 1.0.

107. The antibody or antigen-binding portion according to claim 104, that specifically binds the LPS O-specific side chain of one strain and does not bind to the LPS O-specific side chain of the other four strains.

108. A human monoclonal antibody or antigen-binding portion thereof that specifically binds *Pseudomonas aeruginosa* strain PA01 LPS O-specific side chain.

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109. The human monoclonal antibody or antigen-binding portion according to claim 108, which is an IgM antibody.

110. The human monoclonal antibody or antigen-binding portion according to claim 108, that is produced by the hybridoma cell line having ATCC accession # _____ or ATCC accession # _____.

111. A human monoclonal antibody or antigen-binding portion thereof that specifically binds *Pseudomonas aeruginosa* strain 170003 LPS O-specific side chain.

112. The human monoclonal antibody or antigen-binding portion according to claim 111, which is an IgM antibody.

113. The human monoclonal antibody or antigen-binding portion according to claim 111, that is produced by the hybridoma cell line having ATCC accession # _____.

114. A human monoclonal antibody or an antigen-binding portion thereof, that inhibits the binding of an antibody according to any one of claim 108-113.

115. An antibody or antigen-binding portion thereof that specifically binds *Pseudomonas aeruginosa* LPS O-specific side chain, wherein said antibody has comprises a heavy chain that includes one or more of CDR1, CDR2 and CDR3 shown in Figure 9.

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116. The antibody or antigen-binding portion according to claim 115, wherein said heavy chain comprises the CDR3 shown in Figure 9.

117. The antibody or antigen-binding portion according to claim 115 comprising the amino acid sequence shown in SEQ ID NO: 2.

118. An antibody or antigen-binding portion thereof that specifically binds *Pseudomonas aeruginosa* LPS O-specific side chain, wherein said antibody has comprises a light chain that includes one or more of CDR1, CDR2 and CDR3 shown in Figure 10.

119. The antibody or antigen-binding portion according to claim 118, wherein said heavy chain comprises the CDR3 shown in Figure 10.

120. The antibody or antigen-binding portion according to claim 118 comprising the amino acid sequence shown in SEQ ID NO: 4.

121. A passive vaccine for preventing or inhibiting *Pseudomonas aeruginosa* infection comprising one or more human monoclonal antibodies or an antigen-binding portion thereof, selected from the group consisting of the antibody or portion according to claim 1, claim 102, claim 103, and claims 115-120.

122. The passive vaccine according to claim 121 comprising two or more human monoclonal antibodies or an antigen-binding portion thereof, wherein said human monoclonal antibodies or portions specifically

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bind different strains of *Pseudomonas aeruginosa*.

123. A method for detecting the presence of
Pseudomonas aeruginosa in a biological sample
comprising the steps of contacting said sample with an
5 antibody or antigen-binding portion thereof according
to any one of claims 1, 102, 103 or 115-120.

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Figure 1: S20 Monoclonal Antibody Gene Usage

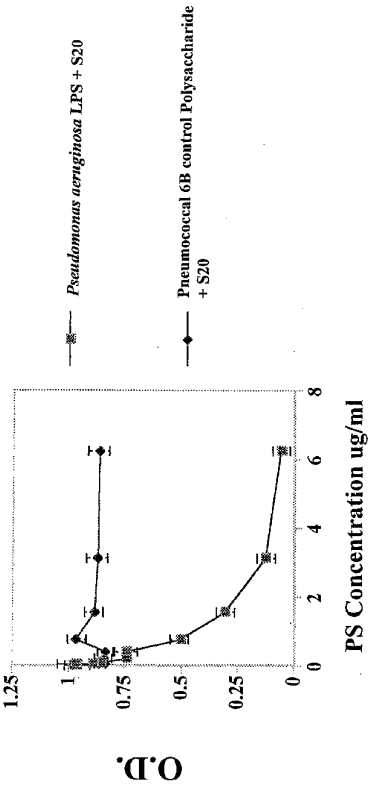
Heavy Chain Gene Segments		
<u>VH</u>	<u>D</u>	<u>JH</u>
V _H 3/V3-33	D2-8	J _H 4b
Light Chain Gene Segments		
<u>VL</u>	<u>JL</u>	
V _K 2/A2	J _K 1	

