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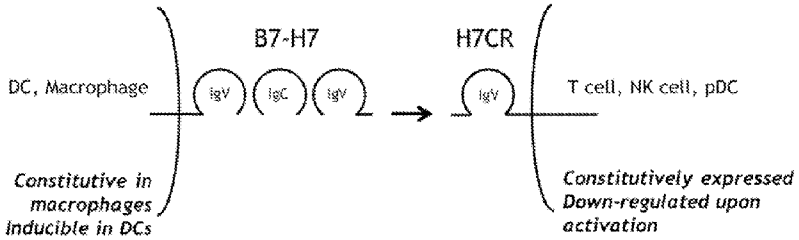

FIG. 1

(57) Abstract: The present invention relates to antibodies and their antigen-binding fragments and to other molecules that are capable of immunospecifically binding to the B7-H5 ligand of the B7-H5:CD28H pathway, and to the uses of such molecules in the treatment and diagnosis of autoimmune disease, transplant rejection and other inflammatory diseases.

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ANTI-B7-H5 ANTIBODIES AND THEIR USES

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0001] This invention was made with government support under award numbers ROl CA97085 and ROl A172592 by the National Institutes of Health (NIH), and U19 CA113341 by the National Cancer Institute (NCI). The government has certain rights in the invention.

REFERENCE TO SEQUENCE LISTING

[0002] This application includes one or more Sequence Listings pursuant to 37 C.F.R. § 1.821 *et seq.*, which are disclosed in both paper and computer-readable media, and which paper and computer-readable disclosures are herein incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0003] The present invention relates to antibodies and their antigen-binding fragments and to other molecules that are capable of binding to the B7-H5 ligand of the B7-H5:CD28H pathway, and to the uses of such molecules in the treatment and diagnosis of autoimmune disease, transplant rejection and other inflammatory diseases.

BACKGROUND OF THE INVENTION

[0004] The immune system of humans and other mammals is responsible for providing protection against infection and disease. Such protection is provided both by a humoral immune response and by a cell-mediated immune response. The humoral response results in the production of antibodies and other biomolecules that are capable of recognizing and neutralizing foreign targets (antigens). In contrast, the cell-mediated immune response involves the activation of macrophages, natural killer cells (NK), and antigen-specific cytotoxic T-lymphocytes by T cells, and the release of various cytokines in response to the recognition of an antigen (Dong, C. *et al.* (2003) "*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48).

[0005] The ability of T cells to optimally mediate an immune response against an antigen requires two distinct signaling interactions (Viglietta, V. *et al.* (2007) "*Modulating Co-Stimulation*," Neurotherapeutics 4:666-675; Korman, A.J. *et al.* (2007) "*Checkpoint Blockade in Cancer Immunotherapy*," Adv. Immunol. 90:297-339). First, antigen that has been arrayed on the surface of antigen-presenting cells (APC) must be presented to an antigen-specific naive $CD4^+$ T cell. Such presentation delivers a signal via the T cell receptor (TCR) that directs the T cell to initiate an immune response that will be specific to the presented antigen. Second, a series of co-stimulatory and co-inhibitory signals, mediated through interactions between the APC and distinct T cell surface molecules, triggers first the activation and proliferation of the T cells and ultimately their inhibition. Thus, the first signal confers specificity to the immune response whereas the second signal serves to determine the nature, magnitude and duration of the response.

[0006] The immune system is tightly controlled by co-stimulatory and co-inhibitory ligands and receptors. These molecules provide the second signal for T cell activation and provide a balanced network of positive and negative signals to maximize immune responses against infection while limiting immunity to self (Wang, L. *et al.* (March 7, 2011) "*VISTA, A Novel Mouse Ig Superfamily Ligand That Negatively Regulates T Cell Responses*," J. Exp. Med. 10.1084/jem.20100619:1-16; Lepenies, B. *et al.* (2008) "*The Role Of Negative Costimulators During Parasitic Infections*," Endocrine, Metabolic & Immune Disorders - Drug Targets 8:279-288). Of particular importance is binding between the B7.1 (CD80) and B7.2 (CD86) ligands of the Antigen Presenting Cell and the CD28 and CLTA-4 receptors of the $CD4^+$ T-lymphocyte (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126; Dong, C. et al. (2003) "*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48; Lindley, P.S. et al. (2009) "*The Clinical Utility Of Inhibiting CD28-Mediated Costimulation*," Immunol. Rev. 229:307-321). Binding of B7.1 or of B7.2 to CD28 stimulates T cell activation; binding of B7.1 or B7.2 to CTLA4 inhibits such activation (Dong, C. *et al.* (2003) "*Immune Regulation by Novel*

Costimulatory Molecules," Immunolog. Res. 28(1):39-48; Lindley, P.S. *et al.* (2009) "*The Clinical Utility Of Inhibiting CD28-Mediated Costimulation*," Immunol. Rev. 229:307-321; Greenwald, R.J. *et al.* (2005) "*The B7 Family Revisited*," Ann. Rev. Immunol. 23:515-548). CD28 is constitutively expressed on the surface of T cells (Gross, J., *et al.* (1992) "*Identification And Distribution Of The Costimulatory Receptor CD28 In The Mouse*," J. Immunol. 149:380–388), whereas CTLA4 expression is rapidly up-regulated following T-cell activation (Linsley, P. *et al.* (1996) "*Intracellular Trafficking Of CTLA4 And Focal Localization Towards Sites Of TCR Engagement*," Immunity 4:535–543). Since CTLA4 is the higher affinity receptor (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126), binding first initiates T cell proliferation (via CD28) and then inhibits it (via nascent expression of CTLA4), thereby dampening the effect when proliferation is no longer needed.

[0007] Further investigations into the ligands of the CD28 receptor have led to the identification and characterization of a set of related B7 molecules (the "B7 Superfamily") (Coyle, A.J. *et al.* (2001) "*The Expanding B7 Superfamily: Increasing Complexity In Costimulatory Signals Regulating T Cell Function*," Nature Immunol. 2(3):203-209; Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126; Greenwald, R.J. *et al.* (2005) "*The B7 Family Revisited*," Ann. Rev. Immunol. 23:515-548; Collins, M. *et al.* (2005) "*The B7 Family Of Immune-Regulatory Ligands*," Genome Biol. 6:223.1-223.7; Loke, P. *et al.* (2004) "*Emerging Mechanisms Of Immune Regulation: The Extended B7 Family And Regulatory T Cells*." Arthritis Res. Ther. 6:208-214; Korman, A.J. *et al.* (2007) "*Checkpoint Blockade in Cancer Immunotherapy*," Adv. Immunol. 90:297-339; Flies, D.B. *et al.* (2007) "*The New B7s: Playing a Pivotal Role in Tumor Immunity*," J. Immunother. 30(3):251-260; Agarwal, A. *et al.* (2008) "*The Role Of Positive Costimulatory Molecules In Transplantation And Tolerance*," Curr. Opin. Organ Transplant. 13:366-372; Lenschow, D.J. *et al.* (1996) "*CD28/B7 System of T Cell Costimulation*," Ann. Rev. Immunol. 14:233-258; Wang, S. *et al.* (2004) "*Co-Signaling Molecules Of The B7-CD28 Family In Positive And Negative Regulation Of T Lymphocyte*

Responses," Microbes Infect. 6:759-766). There are currently nine known members of the family: B7.1 (CD80), B7.2 (CD86), the inducible co-stimulator ligand (ICOS-L, B7-H2), the programmed death-1 ligand (PD-L1; B7-H1), the programmed death-2 ligand (PD-L2; B7-DC), B7-H3, B7-H4 (also referred to as B7x, B7-H6 and B7S1; Sica, G.L. *et al.* (2003) "*B7-4, A Molecule Of The B7 Family, Negatively Regulates T Cell Immunity*," Immunity18:849-861; Zang, X. *et al.* (2003) *B7x: A Widely Expressed B7 Family Member That Inhibits T Cell Activation*," Proc. Natl. Acad. Sci. (USA) 100:10388-10392; Prasad, D.V. *et al.* (2003) *B7S1, A Novel B7 Family Member That Negatively Regulates T Cell Activation*," Immunity 18:863-873), B7-H6 (Collins, M. *et al.* (2005) "*The B7 Family Of Immune-Regulatory Ligands*," Genome Biol. 6:223.1-223.7) and B7-H5 (Flajnik, M.F. *et al.* (2012) "*Evolution Of The B7 Family: Co-Evolution Of B7H6 And Nkp30, Identification Of A New B7 Family Member, B7H7, And Of B7's Historical Relationship With The MHC*," Immunogenetics 64:571–590). The B7 family of genes is essential in the regulation of the adaptive immune system. Most B7 family members contain both variable (V)- and constant (C)-type domains of the immunoglobulin superfamily (IgSF).

[0008] B7 ligands are expressed on the cell surface of many different cell types including antigen presenting cells (APCs) and their interaction with receptor molecules on T cells provide activating and/or inhibitory signals that regulate T cell activation and tolerance (Collins, M. *et al.* (2005) "*The B7 Family Of Immune-Regulatory Ligands*," Genome Biol. 6:223.1-223.7). Some inhibitory B7 ligands are also expressed on tumor cells, resulting in suppression of immune responses (Keir, M.E. *et al.* (2008) "*PD-1 And Its Ligands In Tolerance And Immunity*," Annu. Rev. Immunol. 26:677-704; Zou, W. *et al.* (2008) "*Inhibitory B7-Family Molecules In The Tumour Microenvironment*," Nat. Rev. Immunol. 8:467-477). Therefore, stimulating or attenuating the interactions of B7 ligands and their receptors holds therapeutic potential for autoimmune diseases, cancer and infectious disease (WO 2011/020024; Flajnik, M.F. *et al.* (2012) "*Evolution Of The B7 Family: Co-Evolution Of B7H6 And Nkp30, Identification Of A New B7 Family*

Member, B7H7, And Of B7's Historical Relationship With The MHC," Immunogenetics 64:571-590).

[0009] Despite all prior advances in the treatment of inflammatory disease or autoimmune disease, a need remains for compositions capable of providing enhanced immunotherapy for the treatment of such conditions. The present invention is directed to such compositions and their use to treat inflammatory disease, autoimmune disease, and similar diseases and conditions characterized by a hyperactive immune system.

SUMMARY OF THE INVENTION

[0010] Antibodies and their antigen-binding fragments and other molecules that are capable of immunospecifically binding to the B7-H5 ligand of the B7-H5:CD28H pathway, and to the uses of such molecules in the treatment and diagnosis of autoimmune disease, transplant rejection and other inflammatory diseases are provided.

[0011] One embodiment provides an antibody or other molecule having an antigen-binding fragment of an antibody that immunospecifically binds to a mammalian B7-H5 (and in particular, a human B7-H5). The B7-H5 can be, for example, arrayed on the surface of a live cell.

[0012] Exemplary live cells include, but are not limited to macrophage or dendritic cells.

[0013] Another embodiment provides a molecule that immunospecifically binds to B7-H5 and is substantially capable of blocking B7-H5's interaction with CD28H. Still another embodiment provides a molecule that immunospecifically binds to B7-H5 and is substantially incapable of blocking B7-H5's interaction with CD28H.

[0014] The B7-H5 binding molecules can have antigen-binding fragments that contain six CDRs. The CDRs can include at least one consensus CDR of the CDRs of anti-B7-H5 antibodies: 2D3 and 18C3, with all remaining CDRs selected from:

(A) the three light chain and the three heavy chain CDRs of anti-B7-H5 antibody 2D3;or

(B) the three light chain and the three heavy chain CDRs of anti-B7-H5 antibody 18C3.

[0015] Another embodiment provides B7-H5 binding molecules wherein the six CDRs are:

(A) the three light chain and the three heavy chain CDRs of anti-B7-H5 antibody 2D3; or

(B) the three light chain and the three heavy chain CDRs of anti-B7-H5 antibody 18C3.

[0016] In some embodiments, the molecule is a chimeric antibody that includes antigen binding fragments (Fab) from two or more different B7-H5 antibodies.

[0017] The B7-H5 binding molecules can be a monoclonal antibody, a human antibody, a chimeric antibody or a humanized antibody and/or is a bispecific, trispecific or multispecific antibody.

[0018] The B7-H5 binding molecules can be detectably labeled or include a conjugated toxin, drug, receptor, enzyme, or receptor ligand.

[0019] Still another embodiment provides a pharmaceutical composition containing a therapeutically effective amount or a prophylactically effective amount of any of the above-described B7-H5 binding molecules and a physiologically acceptable carrier or excipient.

[0020] In other embodiments, a molecule that is substantially capable of blocking B7-H5's interaction with CD28H is an CD28H fusion protein, preferably an CD28H-Ig fusion protein.

[0021] Methods of using the pharmaceutical compositions in the treatment of a disease are also provided.

[0022] A method for diagnosing a disease in a subject includes assaying cells of the subject for their ability to bind to any of the above-described B7-

H5 binding molecules, wherein the method provides a cytologic assay for diagnosing the presence of a disease in the subject.

[0023] The disclosed B7-H5 binding molecules can be used to an autoimmune disease or transplant rejection.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] **Figure 1** provides a schematic depiction of B7-H5 and its counter-receptor, CD28H.

[0025] **Figure 2**, **Panels A-J**, show that B7-H5 is constitutively expressed on macrophages and inducible on dendritic cells.

[0026] **Figure 3**, **Panels A-D**, show that CD28H is expressed on T cells and natural killer (NK) cells, but not on B cells.

[0027] **Figures 4A-4B** show the ability of the antibodies produced by clones 2D3 and 18C3 to bind human B7-H5 expressed by a CHO transfectants.

[0028] **Figures 5A-5C** show the abilities of the isolated antibodies and control Ig to block the B7-H5:CD28H interaction.

[0029] **Figure 6** shows the ability of a B7-H5 Ig fusion to stimulate a T cell response.

[0030] **Figure 7** shows the ability of the B7-H5 Ig to induce the expression of cytokines: IL-2, IFN-γ and IL-10.

[0031] **Figure 8** shows that the anti-B7-H5 antibody 2D3 of the present invention is capable of binding to B7-H5 so as to block the capacity of B7-H5 to interact with its CD28H counter-receptor.

[0032] **Figure 9** shows the ability of anti-B7-H5 antibody 2D3 to inhibit an allogeneic T cell response.

[0033] **Figure 10A** and **Figure 10B** show the effect of anti-human B7-H5 antibody on the percentage of $CD28H^{+}$ cells among activated ($CD45RO^{+}$)

human T cells in the spleen (**Figure 10A**) and peripheral blood (**Figure 10B**) in NSG mice implanted with human PBMC.

[0034] **Figure 11A** and **Figure 11B** show the effect of anti-human B7-H5 antibody on the percentage of $CD28H^{+}$ cells among naïve ($CD45RO^{-}$) human T cells in the spleen (**Figure 11A**) and peripheral blood (**Figure 11B**) in NSG mice implanted with human PBMC.

[0035] **Figures 12A-12C** show binding properties of recombinant anti-human B7-H5 chimeric antibodies (2D3 and 18C3). **Figures 12A-12C** show the ability of anti-human B7-H5 antibodies (2D3 and 18C3) that had been recombinantly converted from their murine IgG isotype to a human IgG4 isotype to bind human B7-H5 expressed by CHO cells.

[0036] **Figure 13 (Panels A-C)** shows the ability of recombinant anti-human B7-H5 chimeric antibodies (2D3 and 18C3) to completely block the binding of CD28H fusion protein to CHO B7-H5 transfectants. Human B7-H5 FL CHO transfectants were pre-incubated with control supernatant (**Panel A**) or recombinant chimeric 2D3 (**Panel B**) and 18C3 supernatants (**Panel C**), and subsequently stained with biotinylated CD28HhIg fusion protein.

[0037] **Figures 14A-14B** show the epitope recognition site of anti-human B7-H5 antibodies (2D3 and 18C3). **Figure 14A** and **Figure 14B** show that 2D3 and 18C3 recognize the first IgV domain of B7-H5. **Figure 14C** shows B7-H5 IgV domain mediates B7-H5's interaction with CD28H.

[0038] **Figure 15** compares the affinities of antibodies 2D3 and 18C3 for binding to human B7-H5 as expressed on the surface of CHO cells.

[0039] **Figures 16A-16B** show the effect of the endogenous B7-H5:CD28H interaction on the recall of TT-specific memory T cells. **Figure 16A** shows TT vaccine-induced T cell proliferation in the presence of the B7-H5:CD28H interaction blocking antibody 2D3 (**Panel B**) or control Igs (**Panel A**). **Figure 16B** shows the levels of expressed cytokines (**Panel A**) and the BrdU-positive cells (**Panel B**) associated with such response.

[0040] **Figures 17A-17B** show the blockade of endogenous B7-H5:CD28H interaction by 2D3 inhibits the allogeneic proliferative responses of both CD8+ (**Figure 17A**, **Panels A-B**) and CD4+ (**Figure 17B**, **Panels A-B**) T cells in humanized NSG mice.

[0041] **Figure 18** shows simultaneous cross-linking of TCR and B7-H5 fusion protein induced AKT phosphorylation 30 min after stimulation, while TCR cross-linking alone induced minimal AKT phosphorylation. Importantly, inclusion of B7-H5 antibody 2D3 prevented AKT activation, indicating B7-H5 co-stimulation utilizes the AKT pathway to promote T cell response.

[0042] **Figure 19** shows B7-H5-CD28H pathway blockade by decoy receptor fusion protein CD28HIg suppressed the cytotoxic killing activity of allogeneic CD8 T cell against B7-H5 transfected 624Mel melanoma cell line, indicating B7-H5-CD28H pathway promotes cytotoxic killing activity of CD8 T cells on target cells.

[0043] **Figure 20A-B** show simultaneous cross-linking of CD16 and B7-H5 fusion protein on Natural Killer cells (NK) in the presence of soluble recombinant human IL-2 induced NK activation and degranulation (CD107a surface upregulation), whereas CD16 engagement alone did not induce significant NK degranulation. A B7-H5 fusion protein dose dependent NK activation was observed (Figure 20A). Importantly, inclusion of B7-H5 antibody 2D3 prevented NK activation (Figure 20B), indicating B7-H5 co-stimulates NK activation.

Detailed Description of the Invention:

[0044] The present invention relates to antibodies and their antigen-binding fragments and to other molecules that are capable of immunospecifically binding to the B7-H5 ligand of the B7-H5:CD28H pathway, and to the uses of such molecules in the treatment and diagnosis of autoimmune disease, transplant rejection and other inflammatory diseases.

[0045] The B7-H5:CD28H pathway is a cellular immunity pathway that involves the ligand **B7-H5** and its counter-receptor **CD28H** (**Figure 1**). B7-H5 is expressed on antigen presenting cells; it is constitutively expressed on macrophages and inducible on dendritic cells (**Figure 2, Panels A-J**). CD28H is particularly expressed on naïve T cells, NK cells, and plasmacytoid dendritic cells (especially in the spleen, lymph node and thymus), and its expression is down-regulated on matured or activated cells. It is not expressed on γδ T cells or B cells (**Figure 3**), but is expressed on T_n, T_{CM}, T_{EM}, and T_{EMRA} T cell subsets. Human cord blood T cells express CD28H as do $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ thymocytes. B7-H5 interacts with the CD28H counter-receptor to stimulate the immune system, thereby promoting enhanced immune responses. Down-regulation of CD28H has been found to impair activated/memory T cell survival *in vivo*. Thus, the interaction between B7-H5 and CD28H is important for native T cell priming and activated/memory T cell survival *in vivo*. CD28H is also seen to be down-regulated in chronically antigen-exposed / exhausted T cells. CD28H is constitutively expressed on Natural Killer cell (NK). B7-H5-CD28H pathway promotes NK activation and degranulation in the presence of other NK activation signal, such as CD16 crosslinking.

[0046] The present invention reflects, in part, the recognition that molecules, such as antibodies and their antigen binding fragments that immunospecifically bind to B7-H5 ("**anti-B7-H5 antibodies**"), and particularly anti-B7-H5 antibodies or decoy receptor fusion protein CD28HIg that block the B7-H5:CD28H interaction so as to impair (*i.e.*, prevent or attenuate) the ability of B7-H5 to bind to CD28H, are capable of impairing the ability of B7-H5 to promote enhanced immune responses via the CD28H interaction, and thus are capable of serving as antagonists of T cell proliferation and cytokine production and NK activation. Such molecules are capable of mediating a physiological reduction in immune system activation and thus have utility in the treatment of inflammatory disease and autoimmune disease. In particular, such antibodies are capable of reducing the percentage of activated T cells and NK cells *in vivo*.

A. B7-H5

[0047] The B7-H5 amino acid sequence was found to be similar to a previously discovered human gene, HHLA2 (human endogenous retrovirus-H long terminal repeat-associating protein 2 (HHLA2); Mager, D.L. *et al.* (1999) "*Endogenous Retroviruses Provide The Primary Polyadenylation Signal For Two New Human Genes (HHLA2 And HHLA3*," Genomics 59:255-263), that had no known function (Flajnik, M.F. *et al.* (2012) "*Evolution Of The B7 Family: Co-Evolution Of B7H6 And Nkp30, Identification Of A New B7 Family Member, B7H7, And Of B7's Historical Relationship With The MHC*," Immunogenetics 64:571–590). B7-H5 is also referred to herein and elsewhere as B7-H7. Accordingly, the terms B7-H5 and B7-H7 are used interchangeably herein.

[0048] The human B7-H5 sequence has been found to have homologs in chicken, opossum, hoofed mammals (*e.g.*, horse, pig), salmon, and shark. However, only pseudogenes have been thus far identified in rodents (mouse and rat). The amino acid sequences of such genes reveal a similar domain structures in all species, with conservation of the canonical residues for Ig superfamily domains.

[0049] Human B7-H5 polypeptide is 414 amino acids in length and has been reported to contain the following: a signal sequence, an extracellular domain having 3 immunoglobulin-like (Ig-like) domains, a transmembrane domain, and a cytoplasmic domain. In particular, the human B7-H5 polypeptide has been reported to contain an Ig-like V-type 1 domain, an Ig-like C-1 type domain, and an Ig-like V-type 2 domain. Multiple naturally occurring variants of B7-H5 exist (*e.g.*, Accession No. Q9UM44-1 (*homo sapiens*), NP_009003 (GI:5901964, *homo sapiens*), and AAD48396 (GI:15726285, *homo sapiens*); see WO 2011/020024).

[0050] The term "native-B7-H5" (also "native-B7-H7") refers to any naturally occurring B7-H5 amino acid sequence, including immature or precursor and mature forms. The amino acid sequence of a representative human B7-H5, Accession No. Q9UM44-1, is (**SEQ ID NO:1**):

[0051]

MKAQTALSFF LILITSLSGS QGIFPLAFFI YVPMNEQIVI GRLDEDIILP
SSFERGSEVV **IHWKYQDSYK VHSYYKGSDH LESQDPRYAN RTSLFYNEIQ**
NGNASLFFRR VSLLDEGIYT CYVGTAIQVI TNKVVLKVGV FLTPVMKYEK
RNTNSFLICS VLSVYPRPII TWKMDNTPIS ENNMEETGSL DSFSINSPLN
ITGSNSSYEC TIENSLLKQT WTGRWTMKDG LHKMQSEHVS LSCQPVNDYF
SPNQDFKVTW SRMKSGTFSV LAYYLSSSQN TIINESRFSW NKELINQSDF
SMNLMDLNLS DSGEYLCNIS SDEYTLLTIH TVHVEPSQET ASHNKGLWIL
VPSAILAAFL LIWSVKCCRA QLEARRSRHP ADGAQQERCC VPPGERCPSA
PDNGEENVPL SGKV

[0052] The human B7-H5 has been reported to contain the following: a signal sequence at approximately amino acid residues 1 to 22 of **SEQ ID NO:1**, an Ig-like V-type 1 domain (shown underlined above) at approximately amino acid residues 61 to 131 of **SEQ ID NO:1**, an Ig-like C-1 type domain at approximately amino acid residues 138 to 222 of **SEQ ID NO:1**, an Ig-like V-type 2 domain at approximately amino acid residues 235 to 328 of **SEQ ID NO:1**, and a transmembrane domain at approximately amino acid residues 345 to 365 of **SEQ ID NO:1**. The predicted dimer interface for human B7-H5 polypeptide is amino acid residues 141-144, 156, 158, 160, 162, 193-196, 198, 200, 201, 224, and 225 of **SEQ ID NO:1**. The predicted N-linked glycosylation sites for human B7-H5 polypeptide are at amino acid residues 90, 103, and 318 of **SEQ ID NO:1**. Natural variations of human B7-H5 polypeptide include BOT, N344K, and S346R (UniProt Q9UM44) (see, WO 2011/020024, which reference is herein incorporated by reference in its entirety for its teaching of the structure and sequence of human B7-H5).

[0053] A DNA sequence encoding human B7-H5 (**SEQ ID NO:1**) is (**SEQ ID NO:2**):

atgaaggcac agacagcact gtctttcttc ctcattctca taacatctct
gagtggatct caaggcatat tccctttggc tttcttcatt tatgttccta
tgaatgaaca aatcgtcatt ggaagacttg atgaagatat aattctccct
tcttcatttg agaggggatc cgaagtcgta atacactgga agtatcaaga
tagctataag gttcatagtt actacaaagg cagtgaccat ttggaaagcc
aagatcccag atatgcaaac aggacatccc ttttctataa tgagattcaa
aatgggaatg cgtcactatt tttcagaaga gtaagccttc tggacgaagg
aatttacacc tgctatgtag gaacagcaat tcaagtgatt acaaacaaag
tggtgctaaa ggtgggagtt tttctcacac ccgtgatgaa gtatgaaaag
aggaacacaa acagcttctt aatatgcagc gtgttaagtg tttatcctcg
tccaattatc acgtggaaaa tggacaacac acctatctct gaaaacaaca
tggaagaaac agggtctttg gattcttttt ctattaacag cccactgaat
attacaggat caaattcatc ttatgaatgt acaattgaaa attcactgct
gaagcaaaca tggacagggc gctggacgat gaaagatggc cttcataaaa
tgcaaagtga acacgtttca ctctcatgtc aacctgtaaa tgattatttt

tcaccaaacc aagacttcaa agttacttgg tccagaatga aaagtgggac
tttctctgtc ctggcttact atctgagctc ctcacaaaat acaattatca
atgaatcccg attctcatgg aacaaagagc tgataaacca gagtgacttc
tctatgaatt tgatggatct taatctttca gacagtgggg aatatttatg
caatatttct tcggatgaat atactttact taccatccac acagtgcatg
tagaaccgag ccaagaaaca gcttcccata acaaaggctt atggattttg
gtgccctctg cgattttggc agcttttctg ctgatttgga gcgtaaaatg
ttgcagagcc cagctagaag ccaggaggag cagacaccct gctgatggag
cccaacaaga aagatgttgt gtccctcctg gtgagcgctg tcccagtgca
cccgataatg gcgaagaaaa tgtgcctctt tcaggaaaag ta

[0054] The amino acid sequence of a representative human B7-H5 IgV domain human IgG4 fusion protein is (**SEQ ID NO:3**) (B7-H5 sequences are shown in boldface; IgG4 sequences are shown underlined):

IFPLAFFIYV PMNEQIVIGR LDEDIILPSS FERGSEVVIH WKYQDSYKVH
SYYKGSDHLE SQDPRYANRT SLFYNEIQNG NASLFFRRVS LLDEGIYTCY
VGTAIQVITN KVVLKVGVFL TPVMKYEKES KYGPPCPPCP APEFLGGPSV
FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK
PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK
GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN
YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS
LSLSPG

[0055] The fusion protein may additionally comprise an N-terminal leader sequence (such residues 1-22 of **SEQ ID NO:1**, *i.e.*, the naturally occurring B7-H5 leader sequence (**SEQ ID NO:4**):

MKAQTALSFF LILITSLSGS QG

[0056] A DNA sequence encoding the naturally occurring B7-H5 leader sequence is (**SEQ ID NO:5**):

atgaaggccc agaccgccct gtccttcttc ctgatcctga tcacctccct
gtccggcagc caggga

[0057] Although the B7-H5 sequences are shown fused to a human IgG4 region, in accordance with the present invention, such sequences can alternatively be fused to any Ig isotype, or indeed to any other protein.

[0058] A DNA sequence encoding human B7-H5IgV-hIgG4 (**SEQ ID NO:3**) is (**SEQ ID NO:6**):

atcttccctc tggccttctt catctacgtg cccatgaacg agcagatcgt
gatcggccgg ctggacgagg atattatcct gccctccagc ttcgagcggg
gctccgaggt cgtgatccac tggaagtacc aggactccta caaggtgcac
tcctactaca agggctccga ccacctggaa tcccaggacc ccagatacgc
caaccggacc agcctgttct acaacgagat ccagaacggc aacgcctccc
tgttcttccg gcgagtgtcc ctgctggatg agggcatcta cacctgttac
gtgggcaccg ccatccaagt gatcaccaac aaggtggtgc tgaaagtggg
cgtgttcctg acccccgtga tgaagtacga gaaagagtct aagtacggcc
ctccctgccc cccttgtcct gcccctgaat ttctgggcgg accctctgtg
ttcctgttcc ccccaaagcc caaggacacc ctgatgatct cccggacccc
cgaagtgacc tgcgtggtgg tggatgtgtc ccaggaagat cccgaggtgc

agttcaattg gtacgtggac ggcgtggaag tgcacaacgc caagaccaag
cccagagagg aacagttcaa ctccacctac cgggtggtgt ccgtgctgac
cgtgctgcac caggattggc tgaacggcaa agagtacaag tgcaaggtgt
ccaacaaggg cctgcccagc tccatcgaaa agaccatctc caaggccaag
ggccagcccc gggaacccca ggtgtacaca ctgcctccaa gccaggaaga
gatgaccaag aaccaggtgt ccctgacctg tctcgtgaag ggcttctacc
cctccgatat cgccgtggaa tgggagtcca acggccagcc tgagaacaac
tacaagacca cccccctgt gctggactcc gacggctctt tcttcctgta
ctcccgcctg accgtggaca agtccagatg gcaggaaggc aacgtgttct
cctgctccgt gatgcacgag gccctgcaca accactacac ccagaagtcc
ctgtccctga gccccggc

[0059] An amino acid sequence of a representative human B7-H5 human IgG4P fusion protein is including the extracellular domain of B7-H5 is (**SEQ ID NO:24**) (B7-H5ECD-hIgG4P with B7-H5 ECD aa1-340 underlined, and the Serine 228 to Proline mutation double underline)

IFPLAFFIYVPMNEQIVIGRLDEDIILPSSFERGSEVVIHWKYQDSYKVHSYYKGSDHLESQ
DPRYANRTSLFYNEIQNGNASLFFRRVSLLDEGIYTCYVGTAIQVITNKVVLKVGVFLTPVM
KYEKRNTNSFLICSVLSVYPRPIITWKMDNTPISENNMEETGSLDSFSINSPLNITGSNSSY
ECTIENSLLKQTWTGRWTMKDGLHKMQSEHVSLSCQPVNDYFSPNQDFKVTWSRMKSGTFSV
LAYYLSSSQNTIINESRFSWNKELINQSDFSMNLMDLNLSDSGEYLCNISSDEYTLLTIHTV
HVEPSQETESSKYGPP**CP**S**CP**APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPE
VQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPG

[0060] The signal sequence can be the native signal sequence (**SEQ ID NO:29**)

MKAQTALSFFLILITSLSGSQG

[0061] The fusion protein can be encoded by the nucleic acid sequence (**SEQ ID NO:25**)

atgaaggcccagaccgccctgtccttcttcctgatcctgatcacctccctgtccggcagcca
gggaatcttccctctggccttcttcatctacgtgcccatgaacgagcagatcgtgatcggcc
ggctggacgaggatattatcctgccctccagcttcgagcggggctccgaggtcgtgatccac
tggaagtaccaggactcctacaaggtgcactcctactacaagggctccgaccacctggaatc
ccaggaccccagatacgccaaccggaccagcctgttctacaacgagatccagaacggcaacg
cctccctgttcttccggcgagtgtccctgctggatgagggcatctacacctgttacgtgggc
accgccatccaagtgatcaccaacaaggtggtgctgaaagtgggcgtgttcctgacccccgt
gatgaagtacgagaagcggaataccaactctttcctgatctgctccgtgctgtccgtgtacc
ctcggcccatcatcacctggaagatggacaacaccccccatctccgagaacaacatggaagag
acaggctccctggactccttctccatcaactcccccctgaacattaccggctccaactcctc

ctacgagtgcaccatcgagaactccctgctgaagcagacctggaccggcagatggactatga
aggacggcctgcacaagatgcagtccgagcacgtgtccctgtcctgccagcccgtgaacgac
tacttcagccccaaccaggacttcaaagtgacctggtcccggatgaagtccggcaccttcag
cgtgctggcctactacctgtccagctcccagaacaccatcatcaacgagtcccggttctcct
ggaacaaagagctgatcaaccagtccgacttctccatgaacctgatggacctgaacctgtcc
gacagcggcgagtacctgtgcaacatctccagcgacgagtacaccctgctgaccatccacac
cgtgcacgtggaaccctcccaggaaaccgagtctaagtacggccctccctgcccaccttgtc
ccgcccctgaatttctgggcggaccctctgtgttcctgttccccccaaagcccaaggacacc
ctgatgatctcccggaccccgaagtgacatgcgtggtggtggatgtgtcccaggaagatcc
cgaggtgcagttcaattggtacgtggacggcgtggaagtgcacaacgccaagaccaagccca
gagaggaacagttcaactccacctaccgggtggtgtctgtgctgaccgtgctgcaccaggac
tggctgaacggcaaagagtacaagtgcaaggtgtccaacaagggcctgcccagctccatcga
aaagaccatctccaaggccaagggccagccccgggaaccccaggtgtacacactgcctccaa
gccaggaagagatgaccaagaaccaggtgtccctgacttgcctcgtgaagggcttctacccc
tccgatatcgccgtggaatgggagtccaacggccagcctgagaacaactacaagaccacccc
ccctgtgctggactccgacggctctttcttcctgtactcccgcctgaccgtggacaagtcca
gatggcaggaaggcaacgtgttctcctgcagcgtgatgcacgaggccctgcacaaccactac
acccagaagtccctgagcctgtcccccggctga

[0062] In contrast to human B7-H4 which is widely expressed, human B7-H5 is found to exhibit more limited expression (*e.g.*, expressed in the gut, kidney, lung, epithelial cells and lymphocytes). Human HHLA2 is found on chromosome 3q13.33 near B7.1 and B7.2. B7-H5 is constitutively expressed on macrophages and inducible on dendritic cells (DC).