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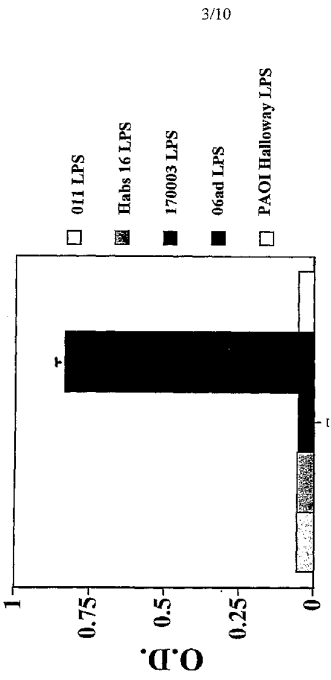
Figure 2: S20 Monoclonal Antibody Specifically Binds Solid Phase *Pseudomonas aeruginosa* LPS



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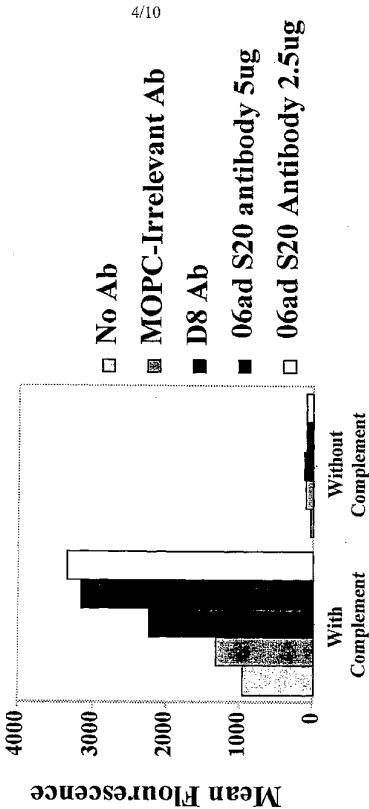
Figure 3: S20 Monoclonal Antibody Specifically Binds *Pseudomonas aeruginosa* 06ad LPS



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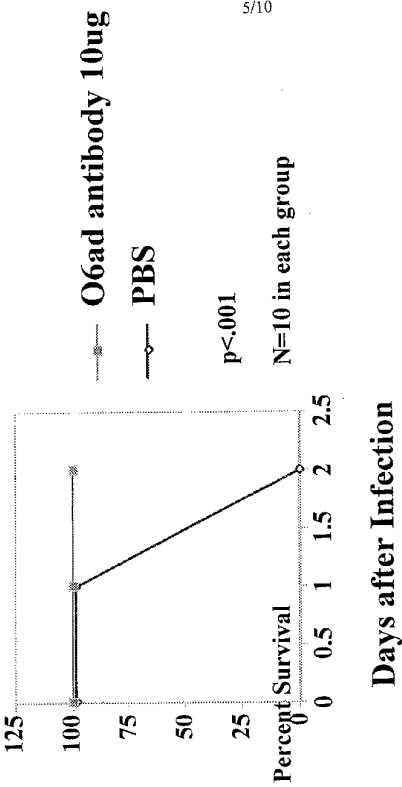
Figure 4: S20 Monoclonal Antibody Opsonization Promotes Complement-Dependent Phagocytosis



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Figure 5: S20 Monoclonal Antibody Protects Neutropenic Mice From Fatal *Pseudomonas aeruginosa* Sepsis



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Heavy Chain Variable Region Sequence (

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTC
TCCTGTGCAGCGTCTGGATTACCTTCAGTAGCTATGGCATCGACTGGGTCCGCCAGGCT
CCAGGCAAGGGGCTGGAGTGGGTGGGACGTATATGGTATAGTGAAGTAATAAATACTAT
GCAGACTCCGTGAAGGGCCGATTACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT
CTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATCCC
CACTATACCCGGGGTTTGTACTACTGGGGCAAGGGAACCTGGTCACCGTCTCCTCAGCC
TCCACCAAGGGCCCATCGGTCTTCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGC
ACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCGCCGAA

Heavy Chain Variable Region Sequence (SEQ ID:2)

QVQLVESGGGVVQPRSLRLSCAASGFTFSSYIDWVRQAPGKLEWVGRVWYSGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRADTAVYYCARDPHYTRGFDYWGKGLVTVSSA
STKGPIGLPLAPCSRSTSESTAALGCLVKDYFAE

Light Chain Variable Region Sequence (SEQ ID:3)