B. CD28H

[0063] CD28H, also referred to herein and elsewhere as H7CR, is the counter-receptor for B7-H5. Accordingly, the terms CD28H and H7CR are used interchangeably herein. As used herein, the term "native CD28H" (also "native H7CR") refers to the naturally occurring counter-receptor of B7-H5, or variations thereof. CD28H is expressed by T cells, NK cells, and plasmacytoid dendritic cells. The human CD28H polypeptide is otherwise referred to as transmembrane and immunoglobulin domain containing 2 (TMIGD2) in the literature/databases (Rahimi, N. *et al.* (Epub 2012 Mar 14) "*Identification Of IGPR-1 As A Novel Adhesion Molecule Involved In Angiogenesis*," Molec. Biol. Cell. 23(9):1646-1656) but the function of CD28H was not previously elucidated. Non-limiting examples of Accession

Numbers for the amino acid sequence of such native CD28H molecules include: Q96BF3-1 (homo sapiens), Q96BF3-2 (homo sapiens), NP_653216.1 (GI:21389429; homo sapiens) and NP_653216.2 (GI:281306838; homo sapiens). A representative amino acid sequence (Q96BF3-2) of the native CD28H molecule is provided below as **SEQ ID NO:7**:

```
MGSPGMVLGL LVQIWALQEA SSLSVQQGPN LLQVRQGSQA TLVCQVDQAT
AWERLRVKWT KDGAILCQPY ITNGSLSLGV CGPQGRLSWQ APSHLTLQLD
PVSLNHSGAY VCWAAVEIPE LEEAEGNITR LFVDPDDPTQ NRNRIASFPG
FLFVLLGVGS MGVAAIVWGA WFWGRRSCQQ RDSGNSPGNA FYSNVLYRPR
GAPKKSEDCS GEGKDQRGQS IYSTSFPQPA PRQPHLASRP CPSPRPCPSP
RPGHPVSMVR VSPRPSPTQQ PRPKGFPKVG EE
```