GATGTTGTGATGACCCAGACTCCACTCTCTCTGTCCGTACCCCTGGACAGCCGGCCTCC
ATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCATAGTGATGGAATGACCTATTTTCTTGG
TATTTGCAGAAGCCAGGCCAGCCTCCACAGCTCCTGATCTATGAAGTTTCCAACCGGTTT
TCTGGAGTGCCAGATAGGTTTCACTGGCAGCGGTCAGGGACAGATTTACACTGAAATC
AGCCGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAAATATACAGCTTCCG
TGGACGTTCCGCCAAGGGACCAAGGTGGAATCAAACGAACGTGTGGCTGCACCATCTGTC
TTCATCTTCCGCCATCTGAT

Light Chain Variable Region Sequence (SEQ ID:4)

DVVMQTPLSLSVTPGQPASISCRSSQSLHSDGMTYFSWYLQKPGQPPQQLLIYEVSNRF
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQNIQLPWTFGGQGTKVEIKRTVAAPSV
FIIPPSD

FIGURE 6

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Inhibition of binding of PA01 IgM human monoclonal antibody with pure PA01 polysaccharide (PS)

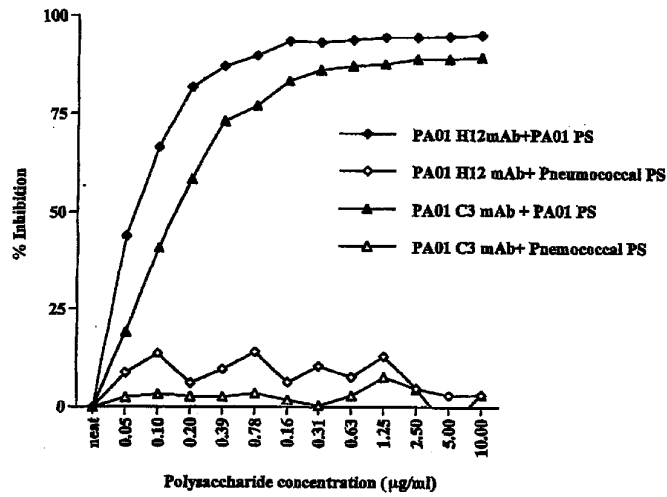


Figure 7

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Inhibition of binding of 170003 IgM human monoclonal antibody with pure 170003 polysaccharide (PS)

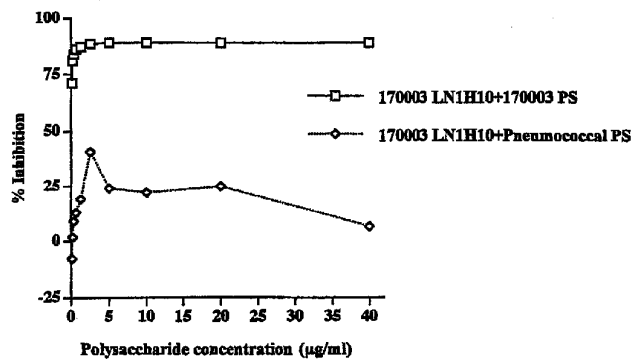


Figure 8

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Heavy Chain Variable Region Sequence**Framework 1**

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCC
Q V Q L V E S G G G V V Q P G R S

CDR1

CTGAGACTCTCTGTGCAGCGTCTGGATTACCTTCAGTAGCTATGGCATG
L R L S C A A S G F T F S S Y G M

Framework 2

CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGCT
H W V R Q A P G K G L E W V A A

CDR2

ATATGGTATAGTGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGA
I W Y S G S N K Y Y A D S V K G R

Framework 3

TTCACCATCTCCAGAGACAATTCGAAGAACACGCTGTATCTGCAATGAAC
F T I S R D N S K N T L Y L Q M N

CDR3

AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATCCCCAC
S L R A E D T A V Y Y C A R D P H

Framework 4

TATACCCGGGGTTTTGACTACTGGGGCAAGGGAACCTGGTCACCGTCTCC
Y T R G F D Y W G K G T L V T V S

Constant

TCAGCCTCCACCAAAGGGCCCATCGGTCTTCCCTGGCGCCCTGCTCCAGG
S A S T K G P I G L P L A P C S R

AGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC
S T S E S T A A L G C L V K D Y F

GCCGAA

A E

Figure 9

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Light Chain Variable Region Sequence**Framework 1**

GATGTTGTGATGACCCAGACTCCACTCTCTGTCCGTACCCCTGGACAG
D V V M T Q T P L S L S V T P G Q

CDR 1

CCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCATAGTGATGGA
P A S I S C R S S Q S L L H S D G