[0064] A DNA sequence encoding human CD28H (**SEQ ID NO:7**) is (**SEQ ID NO:8**):

```
atggggtccc cgggcatggt gctgggcctc ctggtgcaga tctgggccct
gcaagaagcc tcaagcctga gcgtgcagca ggggcccaac ttgctgcagg
tgaggcaggg cagtcaggcg accctggtct gccaggtgga ccaggccaca
gcctgggaac ggctccgtgt taagtggaca aaggatgggg ccatcctgtg
tcaaccgtac atcaccaacg gcagcctcag cctgggggtc tgcgggcccc
agggacggct ctcctggcag gcacccagcc atctcaccct gcagctggac
cctgtgagcc tcaaccacag cggggcgtac gtgtgctggg cggccgtaga
gattcctgag ttggaggagg ctgagggcaa cataacaagg ctctttgtgg
acccagatga ccccacacag aacagaaacc ggatcgcaag cttcccagga
ttcctcttcg tgctgctggg ggtgggaagc atgggtgtgg ctgcgatcgt
gtggggtgcc tggttctggg gccgccgcag ctgccagcaa agggactcag
gtaacagccc aggaaatgca ttctacagca acgtcctata ccggccccgg
ggggccccaa agaagagtga ggactgctct ggagagggga aggaccagag
gggccagagc atttattcaa cctccttccc gcaaccggcc ccccgccagc
cgcacctggc gtcaagaccc tgccccagcc cgagaccctg ccccagcccc
aggcccggcc accccgtctc tatggtcagg gtctctccta gaccaagccc
cacccagcag ccgaggccaa aagggttccc caaagtggga gaggag
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C. Definitions

[0065] As used herein, a molecule is said to be able to "**immunospecifically bind**" a second molecule if such binding exhibits the specificity and affinity of an antibody to its cognate antigen. Antibodies are said to be capable of "**immunospecifically binding**" to a target region or conformation ("epitope") of an antigen (and in particular, the antigen B7-H5) if such binding involves the antigen recognition site of the immunoglobulin molecule. An antibody that immunospecifically binds to a particular antigen may bind to other antigens with lower affinity if the other antigen has some sequence or conformational similarity that is recognized by the antigen recognition site as determined by, *e.g.*, immunoassays, BIACORE® assays, or other assays known in the art, but would not bind to a totally unrelated

antigen. Preferably, however, antibodies (and their antigen binding fragments) will not cross-react with other antigens. Antibodies may also bind to other molecules in a way that is not immunospecific, such as to FcR receptors, by virtue of binding domains in other regions/domains of the molecule that do not involve the antigen recognition site, such as the Fc region.

[0066] The term "**substantially**," as used in the context of binding or exhibited effect, is intended to denote that the observed effect is physiologically or therapeutically relevant. Thus, for example, a molecule is able to substantially block an activity of B7-H5 if the extent of blockage is physiologically or therapeutically relevant (for example if such extent is greater than 60% complete, greater than 70% complete, greater than 75% complete, greater than 80% complete, greater than 85% complete, greater than 90% complete, greater than 95% complete, or greater than 97% complete). Similarly, a molecule is said to have substantially the same immunospecificity and/or characteristic as another molecule, if such immunospecificities and characteristics are greater than 60% identical, greater than 70% identical, greater than 75% identical, greater than 80% identical, greater than 85% identical, greater than 90% identical, greater than 95% identical, or greater than 97% identical).

[0067] As used herein, the term "**subject**" is intended to denote a mammal such as a non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats etc.) and a primate (*e.g.*, monkey and human), most preferably a human. The term "**patient**" is intended to denote a subject receiving a composition of the present invention for a diagnostic, therapeutic or prophylactic purpose.

[0068] As used herein, the term "**antibody**" is intended to denote an immunoglobulin molecule that possesses a "variable region" antigen recognition site. The term "variable region" is intended to distinguish such domain of the immunoglobulin from domains that are broadly shared by antibodies (such as an antibody Fc domain). The variable region comprises a "hypervariable region" whose residues are responsible for antigen binding. The hypervariable region comprises amino acid residues from a

"Complementarity Determining Region" or "**CDR**" (i.e., typically at approximately residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and at approximately residues 27-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (i.e., residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk, 1987, J. Mol. Biol. 196:901-917). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined. The term antibody includes monoclonal antibodies, multi-specific antibodies, human antibodies, humanized antibodies, synthetic antibodies, chimeric antibodies, camelized antibodies (See e.g., Muyldermans et al., 2001, *Trends Biochem. Sci.* 26:230; Nuttall et al., 2000, *Cur. Pharm. Biotech.* 1:253; Reichmann and Muyldermans, 1999, *J. Immunol. Meth.* 231:25; International Publication Nos. WO 94/04678 and WO 94/25591; U.S. Patent No. 6,005,079), single-chain Fvs (scFv) (see, e.g., see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994)), single chain antibodies, disulfide-linked Fvs (sdFv), intrabodies, and anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id and anti-anti-Id antibodies to the disclosed B7-H5 antibodies). In particular, such antibodies include immunoglobulin molecules of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass.

[0069] As used herein, the term "**antigen binding fragment**" of an antibody refers to one or more portions of an antibody that contain the antibody's Complementarity Determining Regions ("CDRs") and optionally the framework residues that comprise the antibody's "variable region" antigen recognition site, and exhibit an ability to immunospecifically bind antigen. Such fragments include Fab', F(ab')2, Fv, single chain (ScFv),and mutants thereof, naturally occurring variants, and fusion proteins comprising

the antibody's "variable region" antigen recognition site and a heterologous protein (e.g., a toxin, an antigen recognition site for a different antigen, an enzyme, a receptor or receptor ligand, etc.). As used herein, the term "**fragment**" refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, at least 150 contiguous amino acid residues, at least 175 contiguous amino acid residues, at least 200 contiguous amino acid residues, or at least 250 contiguous amino acid residues.

[0070] Human, chimeric or humanized derivatives of anti-human B7-H5 antibodies are particularly preferred for in vivo use in humans, however, murine antibodies or antibodies of other species may be advantageously employed for many uses (for example, in vitro or in situ detection assays, acute in vivo use, etc.). A humanized antibody may comprises amino acid residue substitutions, deletions or additions in one or more non-human CDRs. The humanized antibody derivative may have substantially the same binding, stronger binding or weaker binding when compared to a non-derivative humanized antibody. In specific embodiments, one, two, three, four, or five amino acid residues of the CDR have been substituted, deleted or added (i.e., mutated). Completely human antibodies are particularly desirable for therapeutic treatment of human subjects.

[0071] Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences (see U.S. Patent Nos. 4,444,887 and 4,716,111; and International Publication Nos. WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO

96/33735, and WO 91/10741). Human antibodies can be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized using conventional methodologies with a selected antigen, e.g., all or a portion of a B7-H5 polypeptide. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology (see, e.g., U.S. Patent No. 5,916,771). The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93, which is incorporated herein by reference in its entirety). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., International Publication Nos. WO 98/24893, WO 96/34096, and WO 96/33735; and U.S. Patent Nos. 5,413,923, 5,625,126, 5,633,425, 5,569,825, 5,661,016, 5,545,806, 5,814,318, and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Medarex (Princeton, NJ) can be engaged to provide human antibodies

directed against a selected antigen using technology similar to that described above.

[0072] A "**chimeric antibody**" is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules such as antibodies having a variable region derived from a non-human antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, 1985, Science 229:1202; Oi et al., 1986, *BioTechniques* 4:214; Gillies et al., 1989, *J. Immunol. Methods* 125:191-202; and U.S. Patent Nos. 6,311,415, 5,807,715, 4,816,567, and 4,816,397. Chimeric antibodies comprising one or more CDRs from a non-human species and framework regions from a human immunoglobulin molecule can be produced using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; International Publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, 1991, *Molecular Immunology* 28(4/5):489-498; Studnicka et al., 1994, *Protein Engineering* 7:805; and Roguska et al., 1994, Proc. Natl. Acad. Sci. USA 91:969), and chain shuffling (U.S. Patent No. 5,565,332).

[0073] The invention particularly concerns "**humanized antibodies**" (see, e.g., European Patent Nos. EP 239,400, EP 592,106, and EP 519,596; International Publication Nos. WO 91/09967 and WO 93/17105; U.S. Patent Nos. 5,225,539, 5,530,101, 5,565,332, 5,585,089, 5,766,886, and 6,407,213; and Padlan, 1991, *Molecular Immunology* 28(4/5):489-498; Studnicka *et al.*, 1994, *Protein Engineering* 7(6):805-814; Roguska *et al.*, 1994, *PNAS* 91:969-973; Tan *et al.*, 2002, *J. Immunol.* 169:1119-1125; Caldas *et al.*, 2000, *Protein Eng.* 13:353-360; Morea *et al.*, 2000, *Methods* 20:267-79; Baca *et al.*, 1997, *J. Biol. Chem.* 272:10678-10684; Roguska *et al.*, 1996, *Protein Eng.* 9:895-904; Couto *et al.*, 1995, *Cancer Res.* 55 (23 Supp):5973s-5977s; Couto *et al.*, 1995, *Cancer Res.* 55:1717-22; Sandhu, 1994, *Gene* 150:409-10; Pedersen *et al.*, 1994, *J. Mol. Biol.* 235:959-973; Jones *et al.*, 1986, *Nature* 321:522-525; Reichmann *et al.*, 1988, *Nature* 332:323-329; and Presta, 1992, *Curr. Op. Struct. Biol.* 2:593-596). As used

herein, the term "humanized antibody" refers to an immunoglobulin comprising a human framework region and one or more CDR's from a non-human (usually a mouse or rat) immunoglobulin. The non-human immunoglobulin providing the CDR's is called the "donor" and the human immunoglobulin providing the framework is called the "acceptor." Constant regions need not be present, but if they are, they must be substantially identical to human immunoglobulin constant regions, i.e., at least about 85-90%, preferably about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDR's, are substantially identical to corresponding parts of natural human immunoglobulin sequences. A humanized antibody is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. For example, a humanized antibody would not encompass a typical chimeric antibody, because, e.g., the entire variable region of a chimeric antibody is non-human. One says that the donor antibody has been "humanized," by the process of "humanization," because the resultant humanized antibody is expected to bind to the same antigen as the donor antibody that provides the CDR's. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or a non-human primate having the desired specificity, affinity, and capacity. In some instances, Framework Region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin that immunospecifically binds to

an Fc RIIB polypeptide, that has been altered by the introduction of amino acid residue substitutions, deletions or additions (i.e., mutations).

[0074] DNA sequences coding for preferred human acceptor framework sequences include but are not limited to FR segments from the human germline VH segment VH1-18 and JH6 and the human germline VL segment VK-A26 and JK4. In a specific embodiment, one or more of the CDRs are inserted within framework regions using routine recombinant DNA techniques. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (*see*, *e.g.*, Chothia *et al.*, 1998, "*Structural Determinants In The Sequences Of Immunoglobulin Variable Domain*," *J. Mol. Biol.* 278: 457-479 for a listing of human framework regions).

[0075] A humanized or chimeric B7-H5 antibody can include substantially all of at least one, and typically two, variable domains in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, a B7-H5 antibody also includes at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The constant domains of the B7-H5 antibodies may be selected with respect to the proposed function of the antibody, in particular the effector function which may be required. In some embodiments, the constant domains of the B7-H5 antibodies are (or comprise) human IgA, IgD, IgE, IgG or IgM domains. In a specific embodiment, human IgG constant domains, especially of the IgG1 and IgG3 isotypes are used, when the humanized B7-H5 antibodies is intended for therapeutic uses and antibody effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) activity are needed. In alternative embodiments, IgG2 and IgG4 isotypes are used when the B7-H5 antibody is intended for therapeutic purposes and antibody effector function is not required. The invention encompasses Fc constant domains comprising one or more amino acid modifications which

alter antibody effector functions such as those disclosed in U.S. Patent Application Publication Nos. 2005/0037000 and 2005/0064514.

[0076] In some embodiments, the B7-H5 antibody contains both the light chain as well as at least the variable domain of a heavy chain. In other embodiments, the B7-H5 antibody may further include one or more of the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. The antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. In some embodiments, the constant domain is a complement fixing constant domain where it is desired that the antibody exhibit cytotoxic activity, and the class is typically IgG1. In other embodiments, where such cytotoxic activity is not desirable, the constant domain may be of the IgG2 class. The B7-H5 antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art.

[0077] The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor CDR or the consensus framework may be mutagenized by substitution, insertion or deletion of at least one residue so that the CDR or framework residue at that site does not correspond to either the consensus or the donor antibody. Such mutations, however, are preferably not extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental framework region (FR) and CDR sequences, more often 90%, and most preferably greater than 95%. Humanized antibodies can be produced using variety of techniques known in the art, including, but not limited to, CDR-grafting (European Patent No. EP 239,400; International Publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, *Molecular Immunology* 28(4/5):489-498; Studnicka *et al.*, 1994, *Protein Engineering* 7(6):805-814; and Roguska *et al.*, 1994, *Proc. Natl. Acad. Sci.* 91:969-973), chain shuffling (U.S. Patent No. 5,565,332), and techniques disclosed in, *e.g.*, U.S. Patent Nos.

6,407,213, 5,766,886, 5,585,089, International Publication No. WO 9317105, Tan *et al.*, 2002, *J. Immunol.* 169:1119-25, Caldas *et al.*, 2000, *Protein Eng.* 13:353-60, Morea *et al.*, 2000, *Methods* 20:267-79, Baca *et al.*, 1997, *J. Biol. Chem.* 272:10678-84, Roguska *et al.*, 1996, *Protein Eng.* 9:895-904, Couto *et al.*, 1995, *Cancer Res.* 55 (23 Supp):5973s-5977s, Couto *et al.*, 1995, *Cancer Res.* 55:1717-22, Sandhu, 1994, *Gene* 150:409-10, Pedersen *et al.*, 1994, J. *Mol. Biol.* 235:959-73, Jones *et al.*, 1986, *Nature* 321:522-525, Riechmann *et al.*, 1988, *Nature* 332:323, and Presta, 1992, *Curr. Op. Struct. Biol.* 2:593-596. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (*See, e.g.*, Queen *et al.*, U.S. Patent No. 5,585,089; U.S. Publication Nos. 2004/0049014 and 2003/0229208; U.S. Patent Nos. 6,350,861; 6,180,370; 5,693,762; 5,693,761; 5,585,089; and 5,530,101 and Riechmann *et al.*, 1988, *Nature* 332:323).

[0078] The antibodies used in the methods of the present invention may be monospecific. Also of interest are bispecific antibodies, trispecific antibodies or antibodies of greater multispecificity that exhibit specificity to different targets in addition to B7-H5, such as other molecules of the immune system. For example, such antibodies may bind to both B7-H5 and to an antigen that is important for targeting the antibody to a particular cell type or tissue (for example, to an antigen associated with a cancer antigen of a tumor being treated). In another embodiment, such multispecific antibody binds to molecules (receptors or ligands) involved in alternative or supplemental immunomodulatory pathways, such as CTLA4, TIM3, TIM4, OX40, CD40, GITR, 4-1-BB, CD27/CD70, ICOS, B7-H4, LIGHT, PD-1 or LAG3, in order to diminish further modulate the immunomodulatory effects. Furthermore, the multispecific antibody may bind to effecter molecules such as cytokines (e.g., IL-7, IL-15, IL-12, IL-4 TGF-beta, IL-10, IL-17, IFNg,

Flt3, BLys) and chemokines (e.g., CCL21), which may be particularly relevant for down-modulating both acute and chronic immune responses.

[0079] The antibodies of the present invention may be produced by any method known in the art useful for the production of polypeptides, e.g., in vitro synthesis, recombinant DNA production, and the like. Preferably, the antibodies are produced by recombinant DNA technology. The B7-H5 antibodies may be produced using recombinant immunoglobulin expression technology. The recombinant production of immunoglobulin molecules, including humanized antibodies are described in U.S. Patent No. 4,816,397 (Boss et al.), U.S. Patent Nos. 6,331,415 and 4,816,567 (both to Cabilly et al.), U.K. patent GB 2,188,638 (Winter et al.), and U.K. patent GB 2,209,757. Techniques for the recombinant expression of immunoglobulins, including humanized immunoglobulins, can also be found, in Goeddel et al., Gene Expression Technology Methods in Enzymology Vol. 185 Academic Press (1991), and Borreback, Antibody Engineering, W. H. Freeman (1992). Additional information concerning the generation, design and expression of recombinant antibodies can be found in Mayforth, Designing Antibodies, Academic Press, San Diego (1993).

[0080] An exemplary process for the production of the recombinant chimeric B7-H5 antibodies can include the following: a) constructing, by conventional molecular biology methods, an expression vector that encodes and expresses an antibody heavy chain in which the CDRs and variable region of a murine anti-human B7-H5 monoclonal antibody are fused to an Fc region derived from a human immunoglobulin, thereby producing a vector for the expression of a chimeric antibody heavy chain; b) constructing, by conventional molecular biology methods, an expression vector that encodes and expresses an antibody light chain of the murine anti-human B7-H5 monoclonal antibody, thereby producing a vector for the expression of chimeric antibody light chain; c) transferring the expression vectors to a host cell by conventional molecular biology methods to produce a transfected host cell for the expression of chimeric antibodies; and d)

culturing the transfected cell by conventional cell culture techniques so as to produce chimeric antibodies.

[0081] An exemplary process for the production of the recombinant humanized B7-H5 antibodies can include the following: a) constructing, by conventional molecular biology methods, an expression vector that encodes and expresses an anti-human B7-H5 heavy chain in which the CDRs and a minimal portion of the variable region framework that are required to retain donor antibody binding specificity are derived from a non-human immunoglobulin, such as a murine anti-human B7-H5 monoclonal antibody, and the remainder of the antibody is derived from a human immunoglobulin, thereby producing a vector for the expression of a humanized antibody heavy chain; b) constructing, by conventional molecular biology methods, an expression vector that encodes and expresses an antibody light chain in which the CDRs and a minimal portion of the variable region framework that are required to retain donor antibody binding specificity are derived from a non-human immunoglobulin, such as a murine anti-human B7-H5 monoclonal antibody, and the remainder of the antibody is derived from a human immunoglobulin, thereby producing a vector for the expression of humanized antibody light chain; c) transferring the expression vectors to a host cell by conventional molecular biology methods to produce a transfected host cell for the expression of humanized antibodies; and d) culturing the transfected cell by conventional cell culture techniques so as to produce humanized antibodies.

[0082] With respect to either exemplary method, host cells may be co-transfected with such expression vectors, which may contain different selectable markers but, with the exception of the heavy and light chain coding sequences, are preferably identical. This procedure provides for equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA or both. The host cell used to express the recombinant B7-H5 antibody can be either a bacterial cell such as

Escherichia coli, or more preferably a eukaryotic cell (e.g., a Chinese hamster ovary (CHO) cell or a HEK-293 cell). The choice of expression vector is dependent upon the choice of host cell, and may be selected so as to have the desired expression and regulatory characteristics in the selected host cell. Other cell lines that may be used include, but are not limited to, CHO-K1, NSO, and PER.C6 (Crucell, Leiden, Netherlands).

[0083] Any of the above-described antibodies can be used to generate anti-idiotype antibodies using techniques well known to those skilled in the art (see, e.g., Greenspan, N.S. et al. (1989) "*Idiotypes: Structure And Immunogenicity,*" FASEB J. 7:437-444; and Nisinoff, A. (1991) "*Idiotypes: Concepts And Applications*," J. Immunol. 147(8):2429-2438).

[0084] The binding properties of any of the above antibodies can, if desired, be further improved by screening for variants that exhibit such desired characteristics. For example, such antibodies can be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains, such as Fab and Fv or disulfide-bond stabilized Fv, expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage, including fd and M13. The antigen binding domains are expressed as a recombinantly fused protein to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the immunoglobulins, or fragments thereof, of the present invention include those disclosed in Brinkman, U. *et al.* (1995) "*Phage Display Of Disulfide-Stabilized Fv Fragments*," *J. Immunol. Methods*, 182:41-50, 1995; Ames, R.S. *et al.* (1995) "*Conversion Of Murine Fabs Isolated From A Combinatorial Phage Display Library To Full Length Immunoglobulins*," *J. Immunol. Methods*, 184:177-186; Kettleborough, C.A.

et al. (1994) "*Isolation Of Tumor Cell-Specific Single-Chain Fv From Immunized Mice Using Phage-Antibody Libraries And The Re-Construction Of Whole Antibodies From These Antibody Fragments*," Eur. J. Immunol., 24:952-958, 1994; Persic, L. *et al.* (1997) "*An Integrated Vector System For The Eukaryotic Expression Of Antibodies Or Their Fragments After Selection From Phage Display Librarie*s," *Gene*, 187:9-18; Burton, D.R. *et al.* (1994) "*Human Antibodies From Combinatorial Libraries*," Adv. Immunol. 57:191-280; PCT Publications WO 92/001047; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patents Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108.

[0085] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including humanized antibodies, or any other desired fragments, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art (such as those disclosed in PCT Publication WO 92/22324; Mullinax, R.L. *et al.* (1992) "*Expression Of A Heterodimeric Fab Antibody Protein In One Cloning Step*," *BioTechniques*, 12(6):864-869; and Sawai *et al.* (1995) "*Direct Production Of The Fab Fragment Derived From The Sperm Immobilizing Antibody Using Polymerase Chain Reaction And cDNA Expression Vectors*," *Am. J. Reprod. Immunol.* 34:26-34; and Better, M. *et al.* (1988) "*Escherichia coli Secretion Of An Active Chimeric Antibody Fragment*," Science 240:1041-1043). Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patent Nos. 4,946,778 and 5,258,498; Huston, J.S. *et al.* (1991) "*Protein Engineering Of Single-Chain Fv Analogs And Fusion Proteins*," Methods in Enzymology 203:46-88; Shu, L. *et al.*, "*Secretion Of A Single-Gene-Encoded Immunoglobulin From Myeloma Cells*," Proc. Natl. Acad. Sci. (USA) 90:7995-7999; and Skerra. A. *et al.* (1988) "*Assembly Of A*

Functional Immunoglobulin Fv Fragment In Escherichia coli," Science 240:1038-1040.

[0086] Phage display technology can be used to increase the affinity of an antibody for B7-H5. This technique would be useful in obtaining high affinity antibodies that could be used in the disclosed combinatorial methods. This technology, referred to as affinity maturation, employs mutagenesis or CDR walking and re-selection using such receptors or ligands (or their extracellular domains) or an antigenic fragment thereof to identify antibodies that bind with higher affinity to the antigen when compared with the initial or parental antibody (See, e.g., Glaser, S.M. *et al.* (1992) "*Antibody Engineering By Codon-Based Mutagenesis In A Filamentous Phage Vector System*," J. Immunol. 149:3903-3913). Mutagenizing entire codons rather than single nucleotides results in a semi-randomized repertoire of amino acid mutations. Libraries can be constructed consisting of a pool of variant clones each of which differs by a single amino acid alteration in a single CDR and which contain variants representing each possible amino acid substitution for each CDR residue. Mutants with increased binding affinity for the antigen can be screened by contacting the immobilized mutants with labeled antigen. Any screening method known in the art can be used to identify mutant antibodies with increased avidity to the antigen (*e.g.*, ELISA) (see, *e.g.*, Wu, H. *et al.* (1998) "*Stepwise In Vitro Affinity Maturation Of Vitaxin, An Alphav Beta3-Specific Humanized Mab*," Proc. Natl. Acad. Sci. (USA) 95(11):6037-6042; Yelton, D.E. *et al.* (1995) "*Affinity Maturation Of The BR96 Anti-Carcinoma Antibody By Codon-Based Mutagenesis*," J. Immunol. 155:1994-2004). CDR walking which randomizes the light chain may be used possible (see, Schier *et al.* (1996) "*Isolation Of Picomolar Affinity Anti-C-Erbb-2 Single-Chain Fv By Molecular Evolution Of The Complementarity Determining Regions In The Center Of The Antibody Binding Site*," J. Mol. Biol. 263:551-567).

[0087] The invention thus contemplates the use of random mutagenesis to identify improved CDRs. Phage display technology can alternatively be used to increase (or decrease) CDR affinity. This technology, referred to as

affinity maturation, employs mutagenesis or "CDR walking" and re-selection uses the target antigen or an antigenic fragment thereof to identify antibodies having CDRs that bind with higher (or lower) affinity to the antigen when compared with the initial or parental antibody (see, e.g., Glaser, S.M. et al. (1992) "*Antibody Engineering By Codon-Based Mutagenesis In A Filamentous Phage Vector System*," J. Immunol. 149:3903-3913). Mutagenizing entire codons rather than single nucleotides results in a semi-randomized repertoire of amino acid mutations. Libraries can be constructed consisting of a pool of variant clones each of which differs by a single amino acid alteration in a single CDR and which contain variants representing each possible amino acid substitution for each CDR residue. Mutants with increased (or decreased) binding affinity for the antigen can be screened by contacting the immobilized mutants with labeled antigen. Any screening method known in the art can be used to identify mutant antibodies with increased (or decreased) avidity to the antigen (e.g., ELISA) (see, Wu, H. et al. (1998) "*Stepwise In Vitro Affinity Maturation Of Vitaxin, An Alphav Beta3-Specific Humanized Mab*," Proc. Natl. Acad. Sci. (USA) 95(11):6037-6042; Yelton, D.E. et al. (1995) "*Affinity Maturation Of The BR96 Anti-Carcinoma Antibody By Codon-Based Mutagenesis*," J. Immunol. 155:1994-2004). CDR walking which randomizes the light chain may be used possible (see, Schier et al. (1996) "*Isolation Of Picomolar Affinity Anti-C-Erbb-2 Single-Chain Fv By Molecular Evolution Of The Complementarity Determining Regions In The Center Of The Antibody Binding Site*," J. Mol. Biol. 263:551-567).

[0088] Methods for accomplishing such affinity maturation are described for example in: Krause, J.C. *et al.* (2011) "*An Insertion Mutation That Distorts Antibody Binding Site Architecture Enhances Function Of A Human Antibody*," MBio. 2(1) pii: e00345-10. doi: 10.1128/mBio.00345-10; Kuan, C.T. *et al.* (2010) "*Affinity-Matured Anti-Glycoprotein NMB Recombinant Immunotoxins Targeting Malignant Gliomas And Melanomas*," Int. J. Cancer 10.1002/ijc.25645; Hackel, B.J. *et al.* (2010) "*Stability And CDR Composition Biases Enrich Binder Functionality Landscapes*," J. Mol. Biol. 401(1):84-96; Montgomery, D.L. *et al.* (2009) "*Affinity Maturation And*

Characterization Of A Human Monoclonal Antibody Against HIV-1 gp41," MAbs 1(5):462-474; Gustchina, E. *et al.* (2009) "*Affinity Maturation By Targeted Diversification Of The CDR-H2 Loop Of A Monoclonal Fab Derived From A Synthetic Naïve Human Antibody Library And Directed Against The Internal Trimeric Coiled-Coil Of Gp41 Yields A Set Of Fabs With Improved HIV-1 Neutralization Potency And Breadth*," Virology 393(1):112-119; Finlay, W.J. *et al.* (2009) "*Affinity Maturation Of A Humanized Rat Antibody For Anti-RAGE Therapy: Comprehensive Mutagenesis Reveals A High Level Of Mutational Plasticity Both Inside And Outside The Complementarity-Determining Regions*," J. Mol. Biol. 388(3):541-558; Bostrom, J. *et al.* (2009) "*Improving Antibody Binding Affinity And Specificity For Therapeutic Development*," Methods Mol. Biol. 525:353-376; Steidl, S. *et al.* (2008) "*In Vitro Affinity Maturation Of Human GM-CSF Antibodies By Targeted CDR-Diversification*," Mol. Immunol. 46(1):135-144; and Barderas, R. *et al.* (2008) "Affinity maturation of antibodies assisted by *in silico* modeling," Proc. Natl. Acad. Sci. (USA) 105(26):9029-9034.

[0089] The invention particularly contemplates the production and use of "**derivatives**" of any of the above-described antibodies and their antigen-binding fragments.

[0090] The term "derivative" refers to an antibody or antigen-binding fragment thereof that immunospecifically binds to an antigen but which comprises, one, two, three, four, five or more amino acid substitutions, additions, deletions or modifications relative to a "parental" (or wild-type) molecule. Such amino acid substitutions or additions may introduce naturally occurring (*i.e.*, DNA-encoded) or non-naturally occurring amino acid residues. The term "derivative" encompasses, for example, chimeric or humanized variants of any of antibodies 1.3, 4.5 or 7.8, as well as variants having altered CH1, hinge, CH2, CH3 or CH4 regions, so as to form, for example antibodies, *etc.*, having variant Fc regions that exhibit enhanced or impaired effector or binding characteristics. The term "derivative" additionally encompasses non-amino acid modifications, for example, amino

acids that may be glycosylated (*e.g.*, have altered mannose, 2-N-acetylglucosamine, galactose, fucose, glucose, sialic acid, 5-N-acetylneuraminic acid, 5-glycolneuraminic acid, *etc.* content), acetylated, pegylated, phosphorylated, amidated, derivatized by known protecting/blocking groups, proteolytic cleavage, linked to a cellular ligand or other protein, *etc.* In some embodiments, the altered carbohydrate modifications modulate one or more of the following: solubilization of the antibody, facilitation of subcellular transport and secretion of the antibody, promotion of antibody assembly, conformational integrity, and antibody-mediated effector function. In a specific embodiment the altered carbohydrate modifications enhance antibody mediated effector function relative to the antibody lacking the carbohydrate modification. Carbohydrate modifications that lead to altered antibody mediated effector function are well known in the art (for example, *see* Shields, R.L. *et al.* (2002) "*Lack Of Fucose On Human IgG N-Linked Oligosaccharide Improves Binding To Human Fcgamma RIII And Antibody-Dependent Cellular Toxicity.*," J. Biol. Chem. 277(30): 26733-26740; Davies J. *et al.* (2001) "*Expression Of GnTIII In A Recombinant Anti-CD20 CHO Production Cell Line: Expression Of Antibodies With Altered Glycoforms Leads To An Increase In ADCC Through Higher Affinity For FC Gamma RIII*," Biotechnology & Bioengineering 74(4): 288-294). Methods of altering carbohydrate contents are known to those skilled in the art, *see*, *e.g.*, Wallick, S.C. *et al.* (1988) "*Glycosylation Of A VH Residue Of A Monoclonal Antibody Against Alpha (1----6) Dextran Increases Its Affinity For Antigen*," J. Exp. Med. 168(3): 1099-1109; Tao, M.H. *et al.* (1989) "*Studies Of Aglycosylated Chimeric Mouse-Human IgG. Role Of Carbohydrate In The Structure And Effector Functions Mediated By The Human IgG Constant Region*," J. Immunol. 143(8): 2595-2601; Routledge, E.G. *et al.* (1995) "*The Effect Of Aglycosylation On The Immunogenicity Of A Humanized Therapeutic CD3 Monoclonal Antibody*," Transplantation 60(8):847-53; Elliott, S. *et al.* (2003) "*Enhancement Of Therapeutic Protein In Vivo Activities Through Glycoengineering*," Nature Biotechnol. 21:414-21; Shields, R.L. *et al.* (2002) "*Lack Of Fucose On Human IgG N-Linked Oligosaccharide Improves*

Binding To Human Fcgamma RIII And Antibody-Dependent Cellular Toxicity.," J. Biol. Chem. 277(30): 26733-26740).

[0091] In some embodiments, a humanized antibody is a derivative. Such a humanized antibody comprises amino acid residue substitutions, deletions or additions in one or more non-human CDRs. The humanized antibody derivative may have substantially the same binding, better binding, or worse binding when compared to a non-derivative humanized antibody. In specific embodiments, one, two, three, four, or five amino acid residues of the CDR have been substituted, deleted or added (*i.e.,* mutated).

[0092] A derivative antibody or antibody fragment may be modified by chemical modifications using techniques known to those of skill in the art, including, but not limited to, specific chemical cleavage, acetylation, formulation, metabolic synthesis of tunicamycin, *etc.* In one embodiment, an antibody derivative will possess a similar or identical function as the parental antibody. In another embodiment, an antibody derivative will exhibit an altered activity relative to the parental antibody. For example, a derivative antibody (or fragment thereof) can bind to its epitope more tightly or be more resistant to proteolysis than the parental antibody.

[0093] Derivatized antibodies may be used to alter the half-lives (*e.g.*, serum half-lives) of parental antibodies in a mammal, preferably a human. Preferably such alteration will result in a half-life of greater than 15 days, preferably greater than 20 days, greater than 25 days, greater than 30 days, greater than 35 days, greater than 40 days, greater than 45 days, greater than 2 months, greater than 3 months, greater than 4 months, or greater than 5 months. The increased half-lives of the humanized antibodies of the present invention or fragments thereof in a mammal, preferably a human, results in a higher serum titer of said antibodies or antibody fragments in the mammal, and thus, reduces the frequency of the administration of said antibodies or antibody fragments and/or reduces the concentration of said antibodies or antibody fragments to be administered. Antibodies or fragments thereof having increased *in vivo* half-lives can be generated by techniques known to those of skill in the art. For example, antibodies or fragments thereof with

increased *in vivo* half-lives can be generated by modifying (*e.g.*, substituting, deleting or adding) amino acid residues identified as involved in the interaction between the Fc domain and the FcRn receptor. The humanized B7-H5 antibodies can be engineered to increase biological half-lives (*see, e.g.* U.S. Patent No. 6,277,375). For example, humanized B7-H5 antibodies can be engineered in the Fc-hinge domain to have increased *in vivo* or serum half-lives.

[0094] Antibodies or fragments thereof with increased *in vivo* half-lives can be generated by attaching to said antibodies or antibody fragments polymer molecules such as high molecular weight polyethyleneglycol (PEG). PEG can be attached to said antibodies or antibody fragments with or without a multifunctional linker either through site-specific conjugation of the PEG to the N– or C- terminus of said antibodies or antibody fragments or via epsilon-amino groups present on lysine residues. Linear or branched polymer derivatization that results in minimal loss of biological activity will be used. The degree of conjugation will be closely monitored by SDS-PAGE and mass spectrometry to ensure proper conjugation of PEG molecules to the antibodies. Unreacted PEG can be separated from antibody-PEG conjugates by, *e.g.*, size exclusion or ion-exchange chromatography.

[0095] The B7-H5 antibodies may also be modified by the methods and coupling agents described by Davis *et al.* (*See* U.S. Patent No. 4,179,337) in order to provide compositions that can be injected into the mammalian circulatory system with substantially no immunogenic response.

[0096] One embodiment encompasses modification of framework residues of the humanized B7-H5 antibodies. Framework residues in the framework regions may be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions.

(*See, e.g.*, U.S. Patent No. 5,585,089; and Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies For Therapy*," Nature 332:323-327).

[0097] Yet another embodiment encompasses anti-human B7-H5 antibodies (and more preferably, humanized antibodies) and antigen-binding fragments thereof that are recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a heterologous molecule (*i.e.*, an unrelated molecule). The fusion does not necessarily need to be direct, but may occur through linker sequences.

[0098] In one embodiment such heterologous molecules are polypeptides having at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids. Such heterologous molecules may alternatively be enzymes, hormones, cell surface receptors, drug moieties, such as: toxins (such as abrin, ricin A, pseudomonas exotoxin (*i.e.*, PE-40), diphtheria toxin, ricin, gelonin, or pokeweed antiviral protein), proteins (such as tumor necrosis factor, interferon (*e.g.*, α-interferon, β-interferon), nerve growth factor, platelet derived growth factor, tissue plasminogen activator, or an apoptotic agent (*e.g.*, tumor necrosis factor-α, tumor necrosis factor-β)), biological response modifiers (such as, for example, a lymphokine (*e.g.*, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6")), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or macrophage colony stimulating factor, ("M-CSF")), or growth factors (*e.g.*, growth hormone ("GH"))), cytotoxins (*e.g.*, a cytostatic or cytocidal agent, such as paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof), antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, BiCNU® (carmustine; BSNU) and lomustine (CCNU), cyclothosphamide,

busulfan, dibromomannitol, streptozotocin, mitomycin C, and cisdichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), or anti-mitotic agents (*e.g.*, vincristine and vinblastine).

[0099] Techniques for conjugating such therapeutic moieties to antibodies are well known; *see*, *e.g.*, Arnon *et al.*, "*Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy*", in MONOCLONAL ANTIBODIES AND CANCER THERAPY, Reisfeld *et al.* (eds.), 1985, pp. 243-56, Alan R. Liss, Inc.); Hellstrom *et al.*, "*Antibodies For Drug Delivery*", in CONTROLLED DRUG DELIVERY *(2nd Ed.)*, Robinson *et al.* (eds.), 1987, pp. 623-53, Marcel Dekker, Inc.); Thorpe, "A*ntibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review*", in MONOCLONAL ANTIBODIES '84: BIOLOGICAL AND CLINICAL APPLICATIONS, Pinchera *et al.* (eds.), 1985, pp. 475-506); "*Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy*", in MONOCLONAL ANTIBODIES FOR CANCER DETECTION AND THERAPY, Baldwin *et al.* (eds.), 1985, pp. 303-16, Academic Press; and Thorpe *et al.* (1982) "*The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates*," Immunol. Rev. 62:119-158.

[00100] In one embodiment, the B7-H5 antibodies or B7-H5 fusion molecules include an Fc portion. The Fc portion of such molecules may be varied by isotype or subclass, may be a chimeric or hybrid, and/or may be modified, for example to improve effector functions, control of half-life, tissue accessibility, augment biophysical characteristics such as stability, and improve efficiency of production (and less costly). Many modifications useful in construction of disclosed fusion proteins and methods for making them are known in the art, see for example Mueller, J.P. *et al.* (1997) "*Humanized Porcine VCAM-Specific Monoclonal Antibodies With Chimeric IgG2/G4 Constant Regions Block Human Leukocyte Binding To Porcine Endothelial Cells*," Mol. Immun. 34(6):441-452, Swann, P.G. (2008)

"*Considerations For The Development Of Therapeutic Monoclonal Antibodies*," Curr. Opin. Immun. 20:493-499 (2008), and Presta, L.G. (2008) "*Molecular Engineering And Design Of Therapeutic Antibodies*," Curr. Opin. Immun. 20:460-470. In some embodiments the Fc region is the native IgG1, IgG2, or IgG4 Fc region. In some embodiments the Fc region is a hybrid, for example a chimeric consisting of IgG2/IgG4 Fc constant regions. Modifications to the Fc region include, but are not limited to, IgG4 modified to prevent binding to Fc gamma receptors and complement, IgG1 modified to improve binding to one or more Fc gamma receptors, IgG1 modified to minimize effector function (amino acid changes), IgG1 with altered/no glycan (typically by changing expression host), and IgG1 with altered pH-dependent binding to FcRn, and IgG4 with serine at amino acid resident #228 in the hinge region changed to proline (S228P) to enhance stability. The Fc region may include the entire hinge region, or less than the entire hinge region.

[00101] The therapeutic outcome in patients treated with rituximab (a chimeric mouse/human IgG1 monoclonal antibody against CD20) for non-Hodgkin's lymphoma or Waldenstrom's macroglobulinemia correlated with the individual's expression of allelic variants of Fcγ receptors with distinct intrinsic affinities for the Fc domain of human IgG1. In particular, patients with high affinity alleles of the low affinity activating Fc receptor CD16A (FcγRIIIA) showed higher response rates and, in the cases of non-Hodgkin's lymphoma, improved progression-free survival. In another embodiment, the Fc domain may contain one or more amino acid insertions, deletions or substitutions that reduce binding to the low affinity inhibitory Fc receptor CD32B (FcγRIIB) and retain wild-type levels of binding to or enhance binding to the low affinity activating Fc receptor CD16A (FcγRIIIA).

[00102] Another embodiment includes IgG_{2-4} hybrids and $_{IgG4}$ mutants that have reduce binding to FcR which increase their half-life. Representative IG_{2-4} hybrids and IgG4 mutants are described in Angal, S. *et al.* (1993) "*A Single Amino Acid Substitution Abolishes The Heterogeneity Of Chimeric Mouse/Human (Igg4) Antibody*," Molec. Immunol. 30(1):105-108; Mueller,

J.P. *et al.* (1997) "*Humanized Porcine VCAM-Specific Monoclonal Antibodies With Chimeric Igg2/G4 Constant Regions Block Human Leukocyte Binding To Porcine Endothelial Cells*," Mol. Immun. 34(6):441-452; and U.S. Patent No. 6,982,323. In some embodiments the IgG_1 and/or IgG_2 domain is deleted for example, Angal, s. *et al.* describe IgG_1 and IgG_2 having serine 241 replaced with a proline.

[00103] Substitutions, additions or deletions in the derivatized antibodies may be in the Fc region of the antibody and may thereby serve to modify the binding affinity of the antibody to one or more Fc R. Methods for modifying antibodies with modified binding to one or more Fc R are known in the art, *see*, *e.g.*, PCT Publication Nos. WO 04/029207, WO 04/029092, WO 04/028564, WO 99/58572, WO 99/51642, WO 98/23289, WO 89/07142, WO 88/07089, and U.S. Patent Nos. 5,843,597 and 5,642,821. In one particular embodiment, the modification of the Fc region results in an antibody with an altered antibody-mediated effector function, an altered binding to other Fc receptors (*e.g.*, Fc activation receptors), an altered antibody-dependent cell-mediated cytotoxicity (ADCC) activity, an altered C1q binding activity, an altered complement-dependent cytotoxicity activity (CDC), a phagocytic activity, or any combination thereof.

[00104] In some embodiments, the invention encompasses antibodies whose Fc region will have been modified so that the molecule will exhibit altered Fc receptor (FcR) binding activity, for example to exhibit decreased activity toward activating receptors such as FcγRIIA or FcγRIIIA, or increased activity toward inhibitory receptors such as FcγRIIB. Preferably, such antibodies will exhibit decreased antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC) activities (relative to a wild-type Fc receptor).

[00105] Modifications that affect Fc-mediated effector function are well known in the art (*see* U.S. Patent No. 6,194,551, and WO 00/42072; Stavenhagen, J.B. *et al.* (2007) "*Fc Optimization Of Therapeutic Antibodies Enhances Their Ability To Kill Tumor Cells In Vitro And Controls Tumor Expansion In Vivo Via Low-Affinity Activating Fcgamma Receptors*," Cancer

Res. 57(18):8882-8890; Shields, R.L. *et al.* (2001) "*High Resolution Mapping of the Binding Site on Human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and Design of IgG1 Variants with Improved Binding to the FcγR*," J. Biol. Chem. 276(9):6591-6604). Exemplary variants of human IgG1 Fc domains with reduced binding to FcγRIIA or FcγRIIIA, but unchanged or enhanced binding to FcγRIIB, include S239A, H268A, S267G, E269A, E293A, E293D, Y296F, R301A,V303A, A327G, K322A, E333A, K334A, K338A, A339A, D376A.

[00106] In some embodiments, the invention encompasses antibodies whose Fc region will have been deleted (for example, an Fab or $F(ab)_2$, *etc.*).

[00107] Any of the molecules of the present invention can be fused to marker sequences, such as a peptide, to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I.A. *et al.* (1984) "*The Structure Of An Antigenic Determinant In A Protein*," Cell, 37:767-778) and the "flag" tag (Knappik, A. *et al.* (1994) "*An Improved Affinity Tag Based On The FLAG Peptide For The Detection And Purification Of Recombinant Antibody Fragments*," Biotechniques 17(4):754-761).

[00108] The present invention also encompasses antibodies or their antigen-binding fragments that are conjugated to a diagnostic or therapeutic agent or any other molecule for which serum half-life is desired to be increased. The antibodies can be used diagnostically (*in vivo*, *in situ* or *in vitro*) to, for example, monitor the development or progression of a disease, disorder or infection as part of a clinical testing procedure to, *e.g.*, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody or indirectly, through an intermediate (such as, for example, a

linker known in the art) using techniques known in the art. *See*, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Such diagnosis and detection can be accomplished by coupling the antibody to detectable substances including, but not limited to, various enzymes, enzymes including, but not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic group complexes such as, but not limited to, streptavidin/biotin and avidin/biotin; fluorescent materials such as, but not limited to, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent material such as, but not limited to, luminol; bioluminescent materials such as, but not limited to, luciferase, luciferin, and aequorin; radioactive material such as, but not limited to, bismuth (^{213}Bi), carbon (^{14}C), chromium (^{51}Cr), cobalt (^{57}Co), fluorine (^{18}F), gadolinium (^{153}Gd, ^{159}Gd), gallium (^{68}Ga, ^{67}Ga), germanium (^{68}Ge), holmium (^{166}Ho), indium (^{115}In, ^{113}In, ^{112}In, ^{111}In), iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), lanthanium (^{140}La), lutetium (^{177}Lu), manganese (^{54}Mn), molybdenum (^{99}Mo), palladium (^{103}Pd), phosphorous (^{32}P), praseodymium (^{142}Pr), promethium (^{149}Pm), rhenium (^{186}Re, ^{188}Re), rhodium (^{105}Rh), ruthemium (^{97}Ru), samarium (^{153}Sm), scandium (^{47}Sc), selenium (^{75}Se), strontium (^{85}Sr), sulfur (^{35}S), technetium (^{99}Tc), thallium (^{201}Ti), tin (^{113}Sn, ^{117}Sn), tritium (^{3}H), xenon (^{133}Xe), ytterbium (^{169}Yb, ^{175}Yb), yttrium (^{90}Y), zinc (^{65}Zn); positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions.

[00109] The molecules of the present invention can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980. Such heteroconjugate antibodies may additionally bind to haptens (such as fluorescein, *etc.*), or to cellular markers (*e.g.*, PD-1, 4-1-BB, B7-H4, B7-H5, CD4, CD8, CD14, CD25, CD27, CD40, CD68, CD163, CTLA4, GITR, LAG-3, OX40, TIM3, TIM4, TLR2, LIGHT, *etc.*) or to cytokines (*e.g.*, IL-7, IL-15, IL-12, IL-4 TGF-beta, IL-10, IL-17, IFNg, Flt3, BLys) or chemokines (*e.g.*, CCL21), *etc.*

[00110] The molecules of the present invention may be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen or of other molecules that are capable of binding to target antigen that has been immobilized to the support via binding to an antibody or antigen-binding fragment of the present invention. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[00111] The present invention additionally includes nucleic acid molecules (DNA or RNA) that encode any such antibodies, fusion proteins or fragments, as well as vector molecules (such as plasmids) that are capable of transmitting or of replication such nucleic acid molecules and expressing such antibodies, fusion proteins or fragments in a cell line. The nucleic acids can be single-stranded, double-stranded, may contain both single-stranded and double-stranded portions.

D. Preferred Modulator Compositions of the Present Invention