Framework 2

ATGACCTATTTTCTTGGTATTTGCAGAAGCCAGGCCAGCCTCCACAGCTC
M T Y F S W Y L Q K P G Q P P Q L

CDR 2**Framework 3**

CTGATCTATGAAGTTTCCAACCGGTTCTCTGGAGTGCCAGATAGGTTCACT
L I Y E V S N R F S G V P D R F S

GGCAGCGGGTCAGGGACAGATTTCACACTGAAAATCAGCCGGGTGGAG
G S G S G T D F T L K I S R V E

CDR 3

GCTGAGGATGTTGGGGTTTATTACTGCATGCAAAATATACAGCTTCCG
A E D V G V Y Y C M Q N I Q L P

Framework 4**Constant**

TGGACGTTCCGCCAAGGGACCAAGGTGGAAATCAAACGAACTGTGGCT
W T F G Q G T K V E I K R T V A

GCACCATCTGTCTTCATCTTCCCGCCATCTGAT
A P S V F I F P P S D

Figure 10

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SUBMISSION OF INDICATION RELATED TO
A DEPOSITED MICROORGANISM PURSUANT TO
PCT RULES 13bis.3(a)(iii) AND 13bis.4

Sirs:

Subsequent to the international filing date,
American Type Culture Collection, the depositary
institution, assigned Accession Numbers PTA-4073, PTA-
4074, and PTA-4075 to the microorganism deposits referred
to on pages 51, 69, and 70 of the above-identified
application.

Pursuant to PCT Rules 13bis.3(a)(iii) and
13bis.4, applicants submit herewith Substitute Sheets 51,
69, and 70, on which the accession numbers PTA-4073, PTA-

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4074, and PTA-4075 have been inserted numbers appear on lines 23-26 of page 51, on line 3 of page 69, and lines 7 and 18 of page 70. Applicants request that the International Bureau replace pages 51, 69, and 70 of the above-identified application with the enclosed Substitute Sheets.

The space provided for an accession number that appears on line 24 of page 51 and that appears second on line 7 of page 70 was incorrectly inserted in the application; no such deposit exists. Applicants will amend the application to remove reference to said deposit at the appropriate time.

Applicants further request that these pages be replaced as soon as possible, since the application is scheduled for publication after March 7, 2002.

Please confirm by facsimile your receipt of this letter and enclosures.

【国際調査報告】

INTERNATIONAL SEARCH REPORT		International Application No. PCT/US 01/28019
A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C07K16/12 C07K16/46 A61K39/40 A61P31/04 C12N5/20 C12N5/10 C12N15/13 C12N15/85 A01K67/027 C07K19/00 G01N33/577 G01N33/569		
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 C07K		
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)		
BIOSIS, EMBASE, CHEM ABS Data, WPI Data, PAJ, EPO-Internal, SEQUENCE SEARCH		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. HEMACHANDRA ET AL.: "Human monoclonal antibody (Mab) against Pseudomonas aeruginosa LPS derived from transgenic Xenomouse™ mice is opsonic and highly protective in the neutropenic mouse model of sepsis." CLINICAL INFECTIOUS DISEASES, vol. 31, no. 1, July 2000 (2000-07), page 213 XP008005471 Chicago, IL, USA abstract 10 --- -/--	1-123
☒ 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 (see specification) "O" document referring 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		Date of mailing of the international search report
5 July 2002		18/07/2002
Name and mailing address of the ISA European Patent Office, P.B. 5818 Palantian 2 NL - 2220 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl Fax: (+31-70) 340-2016		Authorized officer Nootj, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US 01/28019

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 33735 A (CELL GENESYS, INC.) 31 October 1996 (1996-10-31) claims	1,2,17, 18,21, 46, 49-51, 57-66, 69-72, 74-91, 94,97, 103,105, 121
A	S. SAWADA ET AL.: "Immunoprotective human monoclonal antibodies against five major serotypes of <i>Pseudomonas aeruginosa</i> ." JOURNAL OF GENERAL MICROBIOLOGY, vol. 133, no. 12, December 1987 (1987-12), pages 3581-3590, XP000574785 abstract	1-123
A	A. LANG ET AL.: "Isolation and characterization of a human monoclonal antibody that recognizes epitopes shared by <i>Pseudomonas aeruginosa</i> immunotype 1, 3, 4, and 6 lipopolysaccharides." INFECTION AND IMMUNITY, vol. 57, no. 12, December 1989 (1989-12), pages 3851-3855, XP000081169 Washington, DC, USA abstract	1-123
A	EP 0 441 395 A (SUMITOMO PHARMACEUTICALS COMPANY, LTD.) 14 August 1991 (1991-08-14) claims	1-123
A	M. MENDEZ ET AL.: "Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice." NATURE GENETICS, vol. 15, February 1997 (1997-02), pages 146-156, XP002067603 New York, NY, USA cited in the application the whole document	1-123