[00112] As used herein the term "**modulate**" relates to a capacity to alter an effect or result. In particular, the invention relates to polypeptides that comprise an anti-human B7-H5 antibody or any of its antigen-binding fragments that immunospecifically binds human B7-H5 or molecules that physiospecifically bind B7-H5 that are capable of modulating the binding between B7-H5 and its cognate ligands and/or of modulating the signal transduction that occurs as a consequence of B7-H5 – cognate counter-receptor binding. Additionally, multi-specific anti-B7-H5 antibodies, anti-B7-H5 antigen-binding fragments and their respective fusion products that have the added ability to bind CD28H or other cellular ligands or receptors have particular utility in facilitating the co-localization of cells expressing such ligands or receptors to cells that express CD28H.

[00113] The invention concerns antibodies, or fragments thereof, or fusion molecules that comprise such antibodies or fragments, that immunospecifically bind to B7-H5 and are capable of substantially blocking B7-H5's interaction with CD28H *in vitro*, or in a recipient subject or patient.

As used herein, a molecule that is "**capable of substantially blocking B7-H5's interaction with CD28H**" denotes that the provision of such molecule attenuates B7-H5-CD28H interactions by more than 50%, more preferably by more than 60%, more than 70%, more than 80%, more than 90%, more than 95%, more than 99% or most preferably completely attenuates such interaction, as measured by any of the assays disclosed herein. Such antibodies, fragments and fusion molecules have particular utility in attenuating the biological effects of B7-H5 – CD28H interactions.

[00114] The invention thus particularly relates to the molecules of such embodiments involving humanized antibodies and fragments or human antibodies and fragments.

[00115] Most preferably, such molecules will possess sufficient affinity and avidity to be able to bind to B7-H5 when expressed at an endogenous concentration and arrayed on the surface of a subject's cells. The term "**endogenous concentration**" refers to the level at which a molecule is natively expressed (*i.e.*, in the absence of expression vectors or recombinant promoters) in a normal, cancer or pathogen-infected cell.

(1) Preferred Anti-Human B7-H5 Antibodies and Their CDRs

[00116] In accordance with the present invention, such molecules can be produced by screening hybridoma lines for those that produce antibody that are immunospecific for human B7-H5, and then optionally screening amongst such lines for those exhibiting modulating activity (*e.g.*, neutralizing activity, agonizing activity, altered signal transducing activity, *etc.*). The invention particularly provides hamster anti-human B7-H5 clones: **2D3** and **18C3**. Antibodies **2D3** and **18C3** are capable of binding to human B7-H5 and are substantially capable of blocking B7-H5's interaction with CD28H. Antibodies that are substantially capable of blocking B7-H5's interaction with CD28H have particular use in the treatment of inflammatory disease and autoimmune disease. Antibodies that are substantially incapable of blocking B7-H5's interaction with CD28H have particular use in

diagnostics, since such antibodies can be used to detect the presence, location and prevalence of B7-H5:CD28H interactions.

[00117] Antibodies expressed by the anti-human B7-H5 clones which were capable of blocking B7-H5's interaction with CD28H were sequenced to reveal their variable domains. CDR sequences of the variable domains are shown in bold and underlined:

Anti-Human B7-H5 Clone 2D3

Heavy Chain Variable Region (SEQ ID NO:9):

QVQLQQSGAE LVKPGASVKL SCKAS**GYTFT** **SHD**INWVRQR PELGLEWIGW
IFPGDGSTKF NEKFKGKATL TTDKSSSTAY IQLSRLTSED SAVYFC**ARNS**
FYSMDYWGQG TSVTVSS

Polynucleotide Encoding Heavy Chain Variable Region (SEQ ID NO:10):

caggttcaac tgcaacagtc tggagctgaa ctggtaaagc ctggggcttc
agtgaagttg tcctgcaagg cttctggcta caccttcaca agccatgata
taaactgggt gaggcagagg cctgaactgg gacttgagtg gattggatgg
atttttcctg gggatggtag tactaagttc aatgagaagt tcaagggcaa
ggccacactg actacagaca aatcctccag cacagcctac atacagctca
gcaggctgac gtctgaggac tctgctgtct atttctgtgc aagaaactcc
ttctactcta tggactattg gggtcaagga acctcagtca ccgtctcctc
a

Light Chain Variable Region (SEQ ID NO:11):

DIVMSQSPSS LAVSAGEKVT MSCKSS**QSLL** **NSRTRKNQ**LA WYQQKPGQSP
KLLIY**WAF**IR ESGVPDRFTG SGSGTDFTLT ISSVQAEDLA VYYC**KQSYNL**
RTFGGGTKLE IK

Polynucleotide Encoding Light Chain Variable Region (SEQ ID NO:12):

gacattgtga tgtcacagtc tccatcctcc ctggctgtgt cagcaggaga
gaaggtcact atgagctgca aatccagtca gagtctgctc aacagtagaa
cccgaaagaa ccagttggct tggtaccagc agaaaccagg acagtctcct
aaattactga tctactgggc attcattagg gaatctgggg tccctgatcg
cttcacaggc agtggatctg ggacagattt cactctcacc atcagcagtg
tgcaggctga agacctggca gtttattact gcaagcaatc ttataatctt
cggacgttcg gtggaggcac caagctggaa atcaaac

Anti-Human B7-H5 Clone 18C3

Heavy Chain Variable Region (SEQ ID NO:13):

QVQLQQSGAE LVKPGASVKL SCKAS**GYTFT** **SHD**INWVRQR PEQGLEWIGW
IFPGDGSTKF NEKFKGKATL TTDKSSSTAY IQLSRLTSED SAVYFC**ARNS**
FYSMDYWGQG TSVTVSS

Polynucleotide Encoding Heavy Chain Variable Region (SEQ ID NO:14):

caggttcaac tgcagcagtc tggagctgaa ctggtaaagc ctggggcttc
agtgaagttg tcctgcaagg cttctggcta caccttcaca agccatgata
taaactgggt gaggcagagg cctgaacagg gacttgagtg gattggatgg
atttttcctg gggatggtag tactaagttc aatgagaagt tcaagggcaa
ggccacactg actacagaca aatcctccag cacagcctac atacagctca
gcaggctgac atctgaggac tctgctgtct atttctgtgc aagaaactcc
ttctattcta tggactactg gggtcaagga acctcagtca ccgtctcctc
a

Light Chain Variable Region (SEQ ID NO:15):

DIVMSQSPSS LAVSAGEKVT MSCKSS**QSLL** **NSRTRKNQ**LA WYQQKPGQSP
KLLIY**WAS**TR ESGVPDRFTG SGSGTDFTLT ISSVQAEDLA VYYC**KQSYNL**
RTFGGGTKLE IK

Polynucleotide Encoding Light Chain Variable Region (SEQ ID NO:28):

gacattgtga tgtcacagtc tccatcctcc ctggctgtgt cagcaggaga
gaaggtcact atgagctgca aatccagtca gagtctgctc aacagtagaa
cccgaaagaa ccagttggct tggtaccagc agaaaccagg gcagtctcct
aaactgctga tctactgggc atccactagg gaatctgggg tccctgatcg
cttcacaggc agtggatctg ggacagattt cactctcacc atcagcagtg
tgcaggctga agacctggca gtttattact gcaagcaatc ttataatctt
cggacgttcg gtggaggcac caagctggaa atcaaac

(2) Consensus CDRs of the Anti-Human B7-H3 Antibodies of the Present Invention That Block B7-H5:CD28H Interaction

[00118] Analyses of the CDRs of the identified antibodies were conducted in order to identify consensus CDR sequences and likely variant CDR sequences that would provide similar binding attributes. Such variant CDRs were computed using Blosum62.iij analysis according to **Table 1**. **Table 1** presents the Blosum62.iij substitution scores. The higher the score the more conservative the substitution and thus the more likely the substitution will not affect function.

Table 1

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	+4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	+1	0	-3	-2	0
R	-1	+5	0	-2	-3	+1	0	-2	0	-3	-2	+2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	+6	+1	-3	0	0	0	+1	-3	-3	0	-2	-3	-2	+1	0	-4	-2	-3
D	-2	-2	+1	+6	-3	0	+2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	+9	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	+1	0	0	-3	+5	+2	-2	0	-3	-2	+1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	+2	-4	+2	+5	-2	0	-3	-3	+1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	+6	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	+1	-1	-3	0	0	-2	+8	-3	-3	-1	-2	-1	-2	-1	-2	-2	+2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	+4	+2	-3	+1	0	-3	-2	-1	-3	-1	+3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	+2	+4	-2	+2	0	-3	-2	-1	-2	-1	+1
K	-1	+2	0	-1	-3	+1	+1	-2	-1	-3	-2	+5	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	+1	+2	-1	+5	0	-2	-1	-1	-1	-1	+1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	+6	-4	-2	-2	+1	+3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	+7	-1	-1	-4	-3	-2
S	+1	-1	+1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	+4	+1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	+1	+5	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	+1	-4	-3	-2	+11	+2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	+2	-1	-1	-2	-1	+3	-3	-2	-2	+2	+7	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	+3	+1	-2	+1	-1	-2	-2	0	-3	-1	+4

[00119] The present invention permits the formation of novel antibodies and antigen-binding fragments having 1, 2, 3, 4, 5 or 6 variant CDRs. Because the methods of the present invention have identified a substantial number of distinct CDRs, the invention permits a recognition of CDR residues that are likely to be required in any variant of a particular identified CDR. Such residues are shown in boldface in **Table 2** and **Table 3.** For those residues that are found to vary among the compared CDRs, the substitution scores of **Table 1** provide a means for determining the identities of permitted substitutions. For example, if a particular residue of a particular CDR is found to vary as R or S, then since R and S have a substitution score of -1, any substitution of R or S having a substitution score of -1 or greater are as likely as the observed variants (R or S) (or are more likely than R or S) to create a variant CDR having binding attributes that are sufficiently similar to those of the particular CDR to permit the variant CDR to be employed in lieu thereof so as to form a functional anti-B7-H5 antibody or antigen-binding fragment. For each position, the selection of a residue having a higher substitution score is preferred over the selection of a residue having a lower substitution score.

[00120] **Table 2** presents an analysis of the heavy chain CDRs of the anti-B7-H5 antibodies and provides the consensus sequence of the observed and preferred variant light chain ("LC") anti-B7-H5 CDRs of the present invention.

Table 2: Anti-B7-H5 Heavy Chain CDRs															
Heavy Chain CDR1															
Antibody	**Sequence**														**SEQ ID NO**
2D3	G	Y	T	F	T	S	H	D							**16**
18C3	G	Y	T	F	T	S	H	D							**16**
HC CDR1 Consensus Sequence:	**G**	**Y**	**T**	**F**	**T**	**S**	**H**	**D**							**16**
Heavy Chain CDR2															
Antibody	**Sequence**														**SEQ ID NO**
2D3	I	F	P	G	D	G	S	T							**17**
18C3	I	F	P	G	D	G	S	T							**17**
HC CDR2 Consensus Sequence:	**I**	**F**	**P**	**G**	**D**	**G**	**S**	**T**							**17**
Heavy Chain CDR3															
Antibody	**Sequence**														**SEQ ID NO**
2D3	A	R	N	S	F	Y	S	M	D	Y					**18**
18C3	A	R	N	S	F	Y	S	M	D	Y					**18**
HC CDR3 Consensus Sequence:	**A**	**R**	**N**	**S**	**F**	**Y**	**S**	**M**	**D**	**Y**					**18**

[00121] **Table 3** presents an analysis of the light chain CDRs of the anti-B7-H5 antibodies and provides the consensus sequence of the observed and preferred variant anti- B7-H5 heavy chain ("HC") CDRs of the present invention.

Table 3: Anti-B7-H5 Light Chain CDRs															
Light Chain CDR1															
Antibody	**Sequence**														**SEQ ID NO**
2D3	Q	S	L	L	N	S	R	T	R	K	N	Q			**19**
18C3	Q	S	L	L	N	S	R	T	R	K	N	Q			**19**
HC CDR1 Consensus Sequence:	**Q**	**S**	**L**	**L**	**N**	**S**	**R**	**T**	**R**	**K**	**N**	**Q**			**19**
Light Chain CDR2															
Antibody	**Sequence**														**SEQ ID**

Table 3: Anti-B7-H5 Light Chain CDRs										
										NO
2D3	W	A	F							**20**
18C3	W	A	S							**21**
LC CDR2 Consensus Sequence:	**W**	**A**	**X_1**							**22**
X_1 is F or S or a substitution having an equal or greater substitution score (*i.e.*, ≥ -2): A, C, H, I, L, M, F, S, T, W, Y, or V										
Light Chain CDR3										
Antibody	**Sequence**									**SEQ ID NO**
2D3	K	Q	S	Y	N	L	R	T		**23**
18C3	K	Q	S	Y	N	L	R	T		**23**
LC CDR3 Consensus Sequence:	**K**	**Q**	**S**	**Y**	**N**	**L**	**R**	**T**		**23**

[00122] Thus, in addition to antibodies and antigen-binding fragments thereof that possess the CDRs of the anti-B7-H5 antibodies: 2D3 and 13C3, the invention additionally provides antibodies and antigen-binding fragments thereof that possess CDRs having the above-described light and/or heavy chain consensus sequences.

[00123] The present invention encompasses antibodies or fragments thereof comprising an amino acid sequence of a variable heavy chain and/or variable light chain that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of the variable heavy chain and/or light chain of the hamster monoclonal antibody produced by any of the above clones, and which exhibit immunospecific binding to B7-H5. The present invention further encompasses antibodies or fragments thereof that comprise a CDR that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of a CDR of the above-listed clones and which exhibit immunospecific binding to B7-H5. The determination of percent identity of two amino acid sequences can be determined by BLAST protein comparison.

[00124] In a preferred embodiment, the antibody is a immunoglobulin molecule (*e.g.*, an antibody, diabody, fusion protein, *etc.*) that comprises one, two or three light chain CDRs and one, two or three heavy chain CDRs (most preferably three light chain CDRs and three heavy chain CDRs), wherein the **light** chain CDRs include:

(1) the light chain CDR1 of anti-human B7-H5 antibodies 2D3/18C3;

(2) a light chain CDR2 of anti-human B7-H5 antibody 2D3 or anti-human B7-H5 antibody 18C3 or a consensus sequence of the CDR2s of such antibodies; and

(3) the light chain CDR3 of anti-human B7-H5 antibodies 2D3/18C3;

[00125] In an alternative preferred embodiment, the immunoglobulin molecule comprises one, two or three light chain CDRs and one, two or three heavy chain CDRs (most preferably three light chain CDRs and three heavy chain CDRs), wherein the **heavy** chain CDRs include:

(1) the heavy chain CDR1 of anti-human B7-H5 antibodies 2D3/18C3;

(2) the heavy chain CDR2 of anti-human B7-H5 antibodies 2D3/18C3; and

(2) the heavy chain CDR3 of anti-human B7-H5 antibodies 2D3/18C3.

[00126] In a specific embodiment, an antibody or an antigen-binding fragment thereof of the present invention will comprise one, two, three, four, five, or more preferably, all 6 CDRs of the above-described preferred antibodies and will exhibit the ability to bind to human B7-H5.

(3) CD28H Fusion Proteins

[00127] It has been discovered that CD28H fusion proteins can be antagonists of signal transduction between B7-H5 and CD28H. Therefore, CD28H fusion proteins and methods of use thereof to down-modulate the immune system are also disclosed. Therefore, receptor fusion polypeptides

typically block the ability of ligands to bind to transmembrane CD28H, and induce or maintain B7-H5:CD28H mediated signal transduction.

[00128] CD28H fusion polypeptides disclosed herein have a first fusion partner including all or a part of a CD28H polypeptide fused (i) directly to a second polypeptide or, (ii) optionally, fused to a linker peptide sequence that is fused to the second polypeptide. Such fusion proteins may form dimers or multimers. The peptide/polypeptide linker domain can either be a separate domain, or alternatively can be contained within one of the other domains (CD28H polypeptide or second polypeptide) of the fusion protein. Similarly, the domain that functions to dimerize or multimerize the fusion protein can either be a separate domain, or alternatively can be contained within one of the other domains (CD28H polypeptide, second polypeptide or peptide/polypeptide linker domain) of the fusion protein. In one embodiment, the dimerization/multimerization domain and the peptide/polypeptide linker domain are the same.

[00129] Fusion proteins disclosed herein are of formula I: N-R_1-R_2-R_3-C, wherein "N" represents the N-terminus of the fusion protein, "C" represents the C-terminus of the fusion protein. In the preferred embodiment, "R_1" is a CD28H polypeptide, "R_2" is an optional peptide/polypeptide linker domain, and "R_3" is a second polypeptide. Alternatively, R_3 may be a CD28H polypeptide and R_1 may be a second polypeptide.

[00130] **a. First Fusion Partner**

[00131] The receptor fusion proteins can include a full-length CD28H polypeptide, or can contain a fragment or variant of a full length a CD28H. In preferred embodiment the first fusion partner is a soluble fragment of CD28H, for example, part or all of the extracellular domain of CD28H, or a variant thereof. Any mammalian sequence CD28H can be used. As an example, human sequences, as well as known isoforms and variants thereof, are provided in the sequences above. In some embodiments, other mammalian sequences, such as mouse sequences, are known in the art and can be used. Human CD28H polypeptides useful in the disclosed fusion

proteins can have an amino acid sequence least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to SEQ ID NO:7, or preferably 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the extracellular domain of SEQ ID NO:7. Human CD28H polypeptides useful in the disclosed fusion proteins can be encoded by a nucleic acid sequence least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to SEQ ID NO:8, or preferably 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the extracellular domain encoded by the nucleic acid sequence of SEQ ID NO:8.

[00132] **b. Second Fusion Partner**

[00133] The CD28H polypeptide may be fused to a second polypeptide. The presence of the second polypeptide can alter the solubility, stability, affinity and/or valency of the fusion polypeptide. As used herein, "valency" refers to the number of binding sites available per molecule. In one embodiment the second polypeptide is a polypeptide from a different source or different protein.

[00134] In one embodiment, the second polypeptide contains one or more domains of an immunoglobulin heavy chain constant region, preferably having an amino acid sequence corresponding to the hinge, C_H2 and C_H3 regions of a human immunoglobulin Cγ1 chain or to the hinge, C_H2 and C_H3 regions of a murine immunoglobulin Cγ2a chain.

[00135] In a preferred dimeric fusion protein, the dimer results from the covalent bonding of Cys residue in the hinge region of two of the Ig heavy chains that are the same Cys residues that are disulfide linked in dimerized normal Ig heavy chains. Such proteins can be referred to as CD28H-Ig.

[00136] In one embodiment, the immunoglobulin constant domain may contain one or more amino acid insertions, deletions or substitutions that enhance binding to specific cell types, increase the bioavailablity, or increase the stability of the CD28H polypeptides, fusion proteins, or fragments

thereof. Suitable amino acid substitutions include conservative and non-conservative substitutions, as described above.

[00137] In another embodiment the second polypeptide may have a conjugation domain through which additional molecules can be bound to the CD28H fusion proteins. In one such embodiment, the conjugated molecule is capable of targeting the fusion protein to a particular organ or tissue. In another such embodiment the conjugated molecule is another immunomodulatory agent that can enhance or augment the effects of the CD28H fusion protein. In another embodiment the conjugated molecule is Polyethylene Glycol (PEG).

[00138] The Fc portion of the fusion protein may be varied by isotype or subclass, may be a chimeric or hybrid, and/or may be modified, for example to improve effector functions, control of half-life, tissue accessibility, augment biophysical characteristics such as stability, and improve efficiency of production (and less costly). Many modifications useful in construction of disclosed fusion proteins and methods for making them are known in the art, see for example Mueller, et al., *Mol. Immun.*, 34(6):441-452 (1997), Swann, et al., *Cur. Opin. Immun.*, 20:493-499 (2008), and Presta, *Cur. Opin. Immun.* 20:460-470 (2008). In some embodiments the Fc region is the native IgG1, IgG2, or IgG4 Fc region. In some embodiments the Fc region is a hybrid, for example a chimeric consisting of IgG2/IgG4 Fc constant regions. Medications to the Fc region include, but are not limited to, IgG4 modified to prevent binding to Fc gamma receptors and complement, IgG1 modified to improve binding to one or more Fc gamma receptors, IgG1 modified to minimize effector function (amino acid changes), IgG1 with altered/no glycan (typically by changing expression host), and IgG1 with altered pH-dependent binding to FcRn. The Fc region may include the entire hinge region, or less than the entire hinge region.

[00139] The therapeutic outcome in patients treated with rituximab (a chimeric mouse/human IgG1 monoclonal antibody against CD20) for non-Hodgkin's lymphoma or Waldenstrom's macroglobulinemia correlated with the individual's expression of allelic variants of Fcγ receptors with distinct

intrinsic affinities for the Fc domain of human IgG1. In particular, patients with high affinity alleles of the low affinity activating Fc receptor CD16A (FcγRIIIA) showed higher response rates and, in the cases of non-Hodgkin's lymphoma, improved progression-free survival. In another embodiment, the Fc domain may contain one or more amino acid insertions, deletions or substitutions that reduce binding to the low affinity inhibitory Fc receptor CD32B (FcγRIIB) and retain wild-type levels of binding to or enhance binding to the low affinity activating Fc receptor CD16A (FcγRIIIA).

[00140] Another embodiment includes IgG2-4 hybrids and IgG4 mutants that have reduce binding to FcR which increase their half life. Representative IG2-4 hybrids and IgG4 mutants are described in Angal, S. et al., *Molecular Immunology*, 30(1):105-108 (1993); Mueller, J. et al., *Molecular Immonology*, 34(6): 441-452 (1997); and U.S. Patent No. 6,982,323 to Wang et al. In some embodiments the IgG1 and/or IgG2 domain is deleted for example, Angal et al. describe IgG1 and IgG2 having serine 241 replaced with a proline.

[00141] In a preferred embodiment, the Fc domain contains amino acid insertions, deletions or substitutions that enhance binding to CD16A. A large number of substitutions in the Fc domain of human IgG1 that increase binding to CD16A and reduce binding to CD32B are known in the art and are described in Stavenhagen, et al., *Cancer Res.*, 57(18):8882-90 (2007). Exemplary variants of human IgG1 Fc domains with reduced binding to CD32B and/or increased binding to CD16A contain F243L, R929P, Y300L, V305I or P296L substitutions. These amino acid substitutions may be present in a human IgG1 Fc domain in any combination. In one embodiment, the human IgG1 Fc domain variant contains a F243L, R929P and Y300L substitution. In another embodiment, the human IgG1 Fc domain variant contains a F243L, R929P, Y300L, V305I and P296L substitution. In another embodiment, the human IgG1 Fc domain variant contains an N297Q substitution, as this mutation abolishes FcR binding.

[00142]

c. Peptide or polypeptide linker domain

[00143] The disclosed CD28H fusion proteins optionally contain a peptide or polypeptide linker domain that separates the CD28H polypeptide from the second polypeptide.

[00144] **1. Hinge region of antibodies**

[00145] In one embodiment, the linker domain contains the hinge region of an immunoglobulin. In a preferred embodiment, the hinge region is derived from a human immunoglobulin. Suitable human immunoglobulins that the hinge can be derived from include IgG, IgD and IgA. In a preferred embodiment, the hinge region is derived from human IgG. Amino acid sequences of immunoglobulin hinge regions and other domains are well known in the art.

[00146] **2. Other peptide/polypeptide linker domains**

[00147] Other suitable peptide/polypeptide linker domains include naturally occurring or non-naturally occurring peptides or polypeptides. Peptide linker sequences are at least 2 amino acids in length. Preferably the peptide or polypeptide domains are flexible peptides or polypeptides. A "flexible linker" herein refers to a peptide or polypeptide containing two or more amino acid residues joined by peptide bond(s) that provides increased rotational freedom for two polypeptides linked thereby than the two linked polypeptides would have in the absence of the flexible linker. Such rotational freedom allows two or more antigen binding sites joined by the flexible linker to each access target antigen(s) more efficiently. Exemplary flexible peptides/polypeptides include, but are not limited to, the amino acid sequences Gly-Ser, Gly-Ser-Gly-Ser (SEQ ID NO:16), Ala-Ser, Gly-Gly-Gly-Ser (SEQ ID NO:17), $(Gly_4\text{-}Ser)_3$ (SEQ ID NO:18) and $(Gly_4\text{-}Ser)_4$ (SEQ ID NO:19). Additional flexible peptide/polypeptide sequences are well known in the art.

[00148] In one embodiment, the first fusion partner is a fragment of CD28H. In a preferred embodiment, the fusion protein includes the extracellular domain of CD28H, or a fragment thereof, fused to an Ig Fc region. Recombinant CD28H-Ig fusion proteins can be prepared by fusing the coding region of the extracellular domain of a neuropilin or a plexin or a fragment thereof to the Fc region of human IgG1 or mouse IgG2a, or other suitable Ig domain, as described previously (Chapoval, et al., *Methods Mol. Med.*, 45:247-255 (2000)).

d. Exemplary fusion protein

The amino acid sequence of a representative human CD28H-Ig fusion protein is (**SEQ ID NO:20**), a CD28H-hIgG4 (CD28H ECD aa23-140, light chain kappa signal peptide: CD28H sequences are shown in boldface; mutated human IgG4 Fc sequences are shown underlined, with Serine 228 to Proline mutation double underline):

LSVQQGPNLLQVRQGSQATLVCQVDQATAWERLRVKWTKDGAILCQPYITNGSLSLGVCGPQ GRLSWQAPSHLTLQLDPVSLNHSGAYVCWAAVEIPELEEAEGNITRLFVDPDDPTQESKYGP PCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQV YTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRL TVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPGK

The fusion protein may additionally comprise an N-terminal leader sequence (light chain kappa leader sequence) (such residues 1-20 of **SEQ ID NO:21)**,

MSVPTQVLGLLLLWLTDARC

or a naturally occurring CD28H leader sequence such as (**SEQ ID NO:22**):

MGSPGMVLGLLVQIWALQEASS

Although the CD28H sequences are shown fused to a human IgG4 region, in accordance with the present invention, such sequences can alternatively be fused to any Ig isotype, or indeed to any other protein.

A DNA sequence encoding human CD28H-Ig of **SEQ ID NO:20** including the signal sequence of SEQ ID NO:21 is (**SEQ ID NO:23**):

ATGTCCGTGCCCACCCAGGTGCTGGGATTGCTGCTGCTGTGGCTGACCGACGCCAGATGCCT
GTCTGTGCAGCAGGGCCCTAACCTGCTGCAAGTGCGGCAGGGCTCTCAGGCTACACTCGTGT
GTCAGGTGGACCAGGCCACCGCCTGGGAGAGACTGAGAGTGAAGTGGACCAAGGACGGCGCC
ATCCTGTGCCAGCCCTACATCACCAACGGCTCCCTGTCCCTGGGCGTGTGTGGACCTCAGGG
CAGACTGTCTTGGCAGGCCCCTTCTCACCTGACCCTGCAGCTGGACCCTGTGTCCCTGAATC
ACTCCGGCGCCTACGTGTGTTGGGCCGCTGTGGAAATCCCCGAGCTGGAAGAGGCCGAGGGC
AACATCACCCGGCTGTTCGTGGACCCTGACGACCCTACCCAGGAATCTAAGTACGGCCCTCC
CTGCCCTCCTTGCCCAGCCCCTGAATTTCTGGGCGGACCCTCCGTGTTCCTGTTCCCCCCAA
AGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGATGTG
TCCCAGGAAGATCCCGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGC
CAAGACCAAGCCCAGAGAGGAACAGTTCAACTCCACCTACCGGGTGGTGTCCGTGCTGACCG
TGCTGCACCAGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGGCCTG
CCCAGCTCCATCGAAAAGACCATCTCCAAGGCCAAGGGCCAGCCCCGGGAACCCCAGGTGTA
CACACTGCCTCCAAGCCAGGAAGAGATGACCAAGAACCAGGTGTCACTGACCTGTCTCGTGA
AGGGCTTCTACCCCTCCGATATCGCCGTGGAATGGGAGTCCAACGGCCAGCCTGAGAACAAC
TACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCCTTCTTCCTGTACTCCCGCCTGAC
CGTGGACAAGTCCAGATGGCAGGAAGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCC
TGCACAACCACTACACCCAGAAGTCCCTGAGCCTGTCCCCCGGCAAGTGA

E. Therapeutic and Prophylactic Uses of the Preferred Compositions of the Present Invention

[00149] As used herein, the terms "**treat**," "**treating**," "**treatment**" and "**therapeutic use**" refer to the elimination, reduction or amelioration of one or more symptoms of a disease or disorder that would benefit from an increased or decreased immune response. As used herein, a "**therapeutically effective amount**" refers to that amount of a therapeutic agent sufficient to mediate an altered immune response, and more preferably, a clinically relevant altered immune response, sufficient to mediate a reduction or amelioration of a symptom of a disease or condition. An effect is clinically relevant if its magnitude is sufficient to impact the health or prognosis of a recipient subject. A therapeutically effective amount may refer to the amount of therapeutic agent sufficient to reduce or minimize disease progression, *e.g.*, delay or minimize an autoimmune response or an inflammatory response or a transplant rejection. A therapeutically effective amount may also refer to the amount of the therapeutic agent that provides a therapeutic benefit in the treatment or management of a disease. Further, a therapeutically effective amount with respect to a therapeutic agent, B7-H5

antibody or B7-H5 fusion protein or CD28H fusion protein, means that amount of therapeutic agent alone, or in combination with other therapies, that provides a therapeutic benefit in the treatment or management of a disease, *e.g.*, sufficient to enhance the therapeutic efficacy of a therapeutic antibody sufficient to treat or manage a disease.

[00150] As used herein, the term "**prophylactic agent**" refers to an agent that can be used in the prevention of a disorder or disease prior to the detection of any symptoms of such disorder or disease. A "**prophylactically effective**" amount is the amount of prophylactic agent sufficient to mediate such protection. A prophylactically effective amount may also refer to the amount of the prophylactic agent that provides a prophylactic benefit in the prevention of disease. Further, a prophylactically effective amount with respect to a prophylactic agent means that amount of prophylactic agent alone, or in combination with other agents, that provides a prophylactic benefit in the prevention of disease.

[00151] The dosage amounts and frequencies of administration provided herein are encompassed by the terms therapeutically effective and prophylactically effective. The dosage and frequency further will typically vary according to factors specific for each patient depending on the specific therapeutic or prophylactic agents administered, the severity and type of cancer, the route of administration, as well as age, body weight, response, and the past medical history of the patient. Suitable regimens can be selected by one skilled in the art by considering such factors and by following, for example, dosages reported in the literature and recommended in the *Physician's Desk Reference* (56th Ed., 2002).

[00152] The present invention relates to molecules, such as anti-B7-H5 antibodies (and fragments of such antibodies that bind to B7-H5) or CD28H Ig that, by binding to B7-H5 antagonize (*i.e.*, attenuate or impair) B7-H5 function and/or binding to CD28H and thus attenuate or impair T cell proliferation and/or cytokine production. The administration of such molecules to a subject **down-modulates** the immune system of the subject.

[00153] Such down-modulation of the immune system is desirable in the treatment of inflammatory and auto-immune diseases and transplant rejection. Examples of autoimmune disorders that may be treated by administering the antibodies of the present invention include, but are not limited to, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac sprue-dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatrical pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, glomerulonephritis, Graves' disease, Guillain-Barre, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA neuropathy, juvenile arthritis, lichen planus, lupus erthematosus, Ménière's disease, mixed connective tissue disease, multiple sclerosis, Neuromyelitis optica (NMO), type 1 or immune-mediated diabetes mellitus, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychrondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynauld's phenomenon, Reiter's syndrome, Rheumatoid arthritis, sarcoidosis, scleroderma, Sjögren's syndrome, stiff-man syndrome, systemic lupus erythematosus, lupus erythematosus, takayasu arteritis, temporal arteristis/ giant cell arteritis, ulcerative colitis, uveitis, vasculitides such as dermatitis herpetiformis vasculitis, vitiligo, and Wegener's granulomatosis.

[00154] Examples of inflammatory diseases which can be prevented, treated or managed with the disclosed anti-B7-H5 antibodies and antigen binding fragments and CD28H fusion proteins, particularly CD28H-Ig fusion proteins, thereof include, but are not limited to, asthma, encephilitis, inflammatory bowel disease, chronic obstructive pulmonary disease (COPD), allergic disorders, septic shock, pulmonary fibrosis, undifferentiated

spondyloarthropathy, undifferentiated arthropathy, arthritis, inflammatory osteolysis, and chronic inflammation resulting from chronic viral or bacterial infections.

[00155] The anti-B7-H5 antibodies can be employed to produce anti-idiotypic peptides or antibodies (Wallmann, J. *et al.* (2010) "*Anti-Ids in Allergy: Timeliness of a Classic Concept*," World Allergy Organiz. J. 3(6):195–201; Nardi, M. *et al.* (2000) "*Antiidiotype Antibody Against Platelet Anti-GpIIIa Contributes To The Regulation Of Thrombocytopenia In HIV-1-ITP Patients*," J. Exp. Med. 191(12):2093-2100) or mimetics (Zang, Y.C. *et al.* (2003) "*Human Anti-Idiotypic T Cells Induced By TCR Peptides Corresponding To A Common CDR3 Sequence Motif In Myelin Basic Protein-Reactive T Cells*," Int. Immunol. 15(9):1073-1080; Loiarro, M. *et al.* (Epub 2010 Apr 8) "*Targeting TLR/IL-1R Signalling In Human Diseases*," Mediators Inflamm. 2010:674363) of B7-H5. Such molecules serve as surrogates for B7-H5, and thus their administration to a subject down-modulates the immune system of such subject by engaging the CD28H counter-receptor and preventing it from binding to endogenous B7-H5 receptor. Such molecules have utility in the treatment of graft vs. host disease, inflammatory, and auto-immune diseases.

[00156] Thus, the antibodies and antigen-binding fragments and fusion proteins of the present invention have utility in the treatment of graft vs. host disease, inflammatory and auto-immune diseases.

[00157] Similarly, agonist antibodies that enhance binding between such antibodies and such receptor/ligand, and other receptor agonists such a B7-H5 fusion proteins have utility as agonists of B7-H5 signaling, and therefore have utility in the treatment of cancer and infectious disease. Accordingly, the present invention also relates to CD28H agonist molecules, such as B7-H5-Ig fusion proteins that, by binding to CD28H agonize CD28H function (i.e. signal transduction) and/or binding to B7-H5 and thus augment or stimulate T cell proliferation and/or cytokine production. The administration of such molecules to a subject **up-modulates** the immune system of the subject and can be used to treat subjects with cancer or an infectious disease.

As the B7-H5 pathway is involved in T cell activation, such molecules are particularly useful in combination with vaccines.

[00158] The CD28H agonists provided herein are generally useful *in vivo* and *ex vivo* as immune response-stimulating therapeutics. For example, the CD28H agonists are useful for stimulating or enhancing an immune response in host for treating cancer by administering to subject an amount of CD28H agonists. The types of cancer that may be treated with the provided compositions and methods include, but are not limited to, the following: bladder, brain, breast, cervical, colo-rectal, esophageal, kidney, liver, lung, nasopharangeal, pancreatic, prostate, skin, stomach, uterine, ovarian, testicular and hematologic.

[00159] Malignant tumors which may be treated are classified herein according to the embryonic origin of the tissue from which the tumor is derived. Carcinomas are tumors arising from endodermal or ectodermal tissues such as skin or the epithelial lining of internal organs and glands. Sarcomas, which arise less frequently, are derived from mesodermal connective tissues such as bone, fat, and cartilage. The leukemias and lymphomas are malignant tumors of hematopoietic cells of the bone marrow. Leukemias proliferate as single cells, whereas lymphomas tend to grow as tumor masses. Malignant tumors may show up at numerous organs or tissues of the body to establish a cancer.

[00160] The CD28H agonists can be administered for the treatment of local or systemic viral infections, including, but not limited to, immunodeficiency (e.g., HIV), papilloma (e.g., HPV), herpes (e.g., HSV), encephalitis, influenza (e.g., human influenza virus A), and common cold (e.g., human rhinovirus) viral infections. For example, pharmaceutical formulations including the CD28H agonist compositions can be administered topically to treat viral skin diseases such as herpes lesions or shingles, or genital warts. Pharmaceutical formulations of CD28H agonist compositions can also be administered to treat systemic viral diseases, including, but not limited to, AIDS, influenza, the common cold, or encephalitis.

[00161] Representative infections that can be treated, include but are not limited to infections cause by microoganisms including, but not limited to, *Actinomyces, Anabaena, Bacillus, Bacteroides, Bdellovibrio, Bordetella, Borrelia, Campylobacter, Caulobacter, Chlamydia, Chlorobium, Chromatium, Clostridium, Corynebacterium, Cytophaga, Deinococcus, Escherichia, Francisella, Halobacterium, Heliobacter, Haemophilus, Hemophilus influenza type B (HIB), Histoplasma, Hyphomicrobium, Legionella, Leishmania, Leptspirosis, Listeria, Meningococcus A, B and C, Methanobacterium, Micrococcus, Myobacterium, Mycoplasma, Myxococcus, Neisseria, Nitrobacter, Oscillatoria, Prochloron, Proteus, Pseudomonas, Phodospirillum, Rickettsia, Salmonella, Shigella, Spirillum, Spirochaeta, Staphylococcus, Streptococcus, Streptomyces, Sulfolobus, Thermoplasma, Thiobacillus, and Treponema, Vibrio, Yersinia, Cryptococcus neoformans, Histoplasma capsulatum, Candida albicans, Candida tropicalis, Nocardia asteroides, Rickettsia ricketsii, Rickettsia typhi, Mycoplasma pneumoniae, Chlamydial psittaci, Chlamydial trachomatis, Plasmodium falciparum, Plasmodium vivax, Trypanosoma brucei, Entamoeba histolytica, Toxoplasma gondii, Trichomonas vaginalis* and *Schistosoma mansoni*.

[00162] The disclosed CD28H agonists or nucleic acids encoding the same may be administered alone or in combination with any other suitable treatment. In one embodiment the CD28H agonists can be administered in conjunction with, or as a component of, a vaccine composition. Suitable components of vaccine compositions antigens and/or adjuvants. The disclosed CD28H agonists can be administered prior to, concurrently with, or after the administration of a vaccine. In one embodiment the CD28H agonist composition is administered at the same time as administration of a vaccine.

[00163] The disclosed CD28H agonist compositions may be administered in conjunction with prophylactic vaccines, or therapeutic vaccines, which can be used to initiate or enhance a subject's immune response to a pre-existing antigen, such as a tumor antigen in a subject with cancer.

[00164] The desired outcome of a prophylactic, therapeutic or de-sensitized immune response may vary according to the disease, according to principles

well known in the art. Similarly, immune responses against cancer, allergens or infectious agents may completely treat a disease, may alleviate symptoms, or may be one facet in an overall therapeutic intervention against a disease. For example, the stimulation of an immune response against a cancer may be coupled with surgical, chemotherapeutic, radiologic, hormonal and other immunologic approaches in order to affect treatment.

F. Methods of Administration

[00165] Various delivery systems are known and can be used to administer the therapeutic or prophylactic compositions, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or fusion protein, receptor-mediated endocytosis (*see*, *e.g.*, Wu and Wu, 1987, *J. Biol. Chem*. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, *etc.*

[00166] Methods of administering antibodies and fusion proteins include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, the antibodies or fusion proteins are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. *See*, *e.g.*, U.S. Patent Nos. 6,019,968; 5,985, 20; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903. In a specific embodiment, it may be desirable to administer the pharmaceutical compositions locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a

porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering an antibody or fusion protein, care must be taken to use materials to which the antibody or the fusion protein does not absorb.

[00167] In some embodiments, the antibodies or fusions proteins are formulated in liposomes for targeted delivery of the antibodies or fusion proteins. Liposomes are vesicles comprised of concentrically ordered phopsholipid bilayers which encapsulate an aqueous phase. Liposomes typically comprise various types of lipids, phospholipids, and/or surfactants. The components of liposomes are arranged in a bilayer configuration, similar to the lipid arrangement of biological membranes. Liposomes are particularly preferred delivery vehicles due, in part, to their biocompatibility, low immunogenicity, and low toxicity. Methods for preparation of liposomes are known in the art and are encompassed within the invention, *see, e.g.*, Epstein *et al.*, 1985, *Proc. Natl. Acad. Sci. USA*, 82: 3688; Hwang *et al.*, 1980 *Proc. Natl. Acad. Sci. USA*, 77: 4030-4; U.S. Patent Nos. 4,485,045 and 4,544,545.

[00168] Methods of preparing liposomes with a prolonged serum half-life, *i.e.*, enhanced circulation time, such as those disclosed in U.S. Patent No. 5,013,556 can be used to make liposomes-antibody compositions. Preferred liposomes are not rapidly cleared from circulation, *i.e.*, are not taken up into the mononuclear phagocyte system (MPS). The invention encompasses sterically stabilized liposomes which are prepared using common methods known to one skilled in the art. Although not intending to be bound by a particular mechanism of action, sterically stabilized liposomes contain lipid components with bulky and highly flexible hydrophilic moieties, which reduces the unwanted reaction of liposomes with serum proteins, reduces oposonization with serum components and reduces recognition by MPS. Sterically stabilized liposomes are preferably prepared using polyethylene glycol. For preparation of liposomes and sterically stabilized liposome, *see, e.g.,* Bendas *et al.*, 2001 *BioDrugs*, 15(4): 215-224; Allen *et al.*, 1987 *FEBS Lett.* 223: 42-6; Klibanov *et al.*, 1990 *FEBS Lett.*, 268: 235-7; Blum *et al.*,

1990, *Biochim. Biophys. Acta.*, 1029: 91-7; Torchilin *et al.*, 1996, *J. Liposome Res.* 6: 99-116; Litzinger *et al.*, 1994, *Biochim. Biophys. Acta*, 1190: 99-107; Maruyama *et al.*, 1991, *Chem. Pharm. Bull.*, 39: 1620-2; Klibanov *et al.*, 1991, *Biochim Biophys Acta*, 1062; 142-8; Allen *et al.*, 1994, *Adv. Drug Deliv. Rev*, 13: 285-309. The invention also encompasses liposomes that are adapted for specific organ targeting, *see*, *e.g.*, U.S. Patent No. 4,544,545, or specific cell targeting, see, e.g., U.S. Patent Application Publication No. 2005/0074403. Particularly useful liposomes for use in the disclosed compositions and methods can be generated by reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. In some embodiments, a fragment of an antibody, *e.g.*, F(ab'), may be conjugated to the liposomes using previously described methods, *see*, *e.g.*, Martin *et al.*, 1982, *J. Biol. Chem.* 257: 286-288.

[00169] The B7-H5 antibodies, or B7-H5 or CD28H fusion proteins may also be formulated as immunoliposomes. Immunoliposomes refer to a liposomal composition, wherein an antibody or a fragment thereof is linked, covalently or non-covalently to the liposomal surface. The chemistry of linking an antibody to the liposomal surface is known in the art and encompassed within the invention, *see*, *e.g.*, U.S. Patent No. 6,787,153; Allen *et al.*, 1995, Stealth Liposomes, Boca Rotan: CRC Press, 233-44; Hansen *et al.*, 1995, *Biochim. Biophys. Acta*, 1239: 133-144. In most preferred embodiments, immunoliposomes for use in the disclosed methods and compositions are further sterically stabilized. Preferably, the antibodies or fusion proteins are linked covalently or non-covalently to a hydrophobic anchor, which is stably rooted in the lipid bilayer of the liposome. Examples of hydrophobic anchors include, but are not limited to, phospholipids, *e.g.*, phosoatidylethanolamine (PE), phospahtidylinositol (PI). To achieve a covalent linkage between an antibody and a hydrophobic anchor, any of the known biochemical strategies in the art may be used, *see*, *e.g.*, J. Thomas August, *ed.*, 1997, Gene Therapy: Advances in Pharmacology, Volume 40,

Academic Press, San Diego, CA, p. 399-435. For example, a functional group on an antibody molecule may react with an active group on a liposome associated hydrophobic anchor, *e.g.*, an amino group of a lysine side chain on an antibody may be coupled to liposome associated N-glutaryl-phosphatidylethanolamine activated with water-soluble carbodiimide; or a thiol group of a reduced antibody can be coupled to liposomes via thiol reactive anchors, such as pyridylthiopropionylphosphatidylethanolamine. *See*, *e.g.*, Dietrich *et al.*, 1996, *Biochemistry*, 35: 1100-1105; Loughrey *et al.*, 1987, *Biochim. Biophys. Acta*, 901: 157-160; Martin *et al.*, 1982, *J. Biol. Chem.* 257: 286-288; Martin *et al.*, 1981, *Biochemistry*, 20: 4429-38. Although not intending to be bound by a particular mechanism of action, immunoliposomal formulations including an antibody or fusion protein are particularly effective as therapeutic agents, since they deliver the antibody or fusion protein to the cytoplasm of the target cell, *i.e.*, the cell comprising the receptor to which the antibody or fusion protein binds. The immunoliposomes preferably have an increased half-life in blood, specifically target cells, and can be internalized into the cytoplasm of the target cells thereby avoiding loss of the therapeutic agent or degradation by the endolysosomal pathway.

[00170] The immunoliposomal compositions include one or more vesicle forming lipids, an antibody or a fragment or derivative thereof or a fusion protein, and, optionally, a hydrophilic polymer. A vesicle forming lipid is preferably a lipid with two hydrocarbon chains, such as acyl chains and a polar head group. Examples of vesicle forming lipids include phospholipids, *e.g.*, phosphatidylcholine, phosphatidylethanolamine, phosphatidic acid, phosphatidylinositol, sphingomyelin, and glycolipids, *e.g.*, cerebrosides, gangliosides. Additional lipids useful in the formulations are known to one skilled in the art and encompassed within the invention. In some embodiments, the immunoliposomal compositions further comprise a hydrophilic polymer, *e.g.*, polyethylene glycol, and ganglioside GM1, which increases the serum half-life of the liposome. Methods of conjugating hydrophilic polymers to liposomes are well known in the art and encompassed within the invention. For a review of immunoliposomes and

methods of preparing them, *see, e.g.*, U.S. Patent Application Publication No. 2003/0044407; PCT International Publication No. WO 97/38731, Vingerhoeads *et al.*, 1994, *Immunomethods*, 4: 259-72; Maruyama, 2000, *Biol. Pharm. Bull.* 23(7): 791-799; Abra *et al.*, 2002, *Journal of Liposome Research*, 12(1&2): 1-3; Park, 2002, *Bioscience Reports*, 22(2): 267-281; Bendas *et al.*, 2001 *BioDrugs*, 14(4): 215-224, J. Thomas August, *ed.*, 1997, Gene Therapy: Advances in Pharmacology, Volume 40, Academic Press, San Diego, CA, p. 399-435.

[00171] The antibodies and fusion proteins can be packaged in a hermetically sealed container, such as an ampoule or sachette, indicating the quantity of antibody. In one embodiment, the antibodies are supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, the antibodies or fusion proteins are supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, more preferably at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, or at least 75 mg. The lyophilized antibodies or fusion proteins should be stored at between 2 and 8°C in their original container and the antibodies should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, antibodies or fusion proteins are supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the antibody, fusion protein, or conjugated molecule. Preferably, the liquid form of the antibodies or fusion proteins are supplied in a hermetically sealed container at least 1 mg/ml, more preferably at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, at least 25 mg/ml, at least 50 mg/ml, at least 100 mg/ml, at least 150 mg/ml, at least 200 mg/ml of the antibodies of fusion proteins.

[00172] The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and

should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. For antibodies and fusion proteins, the dosage administered to a patient is typically 0.0001 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.0001 mg/kg and 20 mg/kg, 0.0001 mg/kg and 10 mg/kg, 0.0001 mg/kg and 5 mg/kg, 0.0001 and 2 mg/kg, 0.0001 and 1 mg/kg, 0.0001 mg/kg and 0.75 mg/kg, 0.0001 mg/kg and 0.5 mg/kg, 0.0001 mg/kg to 0.25 mg/kg, 0.0001 to 0.15 mg/kg, 0.0001 to 0.10 mg/kg, 0.001 to 0.5 mg/kg, 0.01 to 0.25 mg/kg or 0.01 to 0.10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies or fragments thereof, or fusion proteins may be reduced by enhancing uptake and tissue penetration of the antibodies or fusion proteins by modifications such as, for example, lipidation.

[00173] In yet another embodiment, the compositions can be delivered in a controlled release or sustained release system. Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more antibodies or fusion proteins. *See, e.g.*, U.S. Patent No. 4,526,938; PCT publication WO 91/05548; PCT publication WO 96/20698; Ning *et al.*, 1996, "Intratumoral Radioimmunotheraphy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," *Radiotherapy & Oncology* 39:179-189, Song *et al.*, 1995, "Antibody Mediated Lung Targeting of Long-Circulating Emulsions," *PDA Journal of Pharmaceutical Science & Technology* 50:372-397; Cleek *et al.*, 1997, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," *Pro. Int'l. Symp. Control. Rel. Bioact. Mater.* 24:853-854; and Lam *et al.*, 1997, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," *Proc. Int'l. Symp. Control Rel. Bioact. Mater.* 24:759-760. In one embodiment, a pump may be used in a