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Form PCT/ISA210 (continuation of second sheet) (July 1992)

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *		Relevant to claim No.
A	M. PRESTON ET AL.: "Production and characterization of a set of mouse-human chimeric immunoglobulin G (IgG) subclass and IgA monoclonal antibodies with identical variable regions specific for Pseudomonas aeruginosa serogroup O6 lipopolysaccharide." INFECTION AND IMMUNITY, vol. 66, no. 9, September 1998 (1998-09), pages 4137-4142, XP002204808 Washington, DC, USA abstract -----	1-123
P, X	S. HEMACHANDRA ET AL.: "Human monoclonal antibodies against Pseudomonas aeruginosa lipopolysaccharide derived from transgenic mice containing megabase human immunoglobulin loci are opsonic and protective against fatal Pseudomonas sepsis." INFECTION AND IMMUNITY, vol. 69, no. 4, April 2001 (2001-04), pages 2223-2229, XP002204809 Washington, DC, USA the whole document -----	1-123

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INTERNATIONAL SEARCH REPORT		International application No. PCT/US 01/28019
Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)		
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:		
1. ☒	Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: Although claims 49-56 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.	
2. ☐	Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:	
3. ☐	Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).	
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)		
This International Searching Authority found multiple inventions in this International application, as follows:		
1. ☐	As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.	
2. ☐	As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.	
3. ☐	As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:	
4. ☐	No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:	
Remark on Protest		
☐ The additional search fees were accompanied by the applicant's protest.		
☐ No protest accompanied the payment of additional search fees.		

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/US 01/28019

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9633735 A	31-10-1996	AU 5632296 A	18-11-1996
		CA 2219361 A1	31-10-1996
		EP 0822830 A1	11-02-1998
		JP 11505523 T	21-05-1999
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		EP 0441395 A2	14-08-1991
		JP 4211393 A	03-08-1992

Form PCT/ISA/210 (patent family annex) (July 1992)

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(51)Int.Cl. ⁷	F I	テーマコード (参考)
A 6 1 K 9/72	A 6 1 K 9/72	4 C 0 8 5
A 6 1 K 38/00	A 6 1 K 39/395	D 4 H 0 4 5
A 6 1 K 39/395	A 6 1 K 39/395	R
A 6 1 K 45/00	A 6 1 K 45/00	
A 6 1 P 3/10	A 6 1 P 3/10	
A 6 1 P 9/00	A 6 1 P 9/00	
A 6 1 P 11/00	A 6 1 P 11/00	
A 6 1 P 13/02	A 6 1 P 13/02	
A 6 1 P 17/00	A 6 1 P 17/00	
A 6 1 P 17/02	A 6 1 P 17/02	
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A 6 1 P 19/08	A 6 1 P 19/08	
A 6 1 P 27/02	A 6 1 P 27/02	
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A 6 1 P 37/04	A 6 1 P 37/04	
A 6 1 P 41/00	A 6 1 P 41/00	
A 6 1 P 43/00	A 6 1 P 43/00	1 2 1
C 0 7 K 16/12	C 0 7 K 16/12	
C 0 7 K 19/00	C 0 7 K 19/00	
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C 1 2 N 1/21	C 1 2 N 1/21	
C 1 2 N 5/10	C 1 2 P 21/02	C
C 1 2 N 15/02	C 1 2 P 21/08	
C 1 2 P 21/02	G 0 1 N 33/569	D
C 1 2 P 21/08	G 0 1 N 33/577	B
G 0 1 N 33/569	C 1 2 N 5/00	B
G 0 1 N 33/577	C 1 2 N 15/00	C
	A 6 1 K 37/02	

(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,GQ,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,PH,PL,PT,R O,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,US,UZ,VN,YU,ZA,ZW

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Fターム(参考) 4B024 AA01 BA50 BA53 CA02 DA02 DA05 DA12 GA11 HA15

4B064 AG27 CA02 CA06 CA10 CA19 CA20 CC24 DA01 DA13

4B065 AA42Y AA72X AA90X AA92X AA93X AB01 AB05 AC14 BA02 CA25