controlled release system (*See* Langer, *supra*; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:20; Buchwald *et al.*, 1980, Surgery 88:507; and Saudek *et al.*, 1989, *N. Engl. J. Med.* 321:574). In another embodiment, polymeric materials can be used to achieve controlled release of antibodies (*see e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61; *See* also Levy *et al.*, 1985, *Science* 228:190; During *et al.*, 1989, Ann. Neurol. 25:351; Howard *et al.*, 1989, *J. Neurosurg.* 7 1:105); U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target (*e.g.*, the lungs), thus requiring only a fraction of the systemic dose (*see*, *e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)). In another embodiment, polymeric compositions useful as controlled release implants are used according to Dunn *et al.* (*See* U.S. 5,945,155). This particular method is based upon the therapeutic effect of the in situ controlled release of the bioactive material from the polymer system. The implantation can generally occur anywhere within the body of the patient in need of therapeutic treatment. In another embodiment, a non-polymeric sustained delivery system is used, whereby a non-polymeric implant in the body of the subject is used as a drug delivery system. Upon implantation in the body, the organic solvent of the implant will dissipate, disperse, or leach from the composition into surrounding tissue fluid, and the non-polymeric material will gradually coagulate or precipitate to form a solid, microporous matrix

(*See* U.S. 5,888,533). Controlled release systems are discussed in the review by Langer (1990, *Science* 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents, i.e., B7-H5 antibodies or CD28H fusion proteins. *See, e.g.*, U.S. Patent No. 4,526,938; International Publication Nos. WO 91/05548 and WO 96/20698; Ning *et al.*, 1996, *Radiotherapy & Oncology* 39:179-189; Song *et al.*, 1995, *PDA Journal of Pharmaceutical Science &* Technology 50:372-397; Cleek *et al.*, 1997, *Pro. Int'l. Symp. Control. Rel. Bioact. Mater.* 24:853-854; and Lam *et al.*, 1997, *Proc. Int'l. Symp. Control Rel. Bioact. Mater.* 24:759-760.

[00174] In a specific embodiment wherein the therapeutic or prophylactic composition is a nucleic acid encoding a B7-H5 antibody or an antigen-binding fragment thereof, or a nucleic acid encoding a CD28H fusion protein, for example, CD28H-Ig fusion protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded antibody or fusion, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (*See* U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (*See e.g.*, Joliot *et al.*, 1991, *Proc. Natl. Acad. Sci. USA* 88:1864-1868), *etc.* Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

[00175] Treatment of a subject with a therapeutically or prophylactically effective amount of antibody or fusion protein can include a single treatment or, preferably, can include a series of treatments.

[00176]

G. Pharmaceutical Compositions

[00177] The compositions include bulk drug compositions useful in the manufacture of pharmaceutical compositions (*e.g.*, impure or non-sterile compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) which can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of a prophylactic and/or therapeutic agent disclosed herein or a combination of those agents and a pharmaceutically acceptable carrier. Preferably, the disclosed compositions include a prophylactically or therapeutically effective amount of antibody or fusion protein and a pharmaceutically acceptable carrier.

[00178] In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete and incomplete), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[00179] Generally, the ingredients of compositions are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00180] The compositions can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include, but are not limited to, those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

H. Kits

[00181] One embodiment provides a pharmaceutical pack or kit comprising one or more containers filled with antibody or fusion protein. Additionally, one or more other prophylactic or therapeutic agents useful for the treatment of a disease can also be included in the pharmaceutical pack or kit. One embodiment provides a pharmaceutical pack or kit including one or more containers filled with one or more of the ingredients of the pharmaceutical compositions. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00182] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises one or more antibodies or fusion proteins. In another embodiment, a kit further comprises one or more other prophylactic or therapeutic agents useful for the treatment of cancer, in one or more containers. In another embodiment, a kit further comprises one or more cytotoxic antibodies that bind one or more cancer antigens

associated with cancer. In certain embodiments, the other prophylactic or therapeutic agent is a chemotherapeutic. In other embodiments, the prophylactic or therapeutic agent is a biological or hormonal therapeutic.

I. Diagnostic Methods

[00183] The B7-H5 antibodies and their antigen-binding fragments can be used for diagnostic purposes, such as to detect, diagnose, or monitor diseases, disorders or infections associated with B7-H5 expression. The invention provides for the detection or diagnosis of a disease, disorder or infection, particularly an autoimmune disease comprising: (a) assaying the expression of B7-H5 in cells or in a tissue sample of a subject using one or more antibodies (or fragments thereof) that immunospecifically bind to such antigens; and (b) comparing the level of the antigen with a control level, *e.g.*, levels in normal tissue samples, whereby an increase or decrease in the assayed level of antigen compared to the control level of the antigen is indicative of the disease, disorder or infection. Such antibodies and fragments are preferably employed in immunoassays, such as the enzyme linked immunosorbent assay (ELISA), the radioimmunoassay (RIA) and fluorescence-activated cell sorting (FACS).

[00184] One embodiment relates to the use of such antibodies and fragments, and particularly such antibodies and fragments that bind to human B7-H5, as reagents for IHC analysis in cells of an *in vitro* or *in situ* tissue sample or *in vivo*. Thus, the antibodies and fragments of the present invention have utility in the detection and diagnosis of a disease, disorder, or infection in a human. In one embodiment, such diagnosis comprises: a) administering to a subject (for example, parenterally, subcutaneously, or intraperitoneally) an effective amount of a labeled antibody or antigen-binding fragment that immunospecifically binds to B7-H5; b) waiting for a time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject where B7-H5 is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled antibody in the subject, such that detection of labeled antibody above the

background level indicates that the subject has the disease, disorder, or infection. In accordance with this embodiment, the antibody is labeled with an imaging moiety which is detectable using an imaging system known to one of skill in the art. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

[00185] It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. *In vivo* tumor imaging is described in S.W. Burchiel *et al.*, "*Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments*," (Chapter 13 in TUMOR IMAGING: THE RADIOCHEMICAL DETECTION OF CANCER, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

[00186] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[00187] In one embodiment, monitoring of a disease, disorder or infection is carried out by repeating the method for diagnosing the disease, disorder or infection, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

[00188] Presence of the labeled molecule can be detected in the subject using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the disclosed diagnostic methods include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[00189] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston *et al.*, U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

[00190] Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention unless specified.

Example 1
Isolation and Characterization of Anti-Human B7-H5 Antibodies

[00191] Anti-human B7-H5 antibodies were obtained by immunizing hamsters with B7-H5, and isolating hybridomas that express B7-H5-immunoreactive antibodies. Antibody-producing clones 2D3 and 18C3 were isolated. **Figures 4A-4B** show the ability of the antibodies produced by clones 2D3 and 18C3to bind human B7-H5 expressed by a CHO transfectants.

[00192] The ability of the isolated antibodies to block B7-H5:CD28H interaction was determined by incubating the human B7-H5-expressing CHO transfectants in the presence of such antibodies and increasing concentrations of CD28H Ig (SEQ ID NO:20) and anti-human Ig PE.

[00193] In such an experiment, cells incubated in the presence of non-blocking antibodies are capable of binding the CD28H Ig (seen as a second migration peak) (**Figures 5A-5B**). Antibodies 2D3 and 18C3 were found to block the B7-H5:CD28H interaction.

[00194] An anti-B7-H5 antibody of the present invention (2D3) was tested for its ability to block the B7-H5:CD28H interaction. A B7-H5 Ig fusion

was prepared and found to be capable of binding to CD28H and of thereby stimulating a T cell response (**Figure 6**). The fusion protein is the extracellular domain of B7-H5 (**SEQ ID NO:26**)

IFPLAFFIYVPMNEQIVIGRLDEDIILPSSFERGSEVVIHWKYQDSYKVHSYYKGSDHLESQ
DPRYANRTSLFYNEIQNGNASLFFRRVSLLDEGIYTCYVGTAIQVITNKVVLKVGVFLTPVM
KYEKRNTNSFLICSVLSVYPRPIITWKMDNTPISENNMEETGSLDSFSINSPLNITGSNSSY
ECTIENSLLKQTWTGRWTMKDGLHKMQSEHVSLSCQPVNDYFSPNQDFKVTWSRMKSGTFSV
LAYYLSSSQNTIINESRFSWNKELINQSDFSMNLMDLNLSDSGEYLCNISSDEYTLLTIHTV
HVEPSQET

fused to a murine IgG2a sequence.

An IgG2a sequence can be (**SEQ ID NO:27**)

EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVV
DVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWM
SGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVT
LTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEK
KNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

[00195] mIgG2a does not require the S->P modification (discussed in more detail below and as shown in **SEQ ID NO:20**) to stabilize the Fab arm or deletion of the C-terminal Lys. Fusion proteins can also be made with other Ig sequences, for example, human IgG4. For human IgG4 it can be preferred to include the S->P modification and/or deletion of the final Lys residue, as illustrated in the exemplary hIgG4 proteins discussed above.

[00196] The fusion protein was expressed from a vector encoding the CD33 signal peptide (not the native signal peptide).

[00197] **Figure 7** shows the ability of the B7-H5 Ig to induce the expression of cytokines (IL-2, IFN-γ and IL-10), thereby confirming the ability of the molecule to stimulate the immune system. The provision of the anti-B7-H5 antibody 2D3 inhibited such stimulation (**Figure 8**). The antibody was also found to be capable of inhibiting an allogeneic T cell response (**Figure 9**). These results indicate that those antibodies of the present invention that are capable of binding to B7-H5 so as to block the capacity of B7-H5 to interact with its CD28H counter-receptor are capable of mediating a physiological reduction in immune system activation.

Example 2
Anti-Human B7-H5 Antibodies Reduce the Percentage of Activated T Cells *in vivo*

[00198] In order to investigate the *in vivo* effect of the anti-human B7-H5 antibodies, 20 million peripheral blood mononuclear cells (**PBMC**s) were injected into the peritoneal cavity of NOD scid IL2 receptor gamma chain knockout (**NSG**) mice (see, *e.g.*, Iorns, E. et al. (Epub 2012 Oct 31) "A New Mouse Model For The Study Of Human Breast Cancer Metastasis," PLoS One 7(10):e47995; Misharin, A.V. et al. (2012) "*Development Of A New Humanized Mouse Model To Study Acute Inflammatory Arthritis*," J. Transl. Med. 10:190; Waldron-Lynch, F. et al. (Epub 2012 Aug 17) "*Analysis Of Human Biologics With A Mouse Skin Transplant Model In Humanized Mice*," Amer. J. Transplant. 12(10):2652-2662; Volk, A. *et al.* (2012) "*Comparison Of Three Humanized Mouse Models For Adoptive T Cell Transfer*," J. Gene Med. 14(8):540-548; Racki, W.J. *et al.* (2010) "*NOD-scid IL2rgamma(null) Mouse Model Of Human Skin Transplantation And Allograft Rejection*," Transplantation 89(5):527-536). Phosphate buffered saline (PBS) or anti-human B7-H5 antibody (2D3) was injected into the tail vein of the animals and the percentage of $CD28H^+$ cells among $CD45RO^+$ cells was determined.

[00199] As shown in **Figure 10A** and **Figure 10B**, the percentage of $CD28H^+$ cells among activated ($CD45RO^+$) human T cells in the spleen (**Figure 10A**) and peripheral blood (**Figure 10B**) was reduced upon treatment with the anti-human B7-H5 antibody. Treatment with B7-H5 antibodies thus reduces the percentage of activated T cells *in vivo*. In contrast, such treatment had only a limited effect on naïve ($CD45RO^-$) T cell populations (**Figure 11A** and **Figure 11B**).

Example 3
Recombinant Anti-Human B7-H5 Antibodies Retain Antigen Specificity

[00200] IgG4 antibodies have unique structural and functional properties and undergo "half-antibody exchange" in vivo, resulting in recombined antibodies composed of two different binding specificities (Nirula, A. *et al.* (2011) "W*hat Is Igg4? A Review Of The Biology Of A Unique*

Immunoglobulin Subtype," *Curr. Opin. Rheumatol.* 23(1):119-124; .Aalberse, R.C. *et al.* (2009) "*Immunoglobulin G4: An Odd Antibody*," Clin. Exp. Allergy 39(4):469-477). Ser228 is mutated to Pro to stabilize the arm. A chimeric hIgG4P does not do "half-antibody exchange".

[00201] The human B7-H5-binding antibodies of the present invention (antibodies 2D3 and 18C3) were recombinantly converted to a human IgG4P isotype from their native hamster isotype. CHO transfectants that express human B7-H5 were then incubated in the presence of the recombinant antibodies. As shown in **Figures 12A-C**, both human IgG4P derivative antibodies were found to retain their immunospecific binding ability toward human B7-H5 and blocking capability disrupting the B7-H5-CD28H interaction (**Figure 13, Panels A-C**). **Figures 14A-14B** show that antibodies 2D3 and 18C3 recognize the first IgV domain of B7-H5, which mediates B7-H5's interaction with CD28H (**Figure 14C**). **Figure 15** compares the affinities of antibodies 2D3 and 18C3 for binding to human B7-H5 as expressed on the surface of CHO cells.

Example 4
Blockade of B7-H5:CD28H Interaction Inhibits Tetanus Toxoid (TT)-Specific T Cell Response

[00202] In order to determine the effect of the B7-H5:CD28H interaction on the recall of memory-specific T cells, NSG mice reconstituted with human PBMCs plus autologous dendritic cells were immunized with a tetanus toxoid ("**TT**") vaccine. At the same day, mice were treated with control Ig, or anti-human B7-H5 antibody 2D3. Seven days later, cells in the peritoneal cavity were harvested, and re-stimulated with the TT antigen. T cell division was analyzed by BrdU incorporation. Cytokines in the culture supernatant were examined by a human Th1/Th2 CBA kit.

[00203] The results of such investigations are shown in **Figures 16A-16B**. As shown in these Figures, the endogenous B7-H5:CD28H interaction appears to be important for the recall of TT-specific memory T cells because injection of the mice with B7-H5 blocking mAb 2D3 significantly inhibited TT vaccine-elicited T cell proliferation, as indicated by BrdU incorporation

(**Figure 16A, Panels A-B**). As a result, cytokines in the culture supernatant including IFN-γ, IL-5 and IL-10 were all substantially reduced by the 2D3 mAb (**Figure 16B, Panels A-B**). Taken together, the results indicate that the B7-H5:CD28H interaction plays an important role in the recall of memory T cells.

Example 5
Blockade of B7-H5:CD28H Interaction Inhibits Allogeneic T Cell Response

[00204] In order to determine the effect of the B7-H5:CD28H interaction on the allogeneic response *in vivo*, humanized NSG mice were challenged with allogeneic human macrophages. On the same day, each mouse was inoculated with control or 2D3 mAb. On day 7, splenocytes were harvested and allogeneic CD4 and CD8 T cell proliferations were evaluated by Ki-67 expression by intracellular staining.

[00205] The results of such investigations are shown in **Figure 17**. As shown in **Figure 17**, the endogenous B7-H5:CD28H interaction appears to be important for the allogeneic T cell response because injection of the mice with B7-H5 blocking mAb 2D3 significantly inhibited both CD8+ (**Figure 17A**) and CD4+ (**Figure 17B**) T cell proliferation, as indicated by Ki-67 expression.

Example 6

Blockade of B7-H5:CD28H Interaction Inhibits AKT activation in human T cells

[00206] The results of such investigations are shown in **Figure 18**. As shown in **Figure 18**, the endogenous B7-H5:CD28H interaction appears to be important for AKT pathway activation in the presence of TCR signal. Simultaneous Cross-linking of TCR and B7-H5 fusion protein induced AKT phosphorylation 30 min after stimulation, while TCR cross-linking alone induced minimal AKT phosphorylation. Inclusion of B7-H5 blocking antibody 2D3 prevented AKT activation, indicating B7-H5 blockade could block T cell AKT pathway activation.

Example 7

Blockade of B7-H5:CD28H Interaction Suppress Cytotoxic T lymphocyte Killing against tumor cells

[00207] The results of such investigations are shown in **Figure 19**. As shown in **Figure 19**, the interaction of tumor associated B7-H5 to cytotoxic T cell expressed CD28H costimulates the cytotoxic killing activity, as the blockade of B7-H5-CD28H pathway by decoy receptor fusion protein inhibits the CTL killing at several effector to target (E:T) ratio conditions.

Example 8

Blockade of B7-H5:CD28H Interaction Suppress Natural Killer Cell activation

[00208] The results of such investigations are shown in **Figure 20A-B**. As shown in **Figure 20A**, simultaneous cross-linking of CD16 and B7-H5 fusion protein on Natural Killer cells (NK) in the presence of soluble recombinant human IL-2 induced NK activation and degranulation (CD107a surface upregulation), whereas CD16 engagement alone did not induce significant NK degranulation. B7-H5-CD28H pathway blockade by B7-H5 antibody 2D3 suppressed NK degranulation (Figure 20B), indicating B7-H5 co-stimulates NK activation.

[00209] All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety. While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

We Claim:

1. An antibody or antigen binding fragment thereof comprising six complementarity determining regions (CDRs),

wherein the CDRs comprise

(1) the three light chain CDRs of SEQ ID NO:11 and the three heavy chain CDRs of SEQ ID NO:9, or

(2) the three light chain CDRs of SEQ ID NO:15 and the three heavy chain CDRs of SEQ ID NO:13, and

wherein the antibody or antigen binding fragment thereof binds to B7-H5.

2. The antibody or antigen binding fragment thereof of claim 1, wherein the three light chain CDRs comprise a first light chain CDR comprising amino acids 27-38 of SEQ ID NO:11, a second light chain CDR comprising amino acids 56-58 of SEQ ID NO:11, and a third light chain CDR comprising amino acids 95-102 of SEQ ID NO:11.

3. The antibody or antigen binding fragment thereof of one of claims 1 or 2, wherein the three heavy chain CDRs comprise a first heavy chain CDR comprising amino acids 26-33 of SEQ ID NO:9, a second heavy chain CDR comprising amino acids 51-58 of SEQ ID NO:9, and a third heavy chain CDR comprising amino acids 97-106 of SEQ ID NO:9.

4. The antibody or antigen binding fragment thereof of one of claims 1-3 comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:11.

5. The antibody or antigen binding fragment thereof of one of claims 1-4 comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:9.

6. The antibody or antigen binding fragment thereof of claim 1, wherein the three light chain CDRs comprise a first light chain CDR comprising amino acids 27-38 of SEQ ID NO:15, a second light chain CDR comprising amino acids 56-58 of SEQ ID NO:15, and a third light chain CDR comprising amino acids 95-102 of SEQ ID NO:15.

7. The antibody or antigen binding fragment thereof of one of claims 1 or 2, wherein the three heavy chain CDRs comprise a first heavy chain CDR comprising amino acids 26-33 of SEQ ID NO:13, a second heavy chain CDR

comprising amino acids 51-58 of SEQ ID NO:13, and a third heavy chain CDR comprising amino acids 97-106 of SEQ ID NO:13.

8. The antibody or antigen binding fragment thereof of one of claims 1-3 comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:15.

9. The antibody or antigen binding fragment thereof of one of claims 1-4 comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:13.

10. The antibody or antigen binding fragment thereof of claim 1 comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:11 and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:9.

11. The antibody or antigen binding fragment thereof of claim 1 comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:15 and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:13.

12. The antibody or antigen binding fragment thereof of claim 1, wherein the bound B7-H5 is arrayed on the surface of a live cell or expressed at an endogenous or transfected concentration.

13. The antibody or antigen binding fragment thereof of claim 7, wherein the live cell is a macrophage or dendritic cell.

14. The antibody or antigen binding fragment thereof of claim 1, wherein the B7-H5 is human B7-H5.

15. The antibody or antigen binding fragment thereof of claim 1 wherein the antibody or antigen binding fragment thereof

(A) attenuates the ability of a ligand of B7-H5 to bind to CD28H;

(B) antagonizes signal transduction that occurs as a consequence of B7-H5 binding to CD28H;

(C) inhibits an allogeneic T cell response;

(D) a combination thereof.

16. The antibody or antigen binding fragment thereof of any one of claims 1-15 comprising one or more constant domains from an immunoglobulin constant region (Fc).

17. The antibody or antigen binding fragment thereof of claim 16 wherein the constant domains are human constant domains.

18. The antibody or antigen binding fragment thereof of claim 17 wherein the human constant domains are IgA, IgD, IgE, IgG or IgM domains.

19. The antibody or antigen binding fragment thereof of claim 18 wherein human IgG constant domains are IgG1, IgG2, IgG3, or IgG4 domains.

20. The antibody or antigen binding fragment thereof of any one of claims 1-19 wherein the antibody or antigen binding fragment thereof is detectably labeled or comprises a conjugated toxin, drug, receptor, enzyme, receptor ligand.

21. The antibody or antigen binding fragment thereof of any one of claim 1-20, wherein the antibody is a monoclonal antibody, a human antibody, a chimeric antibody or a humanized antibody.

22. The antibody or antigen binding fragment thereof of any one of claims 1-21, wherein the antibody is a bispecific, trispecific or multispecific antibody.

23. A humanized antibody or antigen binding fragment thereof comprising one or more human IgG4 constant domains and

a light chain variable region comprising the amino acid sequence of SEQ ID NO:11, a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:9, or

a light chain variable region comprising the amino acid sequence of SEQ ID NO:15, a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:13.

24. A pharmaceutical composition comprising the antibody or antigen binding fragment thereof of any one of claims 1-23, or a CD28H-Ig fusion protein and a physiologically acceptable carrier or excipient.

25. The pharmaceutical composition of claim 24 for use in a method of down-modulating the immune system of a subject.

26. The pharmaceutical composition for use of claim 25 wherein the subject has an autoimmune disease.

27. The pharmaceutical composition of claim 24 for use in a method of treating an autoimmune disease.

28. The pharmaceutical composition for use of claim 26 or 27 wherein the autoimmune disease is selected from the group consisting of alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac sprue-dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatrical pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, glomerulonephritis, Graves' disease, Guillain-Barre, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA neuropathy, juvenile arthritis, lichen planus, lupus erthematosus, Ménière's disease, mixed connective tissue disease, multiple sclerosis, Neuromyelitis optica (NMO), type 1 or immune-mediated diabetes mellitus, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychrondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynauld's phenomenon, Reiter's syndrome, Rheumatoid arthritis, sarcoidosis, scleroderma, Sjögren's syndrome, stiff-man syndrome, systemic lupus erythematosus, lupus erythematosus, takayasu arteritis, temporal arteristis/ giant cell arteritis, ulcerative colitis, uveitis, vasculitides such as dermatitis herpetiformis vasculitis, vitiligo, and Wegener's granulomatosis

29. The pharmaceutical composition for use of claim 25 wherein the subject has an inflammatory disease.

30. The pharmaceutical composition of claim 24 for use in a method of treating an inflammatory disease.

31. The pharmaceutical composition for use of claims 29 or 30 wherein the inflammatory disease is selected from the group consisting of asthma, encephilitis, inflammatory bowel disease, chronic obstructive pulmonary

disease (COPD), allergic disorders, septic shock, pulmonary fibrosis, undifferentiated spondyloarthropathy, undifferentiated arthropathy, arthritis, inflammatory osteolysis, and chronic inflammation resulting from chronic viral or bacterial infections.

32. The pharmaceutical composition for use of claim 25 wherein the subject has received or will receive a transplant.

33. The pharmaceutical composition of claim 24 for use in a method of treating or preventing a transplant rejection.

34. Use of the antibody or antigen binding fragment thereof of any of claims 1-23 or a CD28H-Ig fusion protein in manufacture of a medicament for down-modulating the immune system in a subject.

35. Use of the antibody or antigen binding fragment thereof of any of claims 1-23 or a CD28H-Ig fusion protein in manufacture of a medicament for treating an autoimmune disease in a subject.

36. Use of the antibody or antigen binding fragment thereof of any of claims 1-23 or a CD28H-Ig fusion protein in manufacture of a medicament for treating an inflammatory disease in a subject.

37. Use of the antibody or antigen binding fragment thereof of any of claims 1-23 or a CD28H-Ig fusion protein in manufacture of a medicament for treating or preventing transplant rejection in a subject.

38. A method of detection or diagnosis of a disease, disorder or infection, comprising: (a) assaying the expression of B7-H5 in cells or in a tissue sample of a subject using the antibody or antigen binding fragment thereof of any one of claims 1-23 and (b) comparing the level of the B7-H5 with a control level, wherein an increase in the assayed level of B7-H5 compared to the control level is indicative of the disease, disorder or infection.

39. The method of claim 38, wherein the expression of B7-H5 is assayed by enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), or fluorescence-activated cell sorting (FACS).

40. A method for monitoring the progression of a disease, disorder or infection, comprising: (a) assaying the expression of B7-H5 in cells or in a tissue sample of a subject a first time point using the antibody or antigen binding fragment thereof of any one of claims 1-23; and (b) comparing the level of expression of B7-H5 in the cells or in the tissue sample of the subject

at second time point, or over a time course, wherein an increase in the assayed level of B7-H5 is indicative of the progression of disease, disorder or infection.

41. The method of claim 30, wherein the expression of B7-H5 is assayed by enzyme linked immunosorbent assay (ELISA) radioimmunoassay (RIA), or fluorescence-activated cell sorting (FACS).

42. A method for monitoring the response to treatment, comprising: (a) assaying the expression of B7-H5 in cells or in a tissue sample of a subject prior to treatment using the antibody or antigen binding fragment thereof of any one of claims 1-23; and (b) assaying the expression of B7-H5 in cells or in a tissue sample of a subject prior at one or more time points after treatment, and comparing the level of B7-H5 over time, whereby a decrease in the assayed level of B7-H5 compared to the pre-treatment level of B7-H5 is indicative of a response to treatment.

43. The method of claim 42, wherein the expression of B7-H5 is assayed by enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), or fluorescence-activated cell sorting (FACS).

44. A pharmaceutical composition for use in a method of treating a subject for a disease characterized by increased expression of B7-H5, wherein the method comprises the steps of

(i) determining whether the subject has a disease characterized by increased expression of B7-H5 by

(a) assaying the expression of B7-H5 in cells or in a tissue sample of the subject

using the antibody or antigen binding fragment thereof that binds to B7-H5;

(b) comparing the level of the B7-H5 with a control level, wherein an increase in

the assayed level of B7-H5 compared to the control level is indicative that the subject has a disease characterized by increased expression of B7-H5; and

(ii) administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 24 if the subject has a disease characterized by increased expression of B7-H5.

45. The method of claim 44 wherein the antibody or antigen binding fragment thereof is the antibody or antigen binding fragment thereof of any one of claims 1-23.

46. The pharmaceutical compositions for use of claim 44, wherein the disease characterized by increased expression of B7-H5 is an autoimmune disease.

47. The pharmaceutical compositions for use of claim 44, wherein the disease characterized by increased expression of B7-H5 is an inflammatory disease.

48. A pharmaceutical composition for use in a method of treating a subject for a disease characterized by increased expression of CD28H, wherein the method comprises the steps of

(i) determining whether the subject has a disease characterized by increased expression of CD28H by

(a) assaying the expression of CD28H in cells or in a tissue sample of the subject

using an anti- CD28H antibody or an antigen binding fragment thereof;

(b) comparing the level of the CD28H with a control level, wherein an increase in

the assayed level of CD28H compared to the control level is indicative that the subject has a disease characterized by increased expression of CD28H; and

(ii) administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 24 if the subject has a disease characterized by increased expression

of CD28H.

49. The pharmaceutical compositions for use of claim 48, wherein the disease characterized by increased expression of CD28H is an autoimmune disease.

50. The pharmaceutical compositions for use of claim 48, wherein the disease characterized by increased expression of CD28H is an inflammatory disease.

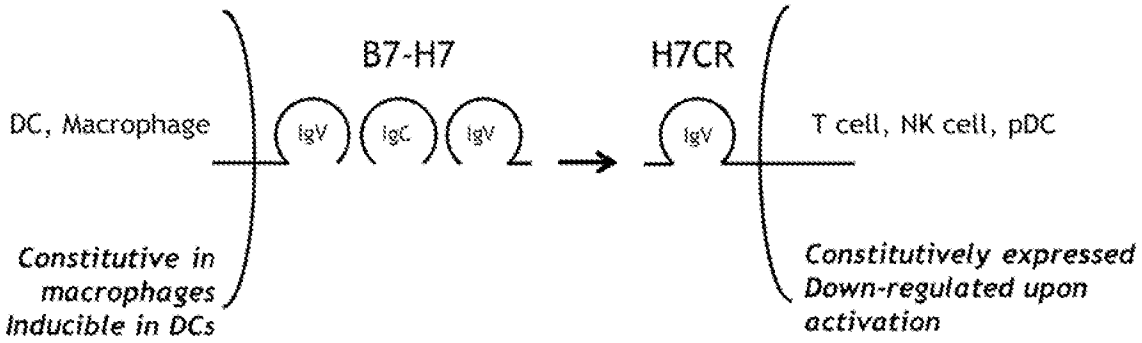

B7-H7
H7CR
DC, Macrophage
IgV
IgC
IgV
IgV
T cell, NK cell, pDC
Constitutive in macrophages Inducible in DCs
Constitutively expressed Down-regulated upon activation

FIG. 1

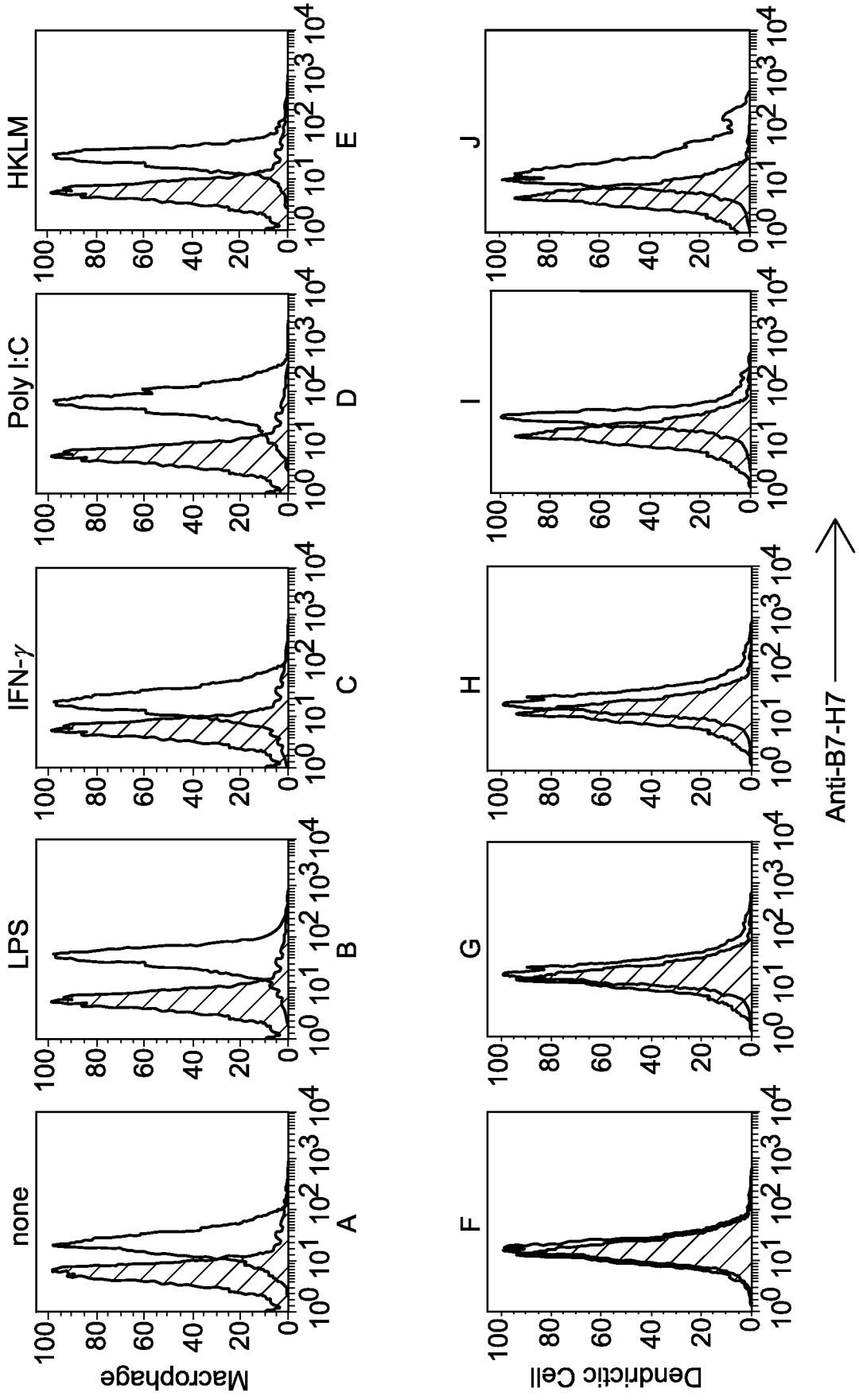

none
LPS
IFN-γ
Poly I:C
HKLM
Macrophage
Dendritic Cell
A
B
C
D
E
F
G
H
I
J
Anti-B7-H7

FIG. 2

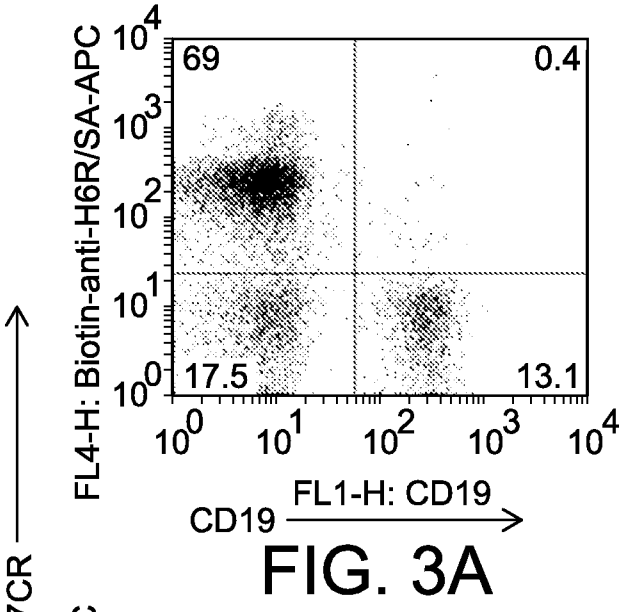


FIG. 3A

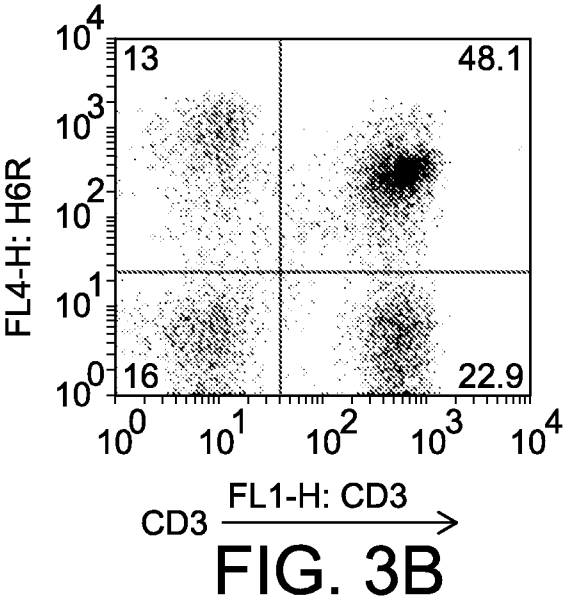


FIG. 3B

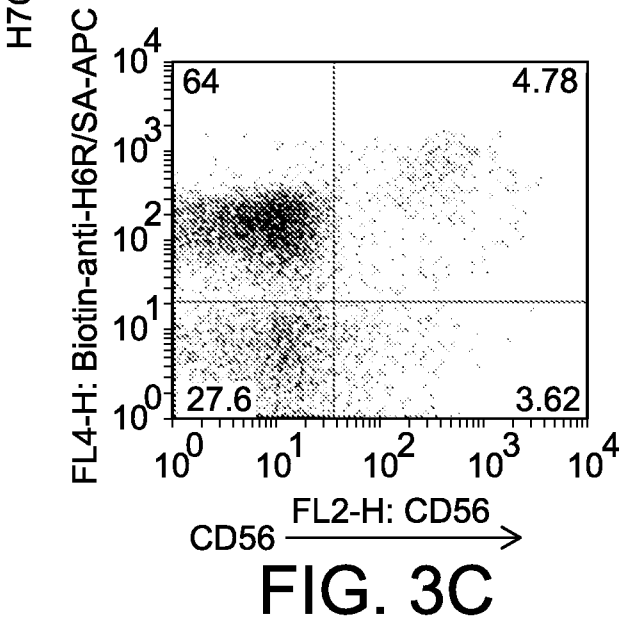


FIG. 3C

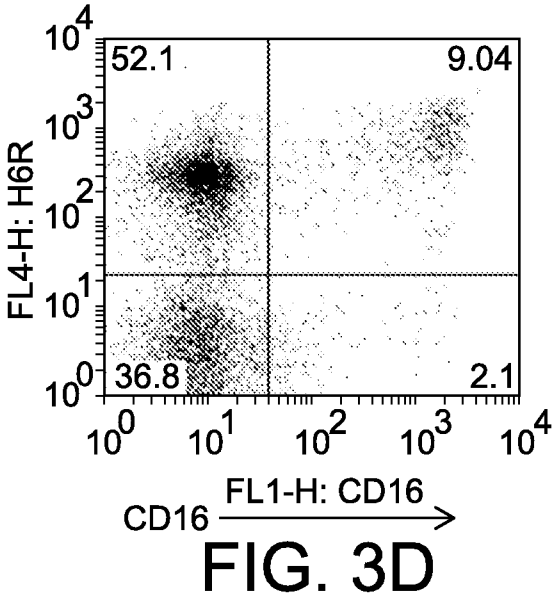


FIG. 3D

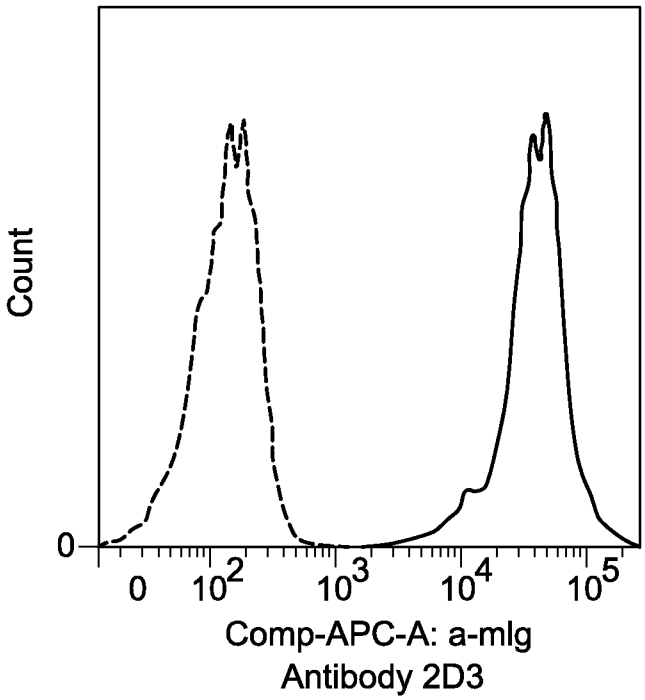

Count
0
0
10^2
10^3
10^4
10^5
Comp-APC-A: a-mlg
Antibody 2D3

FIG. 4A

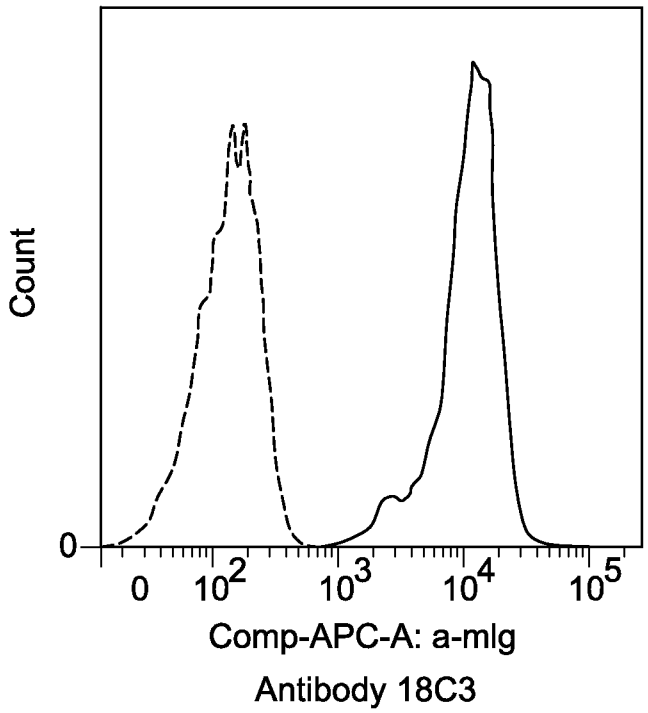

Count
0
0
10^2
10^3
10^4
10^5
Comp-APC-A: a-mlg
Antibody 18C3

FIG. 4B

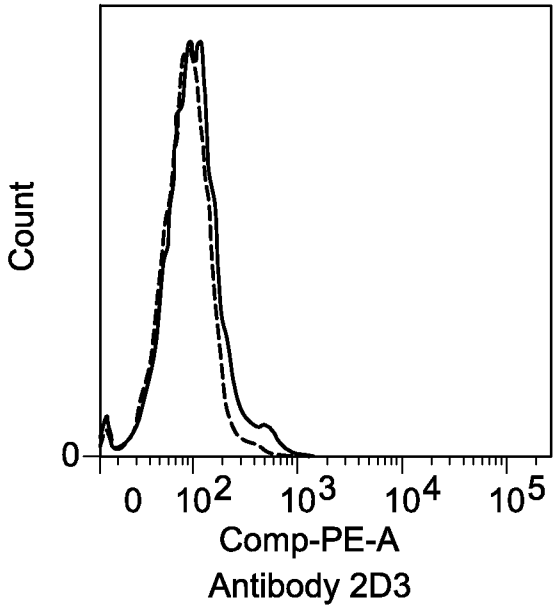


FIG. 5A

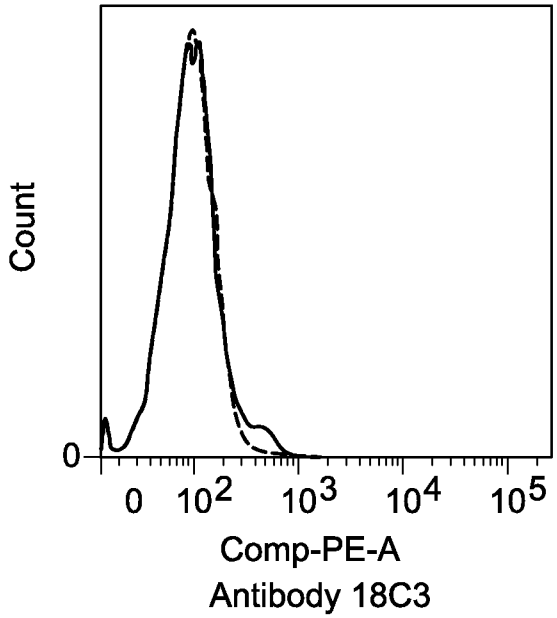


FIG. 5B

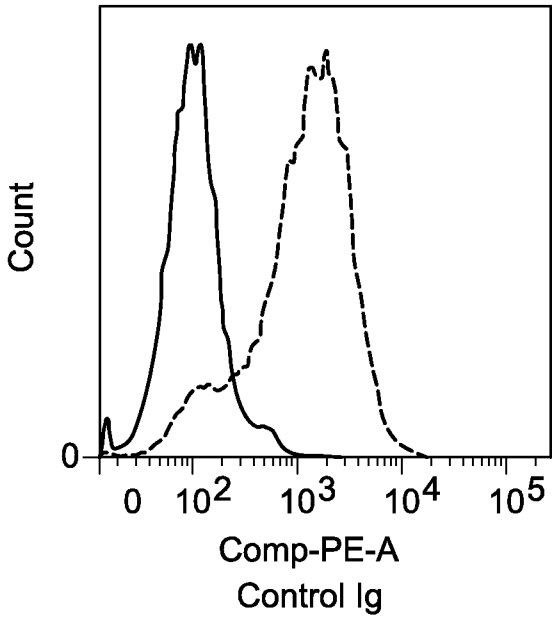


FIG. 5C

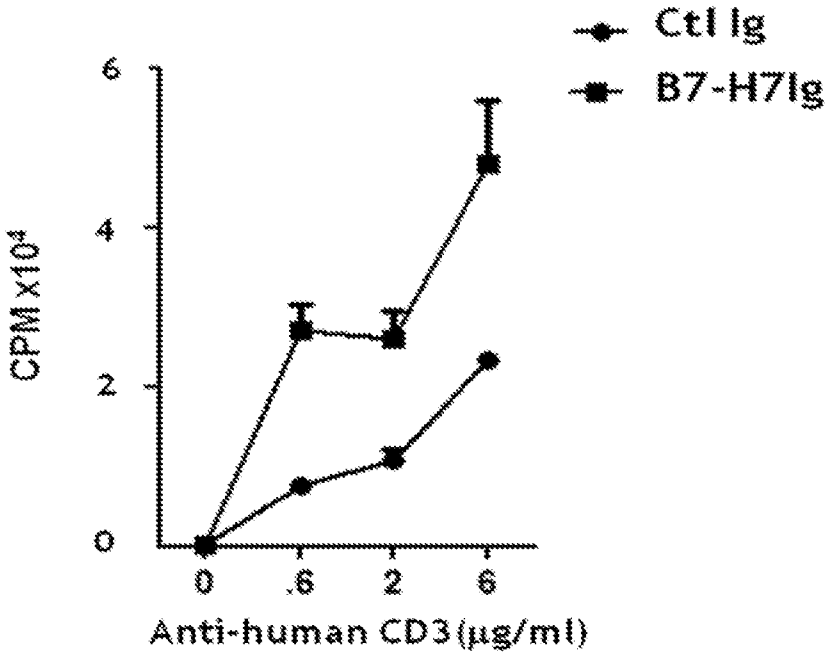

Ctl Ig
B7-H7Ig
CPM x10⁴
6
4
2
0
0
.6
2
6
Anti-human CD3 (μg/ml)

FIG. 6

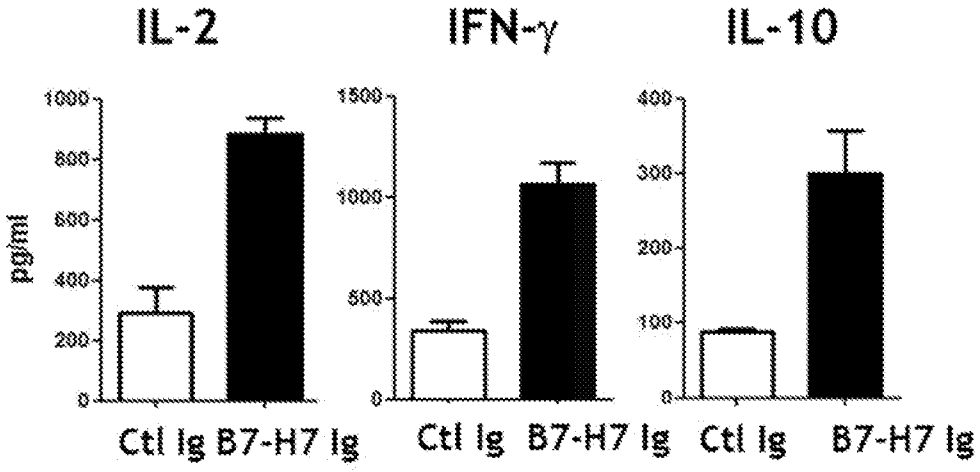

IL-2
IFN-γ
IL-10
pg/ml
1000
800
600
400
200
0
1500
1000
500
0
400
300
200
100
0
Ctl Ig
B7-H7 Ig
Ctl Ig
B7-H7 Ig
Ctl Ig
B7-H7 Ig

FIG. 7

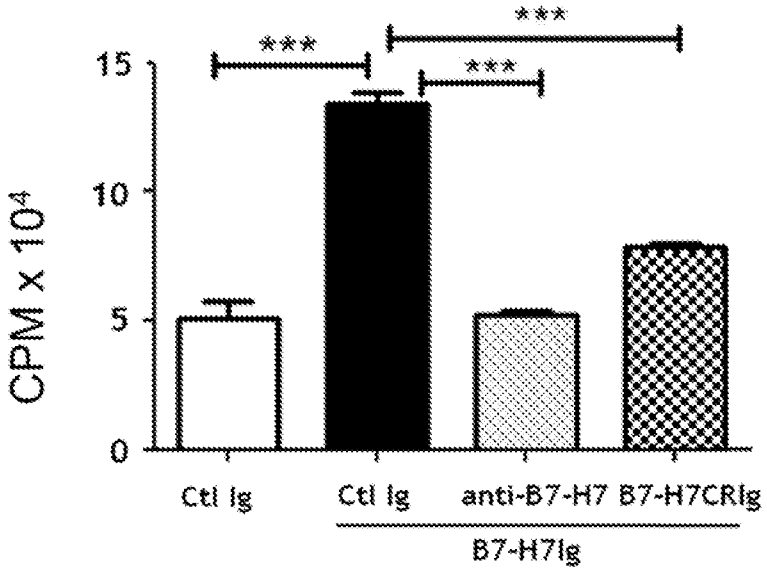

***
***
***
15
10
5
0
CPM x 10⁴
Ctl Ig
Ctl Ig
anti-B7-H7
B7-H7CRIg
B7-H7Ig

FIG. 8

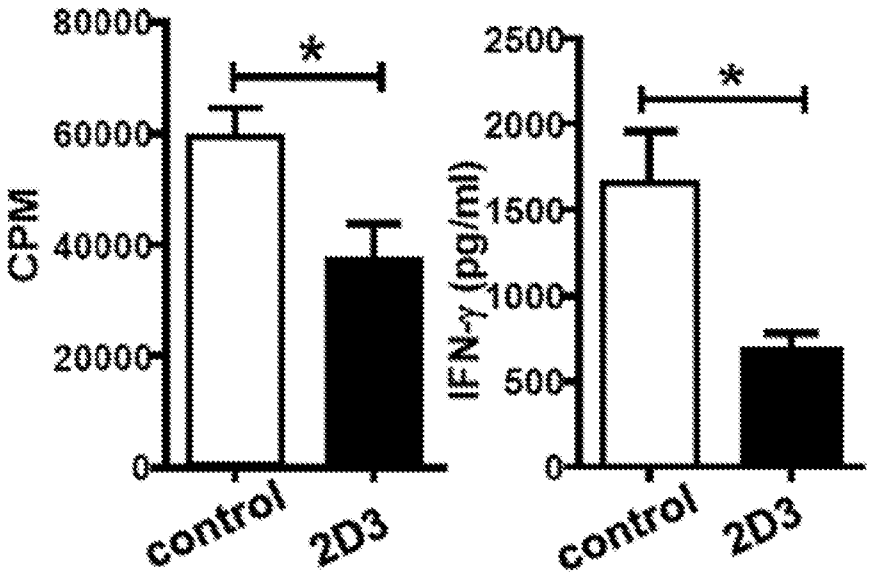

80000
60000
40000
20000
0
CPM
*
control
2D3
2500
2000
1500
1000
500
0
IFN-γ (pg/ml)
*
control
2D3

FIG. 9

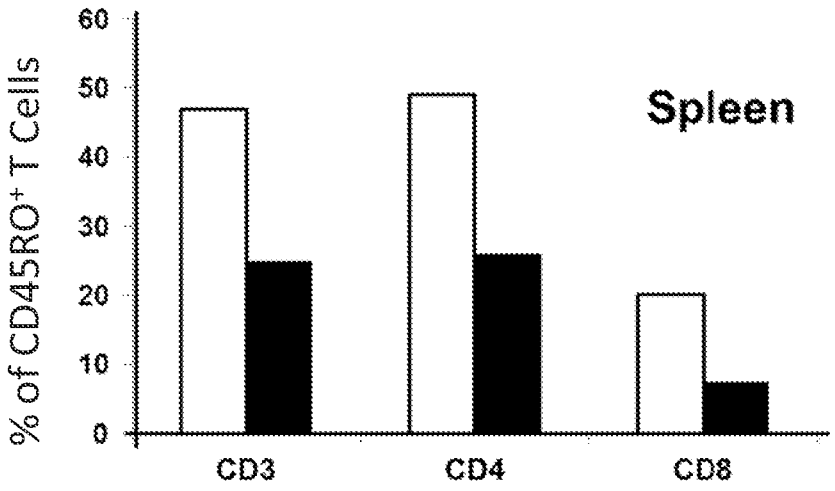


Figure 10A

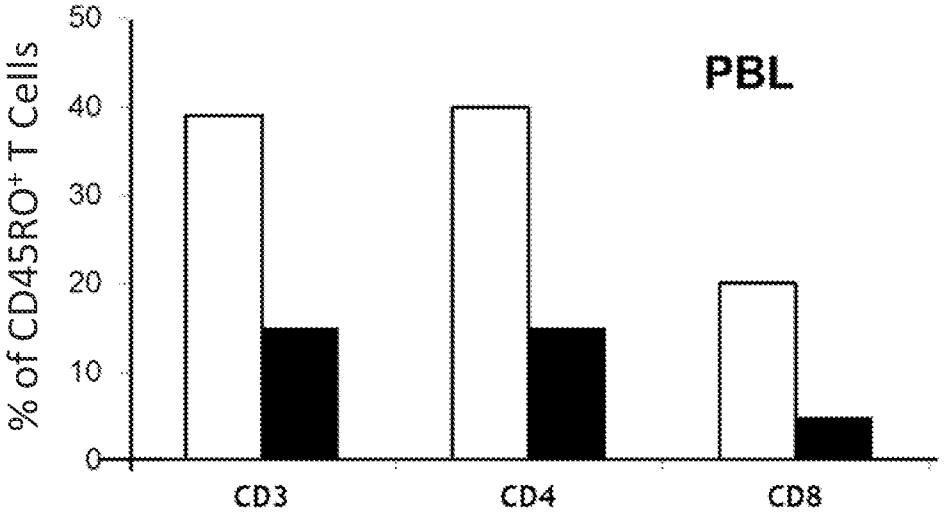
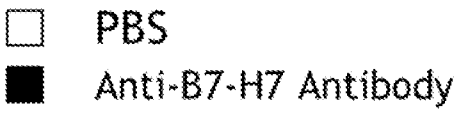


Figure 10B



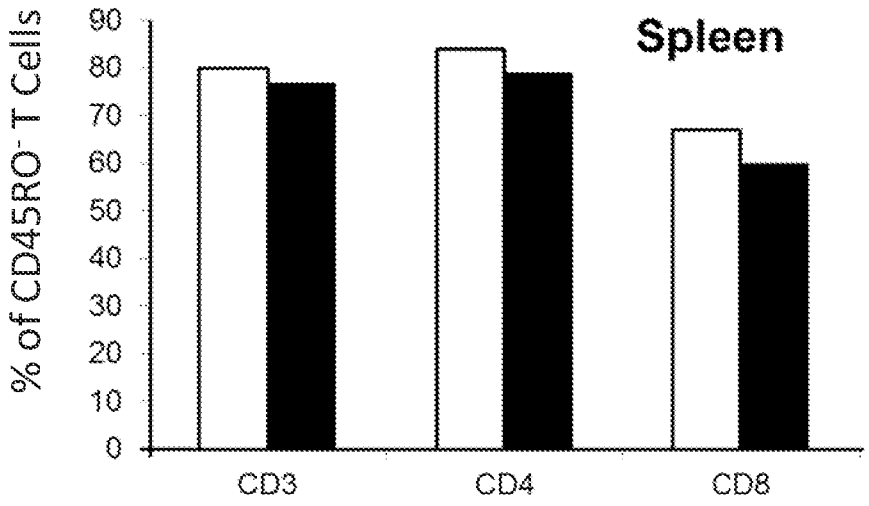


Figure 11A

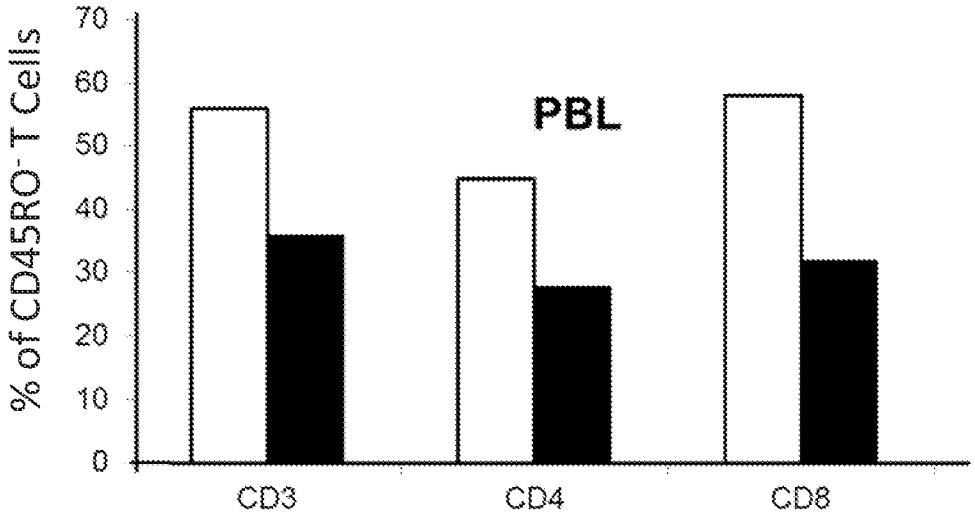
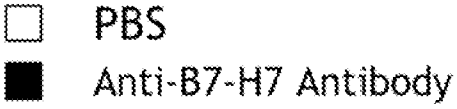
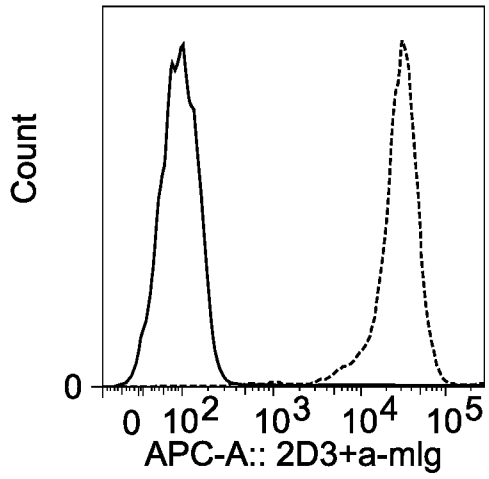


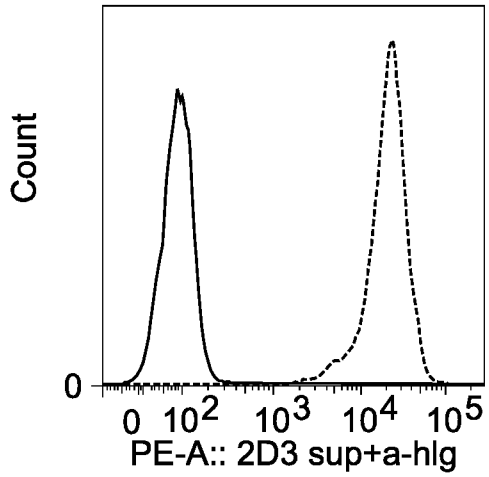
Figure 11B





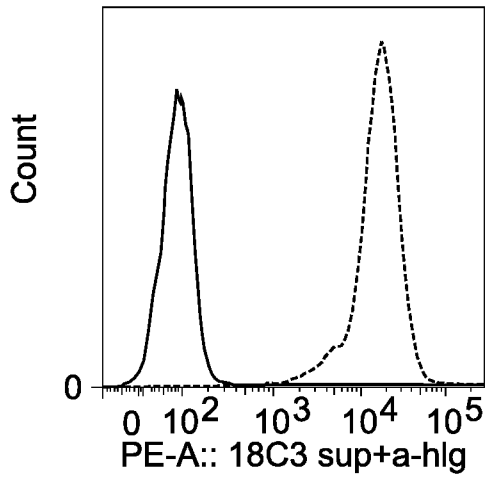
Purified Anti-Human B7-H7 Antibody 2D3

FIG. 12A



Recombinant Anti-Human B7-H7 Chimeric Antibody 2D3 (hIgG4)

FIG. 12B



Recombinant Anti-Human B7-H7 Chimeric Antibody 18C3 (hIgG4)

FIG. 12C

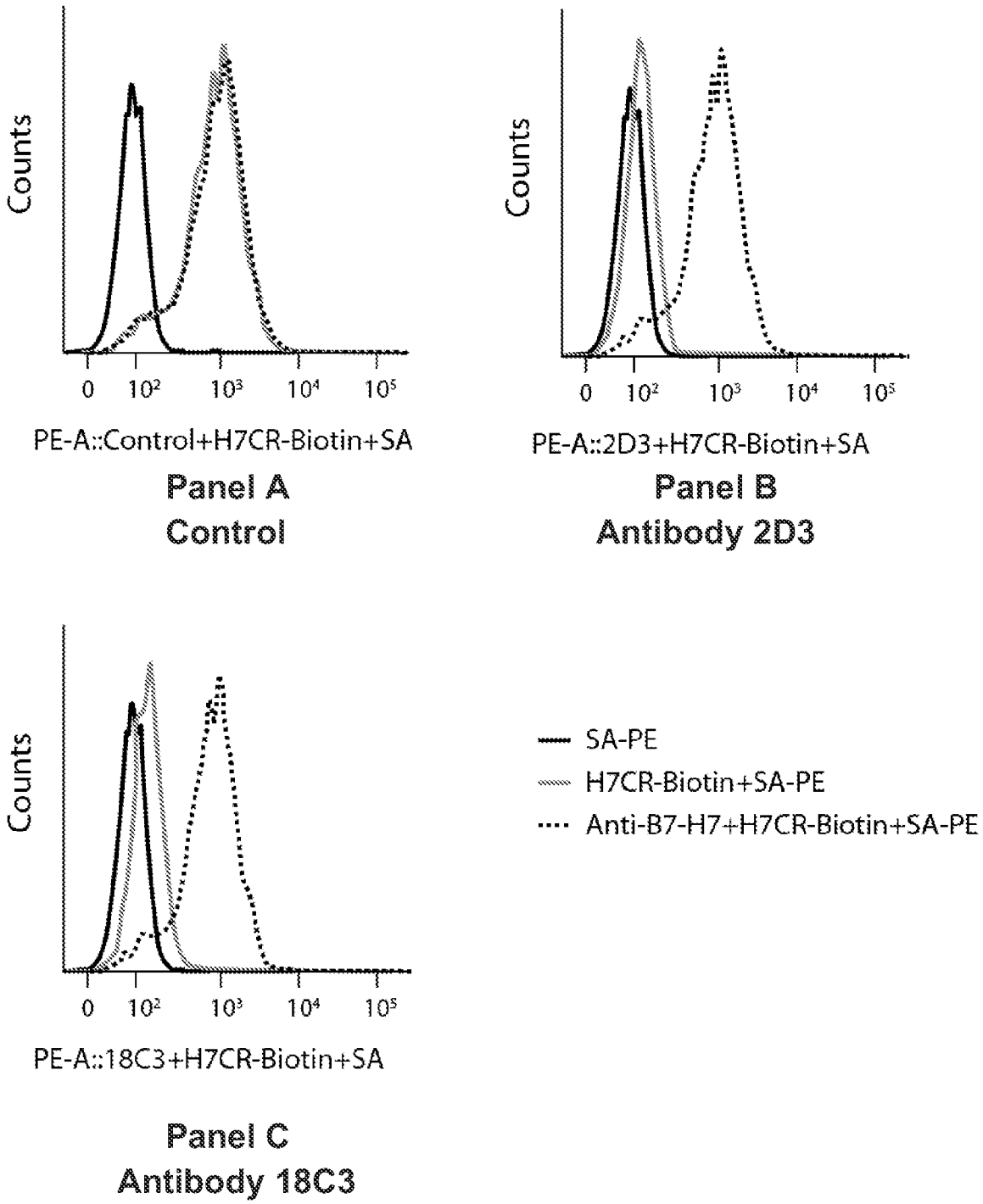

Counts
0
10²
10³
10⁴
10⁵
PE-A::Control+H7CR-Biotin+SA
Panel A
Control
PE-A::2D3+H7CR-Biotin+SA
Panel B
Antibody 2D3
PE-A::18C3+H7CR-Biotin+SA
Panel C
Antibody 18C3
SA-PE
H7CR-Biotin+SA-PE
Anti-B7-H7+H7CR-Biotin+SA-PE

FIG. 13

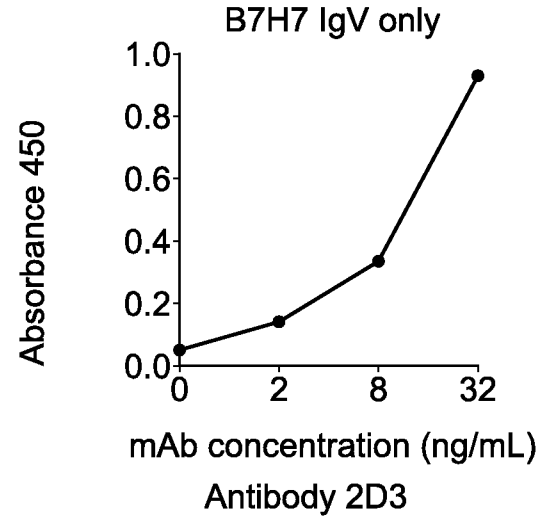


FIG. 14A

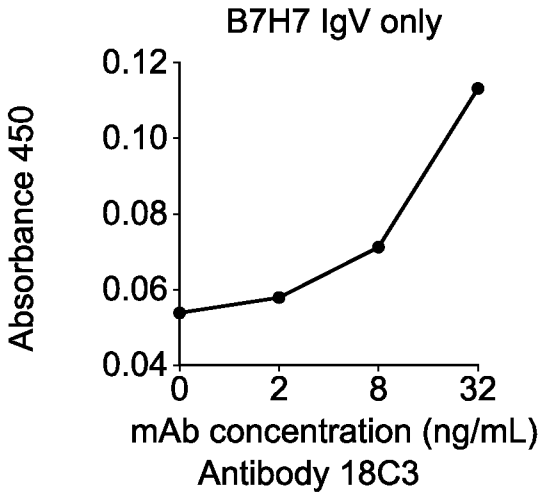


FIG. 14B

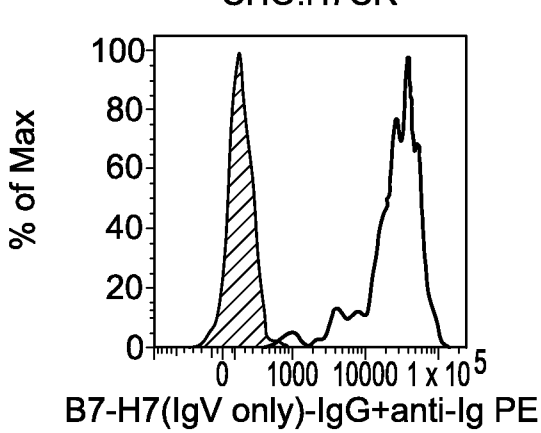


FIG. 14C

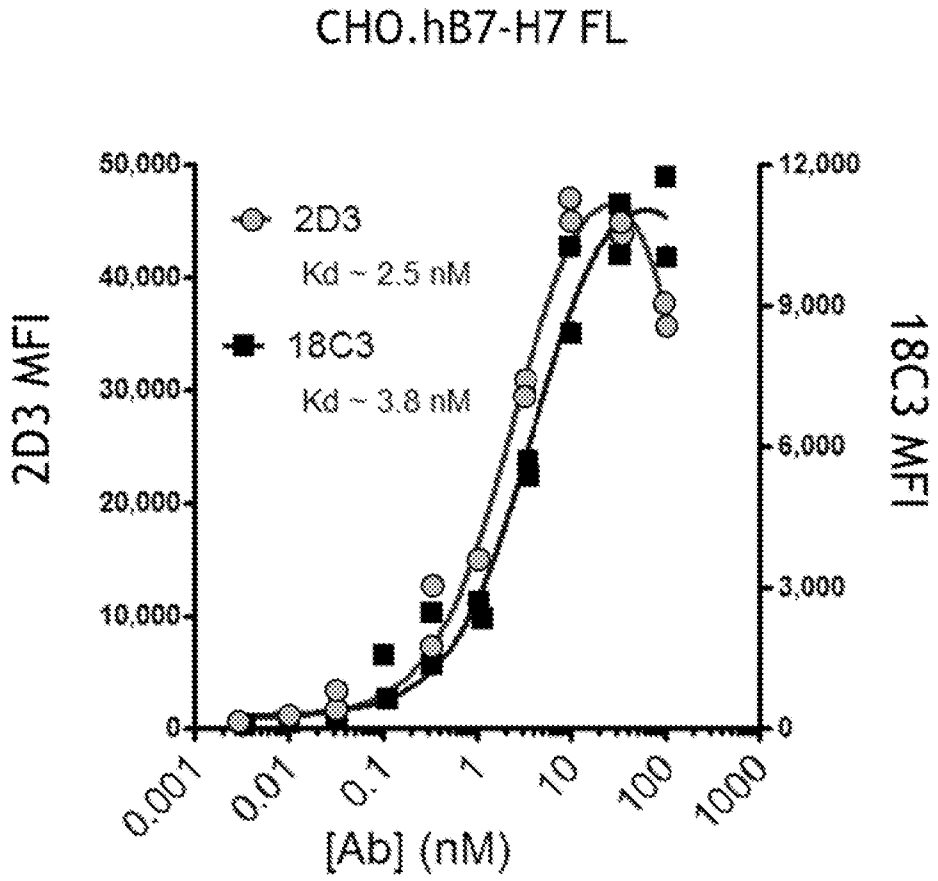
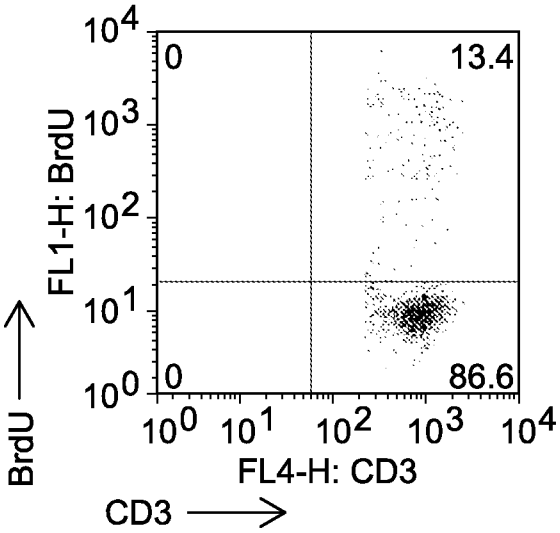
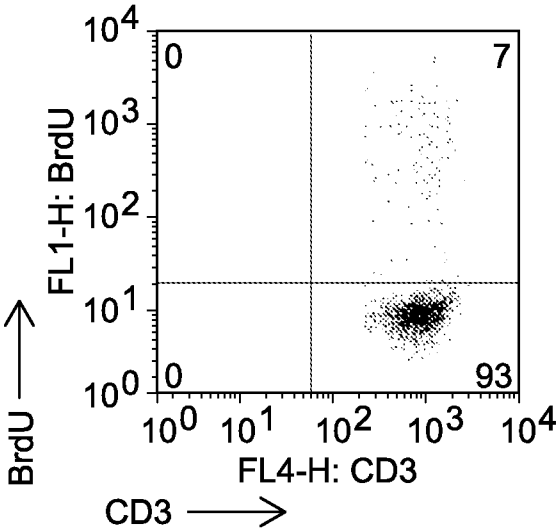

CHO.hB7-H7 FL
2D3
Kd ~ 2.5 nM
18C3
Kd ~ 3.8 nM
2D3 MFI
18C3 MFI
[Ab] (nM)
50,000
40,000
30,000
20,000
10,000
0
12,000
9,000
6,000
3,000
0
0.001
0.01
0.1
1
10
100
1000

FIG. 15

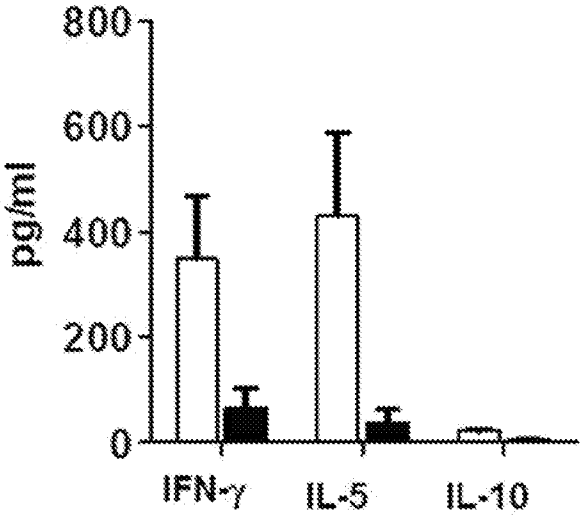


Panel A: Control

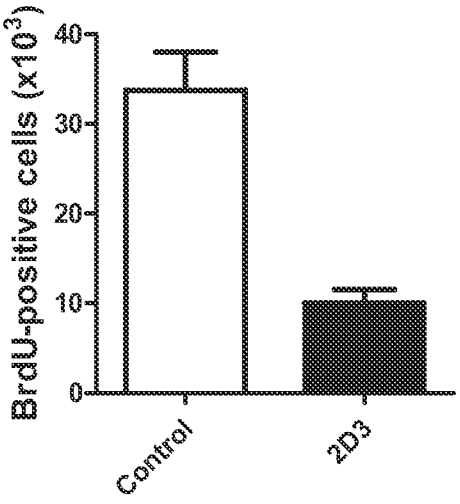


Panel B: Antibody 2D3

FIG. 16A



Panel A: Cytokine Levels



Panel B: BrdU-Positive Cells

FIG. 16B

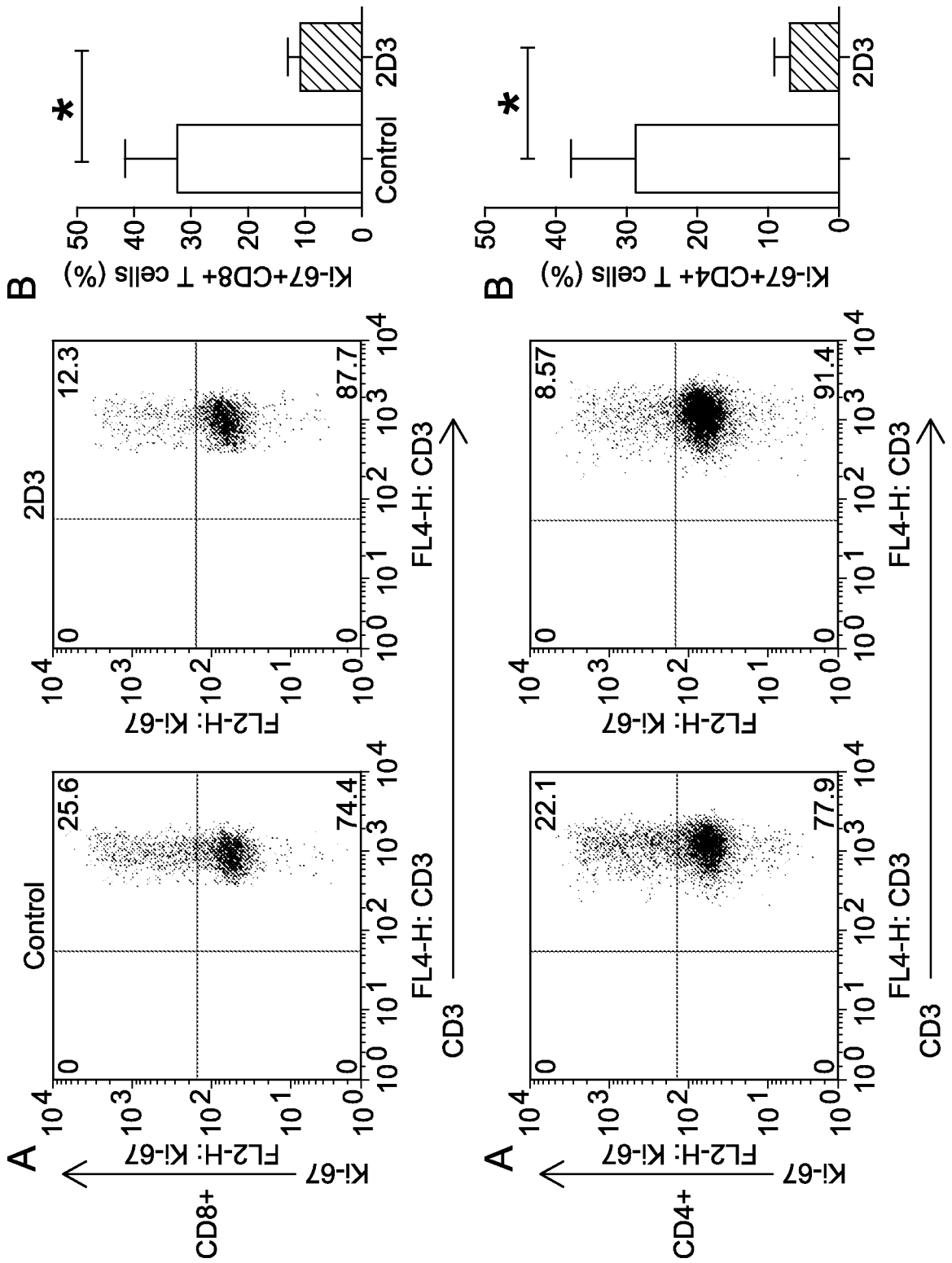

A
B
Control
2D3
CD8+
CD4+
Ki-67
FL2-H: Ki-67
FL4-H: CD3
CD3
25.6
74.4
12.3
87.7
22.1
77.9
8.57
91.4
Ki-67+CD8+ T cells (%)
Ki-67+CD4+ T cells (%)
*

FIG. 17A-B

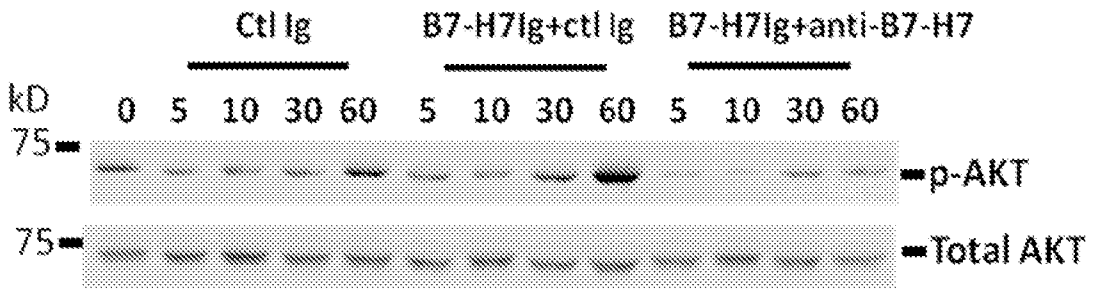

Ctl Ig
B7-H7Ig+ctl Ig
B7-H7Ig+anti-B7-H7
kD
0 5 10 30 60 5 10 30 60 5 10 30 60
75
p-AKT
75
Total AKT

FIG. 18

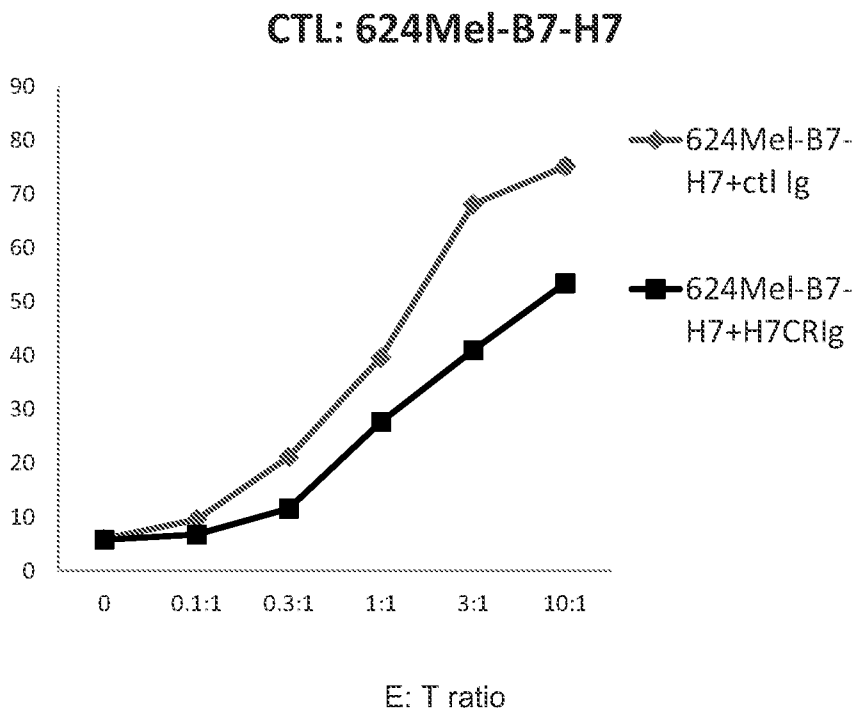

CTL: 624Mel-B7-H7
90
80
70
60
50
40
30
20
10
0
0
0.1:1
0.3:1
1:1
3:1
10:1
624Mel-B7-H7+ctl Ig
624Mel-B7-H7+H7CRIg
E: T ratio

FIG. 19

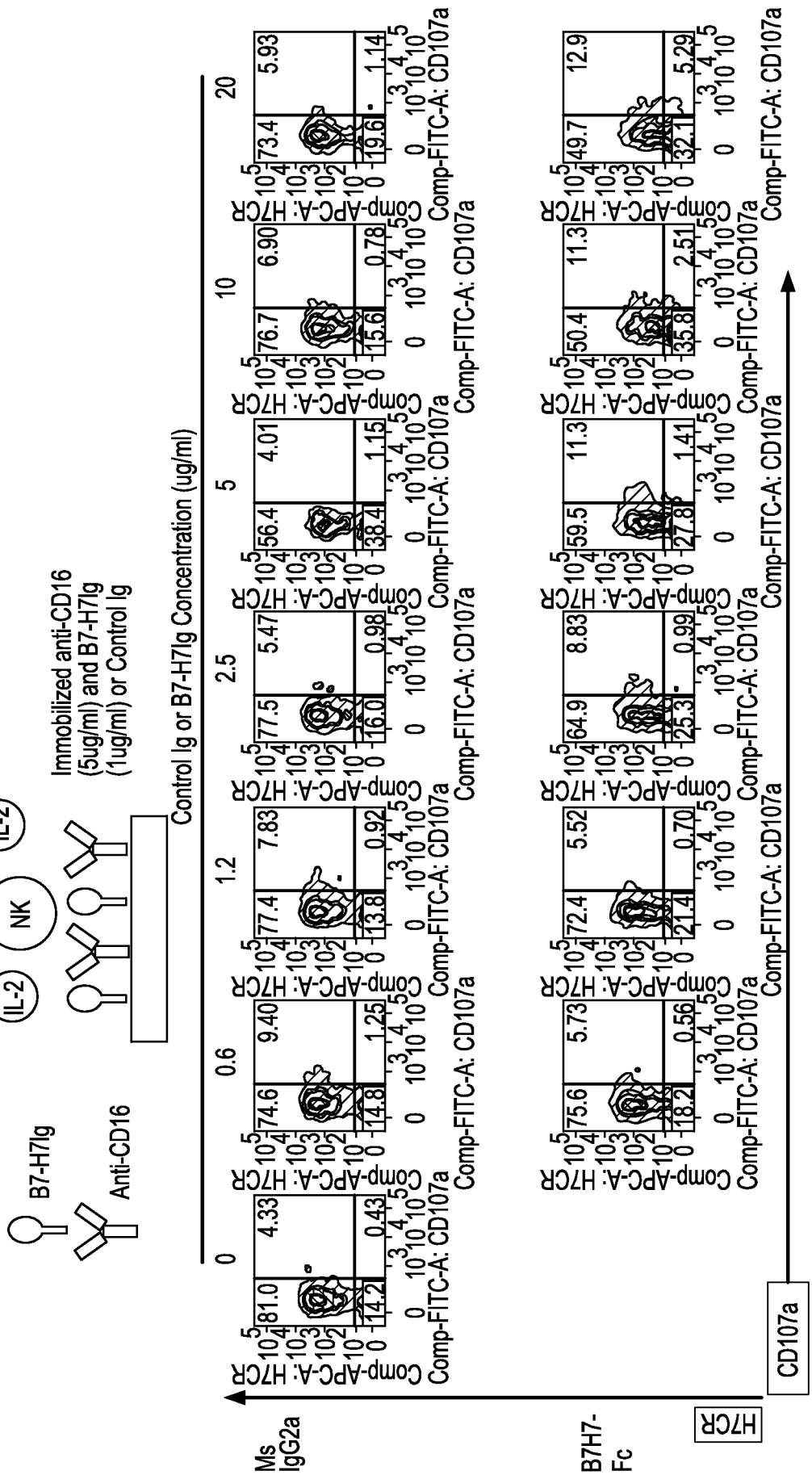

B7-H7Ig
Anti-CD16
IL-2
NK
IL-2
Immobilized anti-CD16
(5ug/ml) and B7-H7Ig
(1ug/ml) or Control Ig
Control Ig or B7-H7Ig Concentration (ug/ml)
0
0.6
1.2
2.5
5
10
20
Ms IgG2a
B7H7-Fc
H7CR
CD107a
Comp-APC-A: H7CR
Comp-FITC-A: CD107a

FIG. 20A

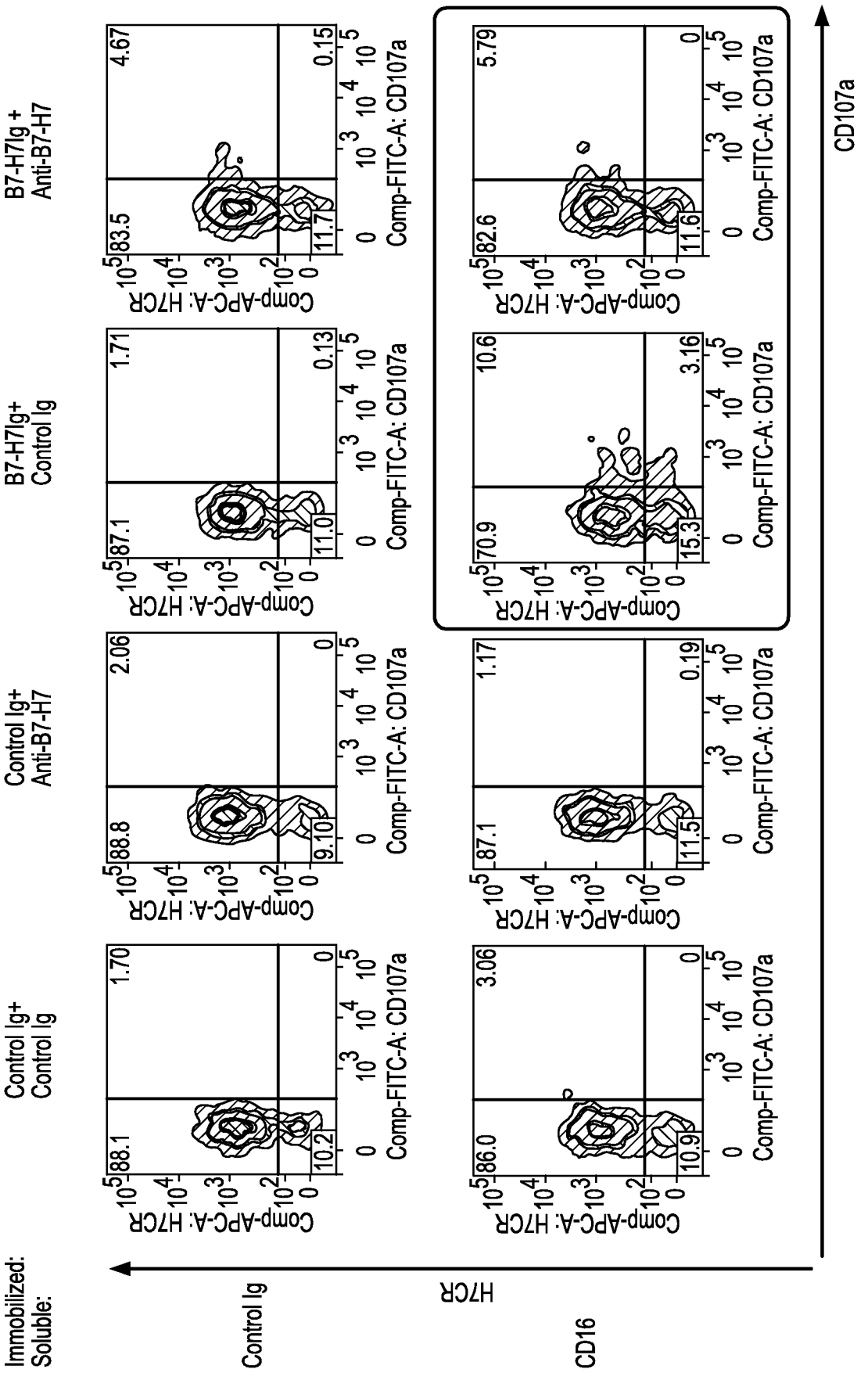

Immobilized:
Soluble:
Control Ig
Control Ig+
Control Ig
Control Ig+
Anti-B7-H7
B7-H7Ig+
Control Ig
B7-H7Ig +
Anti-B7-H7
CD16
H7CR
CD107a
Comp-APC-A: H7CR
Comp-FITC-A: CD107a
88.1
10.2
1.70
0
86.0
10.9
3.06
0
88.8
9.10
2.06
0
87.1
11.5
1.17
0.19
87.1
11.0
1.71
0.13
70.9
15.3
10.6
3.16
83.5
11.7
4.67
0.15
82.6
11.6
5.79
0

FIG. 20B

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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WIPO | PCT

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C07K 14/705 (2006.01) *A61K 39/00* (2006.01)
C07K 16/28 (2006.01)

(21) International Application Number:
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27 May 2014 (27.05.2014)

(25) Filing Language: English

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(30) Priority Data:
61/827,216 24 May 2013 (24.05.2013) US

(71) Applicants: **AMPLIMMUNE, INC.** [US/US]; 45 W. Watkins Mill Road, Gaithersburgh, Maryland 20878 (US). **THE JOHNS HOPKINS UNIVERSITY** [US/US]; 3400 N. Charles Street, Baltimore, Maryland 21218 (US).

(72) Inventors: **CHEN, Lieping**; 51 Canterbury Road, Hamden, Connecticut 06514 (US). **YAO, Sheng**; 11748 Bryce Overlook Court, Columbia, Maryland 21044 (US). **LIU, Linda**; 6512 Tipperary Court, Clarksville, Maryland 21029 (US). **LANGERMANN, Solomon**; 6606 Cross Country Blvd., Baltimore, Maryland 21215 (US).

(74) Agents: **PABST, Patrea L.** et al.; 1545 Peachtree Street, Suite 320, Atlanta, Georgia 30309 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
23 April 2015

(54) Title: ANTI-B7-H5 ANTIBODIES AND THEIR USES

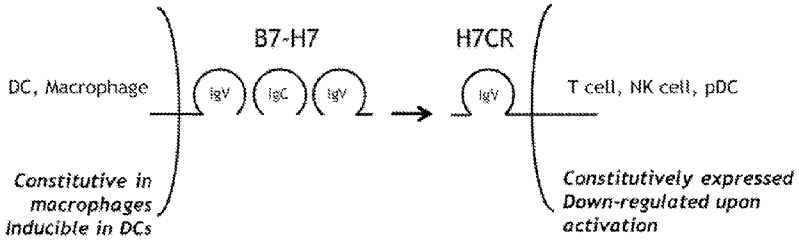

FIG. 1

(57) Abstract: The present invention relates to antibodies and their antigen-binding fragments and to other molecules that are capable of immunospecifically binding to the B7-H5 ligand of the B7-H5:CD28H pathway, and to the uses of such molecules in the treatment and diagnosis of autoimmune disease, transplant rejection and other inflammatory diseases.

WO 2014/190356 A3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/039621

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/705 C07K16/28 A61K39/00
ADD.

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)
C07K A61K

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 practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	L. WANG ET AL: "VISTA, a novel mouse Ig superfamily ligand that negatively regulates T cell responses", NATURE REVIEWS IMMUNOLOGY, vol. 8, no. 6, 14 March 2011 (2011-03-14), pages 467-592, XP055080701, ISSN: 1474-1733, DOI: 10.1038/nri2326 abstract ----- -/--	1-37

[X] Further documents are listed in the continuation of Box C.

[X] See patent family 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 application or patent 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 citation or other special reason (as specified)

"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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 September 2014	06/03/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Turri, Matteo

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/039621

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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:

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 No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

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 fees, this Authority did not invite payment of additional fees.

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.:

1-50(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/039621

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	D. B. FLIES ET AL: "Cutting Edge: A Monoclonal Antibody Specific for the Programmed Death-1 Homolog Prevents Graft-versus-Host Disease in Mouse Models", THE JOURNAL OF IMMUNOLOGY, vol. 187, no. 4, 18 July 2011 (2011-07-18) , pages 1537-1541, XP055140738, ISSN: 0022-1767, DOI: 10.4049/jimmunol.1100660	1-50
A	MOUSTAFA A. SAKR ET AL: "GI24 enhances tumor invasiveness by regulating cell surface membrane-type 1 matrix metalloproteinase", CANCER SCIENCE, vol. 101, no. 11, 13 July 2010 (2010-07-13), pages 2368-2374, XP055140743, ISSN: 1347-9032, DOI: 10.1111/j.1349-7006.2010.01675.x	1-50
A	WO 2011/020024 A2 (UNIV JOHNS HOPKINS [US]; CHEN LIEPING [US]) 17 February 2011 (2011-02-17) paragraph [0153] paragraph [0656] - paragraph [0659]	1-50
A	LINSLEY PETER S ET AL: "The clinical utility of inhibiting CD28-mediated costimulation", May 2009 (2009-05), IMMUNOLOGICAL REVIEWS, VOL. 229, PAGE(S) 307-321, XP055139830, ISSN: 0105-2896	1-50
A	MARTIN F FLAJNIK ET AL: "Evolution of the B7 family: co-evolution of B7H6 and NKp30, identification of a new B7 family member, B7H7, and of B7's historical relationship with the MHC", IMMUNOGENETICS, SPRINGER, BERLIN, DE, vol. 64, no. 8, 11 April 2012 (2012-04-11) , pages 571-590, XP035083875, ISSN: 1432-1211, DOI: 10.1007/S00251-012-0616-2	1-50

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/039621

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011020024 A2	17-02-2011	AU 2010282340 A1	01-03-2012
		CA 2769822 A1	17-02-2011
		EP 2464661 A2	20-06-2012
		JP 2013501814 A	17-01-2013
		US 2012219559 A1	30-08-2012
		WO 2011020024 A2	17-02-2011

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-50(partially)

   All subject-matter relating the antibody binding to B7-H5 and characterized by heavy chain variable region SEQ ID NO:9 and light chain variable region SEQ ID NO:11 (clone 2D3); pharmaceutical compositions comprising the antibodies, uses of the antibodies, methods.

   ---

2. claims: 1-50(partially)

   All subject-matter relating the antibody binding to B7-H5 and characterized by heavy chain variable region SEQ ID NO:13 and light chain variable region SEQ ID NO:15 (clone 18C3); pharmaceutical compositions comprising the antibodies, uses of the antibodies, methods.

   ---

3. claims: 24-37, 48-50(all partially)

   All subject-matter relating to CD28H: pharmaceutical compositions comprising a CD28H-Ig fusion. Use of a CD28H-Ig fusion protein in the manufacture of a medicament. Pharmaceutical compositions for use in methods of treating subjects for diseases characterized by increased expression of CD28H.

   ---

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(21)申请号 201480041706.0

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(85)PCT国际申请进入国家阶段日
2016.01.22

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(87)PCT国际申请的公布数据
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(71)申请人 米迪缪尼有限公司
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申请人 约翰斯霍普金斯大学

(72)发明人 L·陈 S·姚 L·刘 S·兰格曼

(74)专利代理机构 上海专利商标事务所有限公司 31100
代理人 杨昀 陶家蓉

(51)Int.Cl.
C07K 14/705(2006.01)
C07K 16/28(2006.01)
A61K 39/00(2006.01)

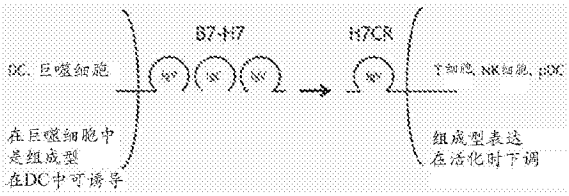
权利要求书4页 说明书46页
序列表23页 附图19页

(54)发明名称

抗-B7-H5抗体及其用途

(57)摘要

本发明涉及抗体和它们的抗原结合片段，并且涉及能够免疫特异性地结合B7-H5:CD28H通路的B7-H5配体的其他分子，并且涉及此类分子在治疗和诊断自身免疫性疾病、移植排斥和其他炎症疾病中的用途。



CN 106414489 A

1.一种包括六个互补决定区(CDR)的抗体或其抗原结合片段，

其中这些CDR包括

(1)SEQ ID NO:11的三个轻链CDR和SEQ ID NO:9的三个重链CDR，或

(2)SEQ ID NO:15的三个轻链CDR和SEQ ID NO:13的三个重链CDR，并且

其中该抗体或其抗原结合片段结合至B7-H5。

2.如权利要求1所述的抗体或其抗原结合片段，其中这三个轻链CDR包括第一轻链CDR、第二轻链CDR、和第三轻链CDR，该第一轻链CDR包括SEQ ID NO:11的氨基酸27-38，该第二轻链CDR包括SEQ ID NO:11的氨基酸56-58，该第三轻链CDR包括SEQ ID NO:11的氨基酸95-102。

3.如权利要求1或2之一所述的抗体或其抗原结合片段，其中这三个重链CDR包括第一重链CDR、第二重链CDR、和第三重链CDR，该第一重链CDR包括SEQ ID NO:9的氨基酸26-33，该第二重链CDR包括SEQ ID NO:9的氨基酸51-58，该第三重链CDR包括SEQ ID NO:9的氨基酸97-106。

4.如权利要求1-3之一所述的抗体或其抗原结合片段，包括轻链可变区，该轻链可变区包括SEQ ID NO:11的氨基酸序列。

5.如权利要求1-4之一所述的抗体或其抗原结合片段，包括重链可变区，该重链可变区包括SEQ ID NO:9的氨基酸序列。

6.如权利要求1所述的抗体或其抗原结合片段，其中这三个轻链CDR包括第一轻链CDR、第二轻链CDR、和第三轻链CDR，该第一轻链CDR包括SEQ ID NO:15的氨基酸27-38，该第二轻链CDR包括SEQ ID NO:15的氨基酸56-58，该第三轻链CDR包括SEQ ID NO:15的氨基酸95-102。

7.如权利要求1或2之一所述的抗体或其抗原结合片段，其中这三个重链CDR包括第一重链CDR、第二重链CDR、和第三重链CDR，该第一重链CDR包括SEQ ID NO:13的氨基酸26-33，该第二重链CDR包括SEQ ID NO:13的氨基酸51-58，该第三重链CDR包括SEQ ID NO:13的氨基酸97-106。

8.如权利要求1-3之一所述的抗体或其抗原结合片段，包括轻链可变区，该轻链可变区包括SEQ ID NO:15的氨基酸序列。

9.如权利要求1-4之一所述的抗体或其抗原结合片段，包括重链可变区，该重链可变区包括SEQ ID NO:13的氨基酸序列。

10.如权利要求1所述的抗体或其抗原结合片段，包括轻链可变区和重链可变区，该轻链可变区包括SEQ ID NO:11的氨基酸序列，该重链可变区包括SEQ ID NO:9的氨基酸序列。

11.如权利要求1所述的抗体或其抗原结合片段，包括轻链可变区和重链可变区，该轻链可变区包括SEQ ID NO:15的氨基酸序列，该重链可变区包括SEQ ID NO:13的氨基酸序列。

12.如权利要求1所述的抗体或其抗原结合片段，其中结合的B7-H5排列在活细胞的表面上或以内源的或转染的浓度进行表达。

13.如权利要求7所述的抗体或其抗原结合片段，其中该活细胞是巨噬细胞或树突细胞。

14.如权利要求1所述的抗体或其抗原结合片段，其中该B7-H5是人类B7-H5。

15.如权利要求1所述的抗体或其抗原结合片段，其中该抗体或其抗原结合片段

(A)衰减B7-H5的配体结合CD28H的能力；

(B)拮抗由于B7-H5结合CD28H而发生的信号传导；

(C)抑制异体T细胞应答；

(D)它们的组合。

16.如权利要求1-15中任一项所述的抗体或其抗原结合片段，包括来自免疫球蛋白恒定区(Fc)的一个或多个恒定结构域。

17.如权利要求16所述的抗体或其抗原结合片段，其中这些恒定结构域是人类恒定结构域。

18.如权利要求17所述的抗体或其抗原结合片段，其中这些人类恒定结构域是IgA、IgD、IgE、IgG或IgM结构域。

19.如权利要求18所述的抗体或其抗原结合片段，其中人类IgG恒定结构域是IgG1、IgG2、IgG3、或IgG4结构域。

20.如权利要求1-19中任一项所述的抗体或其抗原结合片段，其中该抗体或其抗原结合片段被可检测地标记或包括轭合的毒素、药物、受体、酶、受体配体。

21.如权利要求1-20中任一项所述的抗体或其抗原结合片段，其中该抗体是单克隆抗体、人类抗体、嵌合抗体或人源化抗体。

22.如权利要求1-21中任一项所述的抗体或其抗原结合片段，其中该抗体是双特异性抗体、三特异性抗体或多特异性抗体。

23.一种人源化抗体或其抗原结合片段，包括一个或多个人类IgG4恒定结构域以及

包括SEQ ID NO:11的氨基酸序列的轻链可变区，包括SEQ ID NO:9的氨基酸序列重链可变区，或

包括SEQ ID NO:15的氨基酸序列的轻链可变区，包括SEQ ID NO:13的氨基酸序列的重链可变区。

24.一种药用组合物，包括如权利要求1-23中任一项所述的抗体或其抗原结合片段、或CD28H-Ig融合蛋白以及生理学上可接受的载体或赋形剂。

25.如权利要求24所述的药用组合物，用于在下调受试者的免疫系统的方法中使用。

26.用于如权利要求25所述来使用的药用组合物，其中该受试者患有自身免疫性疾病。

27.如权利要求24所述的药用组合物，用于在治疗自身免疫性疾病的方法中使用。

28.用于如权利要求26或27所述来使用的药用组合物，其中该自身免疫性疾病选自下组，该组由以下各项组成：斑形脱发、强直性脊柱炎、抗磷脂综合征、自身免疫性阿狄森氏病、肾上腺的自身免疫性疾病、自身免疫性溶血性贫血、自身免疫性肝炎、自身免疫性卵巢炎以及睾丸炎、自身免疫性血小板减少症、白塞氏病、大疱性类天疱疮、心肌病、乳糜泻性皮炎、慢性疲劳免疫功能紊乱综合征(CFIDS)、慢性发炎性脱髓鞘性多发性神经病、丘-施二氏综合征、瘢痕性类天疱疮、CREST综合征、冷凝集素病、克罗恩氏疾病、盘状狼疮、原发性混合型冷球蛋白血症、纤维肌痛-纤维性肌炎、肾小球肾炎、格拉夫疾病、格林-巴利综合征、桥本氏甲状腺炎、特发性肺纤维化、特发性血小板减少性紫癜(ITP)、IgA神经病、幼年型关节炎、扁平苔癣、红斑狼疮、美尼尔氏病、混合结缔组织病、多发性硬化症、视神经脊髓炎(NMO)、1型或免疫介导的糖尿病、重症肌无力、寻常天疱疮、恶性贫血、结节性多动脉炎、多发性软骨

炎、多腺体综合征、风湿性多肌痛、多发性肌炎以及皮肤肌炎、原发性无丙种球蛋白血症、原发性胆汁性肝硬化、牛皮癣、牛皮癣性关节炎、雷诺氏现象、赖特氏综合征、类风湿性关节炎、类肉瘤病、硬皮病、修格连氏综合征、僵人综合征、全身性红斑狼疮、红斑狼疮、高安氏动脉炎、颞动脉炎/巨细胞性动脉炎、溃疡性结肠炎、葡萄膜炎、血管炎如疱疹样皮炎血管炎、白癜风以及韦格纳氏肉芽肿。

29.用于如权利要求25所述来使用的药用组合物，其中该受试者患有炎症疾病。

30.如权利要求24所述的药用组合物，用于在治疗炎症疾病的方法中使用。

31.用于如权利要求29或30所述来使用的药用组合物，其中该炎症疾病选自下组，该组由以下各项组成：哮喘、脑炎、发炎性肠病、慢性阻塞性肺病(COPD)、过敏性失调、败血性休克、肺纤维化、未分化的脊椎关节病、未分化的关节病、关节炎、炎性骨质溶解以及慢性病毒性或细菌性感染所致的慢性炎症。

32.用于如权利要求25所述来使用的药用组合物，其中该受试者已经接受或将接受移植物。

33.如权利要求24所述的药用组合物，用于在治疗或预防移植排斥的方法中使用。

34.如权利要求1-23中任一项所述的抗体或其抗原结合片段或CD28H-Ig融合蛋白在制造用于下调受试者中的免疫系统的药物中的用途。

35.如权利要求1-23中任一项所述的抗体或其抗原结合片段或CD28H-Ig融合蛋白在制造用于治疗受试者中的自身免疫性疾病的药物中的用途。

36.如权利要求1-23中任一项所述的抗体或其抗原结合片段或CD28H-Ig融合蛋白在制造用于治疗受试者中的炎症疾病的药物中的用途。

37.如权利要求1-23中任一项所述的抗体或其抗原结合片段或CD28H-Ig融合蛋白在制造用于治疗或预防受试者中的移植排斥的药物中的用途。

38.一种疾病、失调或感染的检测或诊断的方法，该方法包括：(a)使用如权利要求1-23中任一项所述的抗体或其抗原结合片段，测定在受试者的细胞或组织样品中的B7-H5的表达，并且(b)将该B7-H5的水平与对照水平进行比较，其中与该对照水平相比B7-H5的测定水平的增加指示该疾病、失调或感染。

39.如权利要求38所述的方法，其中通过酶联免疫吸附测定(ELISA)、放射免疫测定(RIA)或荧光激活细胞分选法(FACS)来测定B7-H5的表达。

40.一种用于监测疾病、失调或感染的进展的方法，该方法包括：(a)使用如权利要求1-23中任一项所述的抗体或其抗原结合片段，在第一时间点测定在受试者的细胞或组织样品中的B7-H5的表达；并且(b)比较在第二时间点或经一个时程在该受试者的细胞或组织样品中的B7-H5的表达水平，其中B7-H5的测定水平中的增加指示疾病、失调或感染的进展。

41.如权利要求30所述的方法，其中通过酶联免疫吸附测定(ELISA)、放射免疫测定(RIA)或荧光激活细胞分选法(FACS)来测定B7-H5的表达。

42.一种用于监测对治疗的反应的方法，该方法包括：(a)使用如权利要求1-23中任一项所述的抗体或其抗原结合片段，测定在治疗前在受试者的细胞或组织样品中的B7-H5的表达；并且(b)在治疗后的一个或多个时间点，测定在受试者的细胞或组织样品中的B7-H5的表达，并且随着时间比较B7-H5的水平，由此与B7-H5的治疗前水平相比B7-H5的测定水平的降低指示对治疗的反应。

43.如权利要求42所述的方法，其中通过酶联免疫吸附测定(ELISA)、放射免疫测定(RIA)或荧光激活细胞分选法(FACS)来测定B7-H5的表达。

44.一种用于在治疗受试者的疾病的方法中使用的药用组合物，该疾病表征为B7-H5的增加的表达，其中该方法包括以下步骤

(i)通过以下方式确定该受试者是否患有表征为B7-H5的增加表达的疾病

(a)使用这种结合至B7-H5的抗体或其抗原结合片段，测定在该受试者的细胞或组织样品中的B7-H5的表达；

(b)将B7-H5的水平与对照水平进行比较，

其中与该对照水平相比B7-H5的测定水平的增加指示该受试者患有表征为B7-H5的增加表达的疾病；

并且

(ii)如果该受试者患有表征为B7-H5的增加表达的疾病，则给予该受试者治疗有效量的如权利要求24所述的药用组合物。

45.如权利要求44所述的方法，其中该抗体或其抗原结合片段是如权利要求1-23中任一项所述的抗体或其抗原结合片段。

46.用于如权利要求44所述来使用的药用组合物，其中这种表征为B7-H5的增加表达的疾病是自身免疫性疾病。

47.用于如权利要求44所述来使用的药用组合物，其中这种表征为B7-H5的增加表达的疾病是炎症疾病。

48.一种用于在治疗受试者的疾病的方法中使用的药用组合物，该疾病表征为CD28H的增加的表达，其中该方法包括以下步骤

(i)通过以下方式确定该受试者是否患有表征为CD28H的增加表达的疾病

(a)使用抗CD28H抗体或其抗原结合片段，测定在该受试者的细胞或组织样品中的CD28H的表达；

(b)将CD28H的水平与对照水平进行比较，

其中与该对照水平相比CD28H的测定水平的增加指示该受试者患有表征为CD28H的增加表达的疾病；

并且

(ii)如果该受试者患有表征为CD28H的增加表达的疾病，则给予该受试者治疗有效量的如权利要求24所述的药用组合物。

49.用于如权利要求48所述来使用的药用组合物，其中这种表征为CD28H的增加表达的疾病是自身免疫性疾病。

50.用于如权利要求48所述来使用的药用组合物，其中这种表征为CD28H的增加表达的疾病是炎症疾病。

抗-B7-H5抗体及其用途

关于联邦资助的研究或开发的声明

[0001] 本发明是用美国国立卫生研究院(NIH)的授予号R01 CA97085和R01 A 172592,以及美国国家癌症研究所(NCI)的U19 CA113341下的政府支持做出的。政府具有本发明的某些权利。

序列表的引用

[0002] 本申请包括依据37 C.F.R.§1.821以及下列等的一个或多个序列表,这些序列表披露于论文和计算机可读介质这二者中,并且通过引用以其全文将这些论文和计算机可读披露结合在此。

发明领域

[0003] 本发明涉及抗体和它们的抗原结合片段,并且涉及能够结合B7-H5:CD28H通路的B7-H5配体的其他分子,并且涉及此类分子在治疗和诊断自身免疫性疾病、移植排斥和其他炎症疾病中的用途。

发明背景

[0004] 人和其他哺乳动物的免疫系统负责提供针对感染和疾病的保护。此类保护是通过体液免疫应答和通过细胞介导的免疫应答二者来提供的。体液应答导致抗体和能够识别并且中和外源靶标(抗原)的其他生物分子的产生。相比之下,细胞介导的免疫应答涉及响应于对抗原的识别,通过T细胞来活化巨噬细胞、自然杀伤细胞(NK)、和抗原特异性细胞毒T淋巴细胞的活化,并且释放不同的细胞因子(董(Dong),C.等人(2003)"通过新颖的共刺激分子的免疫调节(Immune Regulation by Novel Costimulatory Molecules)",免疫学研究(Immunolog.Res.)28(1):39-48)。

[0005] T细胞最佳地介导针对抗原的免疫应答的能力需要两种不同的信号传导相互作用(维格里埃塔(Viglietta),V.等人(2007)"调节共刺激(Modulating Co-Stimulation)",神经治疗学(Neurotherapeutics)4:666-675;科尔曼(Korman),A.J.等人(2007)"癌症免疫疗法中的检查点阻断(Checkpoint Blockade in Cancer Immunotherapy)",免疫学进展(Adv.Immunol.)90:297-339)。第一,已经排列在抗原呈递细胞(APC)的表面上的抗原必须被呈递到抗原特异性原初$CD4^+$T细胞。这种呈递通过T细胞受体(TCR)递送信号,该信号指导T细胞起始免疫应答,该免疫应答对所呈递的抗原将是特异性的。第二,通过APC与不同的T细胞表面分子之间的相互作用介导的一系列共刺激性以及共抑制性信号首先触发T细胞的活化以及增殖并且最终触发它们的抑制。因此,该第一信号为该免疫应答赋予了特异性,而该第二信号用以确定该应答的性质、幅度以及持续时间。

[0006] 免疫系统受共刺激性以及共抑制性配体和受体的紧密控制。这些分子提供了用于T细胞活化的第二信号,并且提供了正信号和负信号的平衡网络,以最大化针对感染的免疫应答同时限制对自身的免疫性(王(Wang),L.等人(2011年3月7日)"VISTA,负向调节T细胞应答的新颖小鼠Ig超家族配体(VISTA,A Novel Mouse Ig Superfamily Ligand That Negatively Regulates T Cell Responses)",实验医学杂志(J.Exp.Med.)10.1084/

jem.20100619:1-16;莱布尼兹(Lepenies),B.等人(2008)“寄生虫感染期间负向共刺激分子的作用(The Role Of Negative Costimulators During Parasitic Infections)”,内分泌、代谢和免疫的失调-药物靶标(Endocrine,Metabolic and Immune Disorders-Drug Targets)8:279-288)。特别重要的是抗原呈递细胞的B7.1(CD80)和B7.2(CD86)配体与$CD4^+$ T淋巴细胞的CD28和CLTA-4受体之间的结合(夏普(Sharpe),A.H.等人(2002)“B7-CD28超家族”,自然综述免疫学(Nature Rev.Immunol.)2:116-126;董(Dong),C.等人(2003)“通过新颖共刺激分子的免疫调节(Immune Regulation by Novel Costimulatory Molecules)”,免疫学研究(Immunolog.Res.)28(1):39-48;林德利(Lindley),P.S.等人(2009)“抑制CD28介导的共刺激的临床应用(The Clinical Utility Of Inhibiting CD28-Mediated Costimulation)”,免疫学评论(Immunol.Rev.)229:307-321)。B7.1或B7.2至CD28的结合刺激T细胞活化;B7.1或B7.2至CTLA4的结合抑制此类活化(董(Dong),C.等人(2003)“通过新颖共刺激分子的免疫调节(Immune Regulation by Novel Costimulatory Molecules)”,免疫学研究(Immunolog.Res.)28(1):39-48;林德利(Lindley),P.S.等人(2009)“抑制CD28介导的共刺激的临床效用(The Clinical Utility Of Inhibiting CD28-Mediated Costimulation)”,免疫学评论(Immunol.Rev.)229:307-321);格林沃尔德(Greenwald),R.J.等人(2005)“再论B7家族(The B7 Family Revisited)”,免疫学年鉴(Ann.Rev.Immunol.)23:515-548)。CD28组成型地表达于T细胞表面上(格罗斯(Gross),J.等人(1992)“小鼠中共刺激受体CD28的鉴定和分布(Identification And Distribution Of The Costimulatory Receptor CD28 In The Mouse)”,免疫学杂志(J.Immunol.)149:380-388),而在T细胞活化后CTLA4表达迅速上调(林斯利(Linsley),P.等人(1996)“CTLA4的细胞内转运和朝向TCR识别的位点的病灶定位(Intracellular Trafficking Of CTLA4 And Focal Localization Towards Sites Of TCR Engagement)”,免疫性(Immunity)4:535-543)。由于CTLA4是更高亲和力受体(夏普(Sharpe),A.H.等人(2002)“B7-CD28超家族”,自然评论:免疫学(Nature Rev.Immunol.)2:116-126),所以结合首先启动T细胞增殖(经由CD28),并且然后抑制它(经由CTLA4的新生表达),由此当不再需要增殖时削弱该作用。

[0007] 对CD28受体的配体的进一步研究已经导致一组相关B7分子的鉴定和表征(“B7超家族”)(科伊尔(Coyle),A.J.等人(2001)“扩大的B7超家族:增加调节T细胞功能的共刺激信号中的复杂性(The Expanding B7 Superfamily:Increasing Complexity In Costimulatory Signals Regulating T Cell Function)”,自然免疫学(Nature Immunol.)2(3):203-209;夏普(Sharpe),A.H.等人(2002)“B7-CD28超家族”,自然评论:免疫学(Nature Rev.Immunol.)2:116-126;格林沃尔德(Greenwald),R.J.等人(2005)“再论B7家族(The B7 Family Revisited)”,免疫学年鉴(Ann.Rev.Immunol.)23:515-548;柯林斯(Collins),M.等人(2005)“免疫调节配体的B7家族(The B7 Family Of Immune-Regulatory Ligands)”,基因组生物学(Genome Biol.)6:223.1-223.7;洛克(Loke),P.等人(2004)“免疫调节的形成机制:扩展的B7家族和调节性T细胞(Emerging Mechanisms Of Immune Regulation:The Extended B7 Family And Regulatory T Cells)”,关节炎研究治疗(Arthritis Res.Ther.)6:208-214;科尔曼(Korman),A.J.等人(2007)“癌症免疫疗法中的检查点阻断(Checkpoint Blockade in Cancer Immunotherapy)”,免疫学进展

(Adv.Immunol.)90:297-339;弗莱斯(Flies),D.B.等人(2007)“新B7:在肿瘤免疫性中起到关键作用(The New B7s:Playing a Pivotal Role in Tumor Immunity)”,免疫治疗学杂志(J.Immunother),30(3):251-260;阿加瓦尔(Agarwal),A.等人(2008)“移植和耐受性中正向共刺激分子的作用(The Role Of Positive Costimulatory Molecules In Transplantation And Tolerance)”,器官移植新见(Curr.Opin.Organ Transplant.)13:366-372;兰斯乔(Lenschow),D.J.等人(1996)“T细胞共刺激的CD28/B7系统)”,免疫学年鉴(Ann.Rev.Immunol.)14:233-258;王(Wang),S.等人(2004)“T淋巴细胞响应的正向和负向调节中B7-CD28家族的共同信号分子(Co-Signaling Molecules Of The B7-CD28 Family In Positive And Negative Regulation Of T Lymphocyte Responses)”,微生物感染(Microbes Infect.)6:759-766)。目前存在该家族的九个已知成员:B7.1(CD80)、B7.2(CD86)、可归纳的共刺激因子配体(ICOS-L、B7-H2),程序化死亡-1配体(PD-L1;B7-H1),程序化死亡-2配体(PD-L2;B7-DC),B7-H3、B7-H4(也称为B7x、B7-H6和B7S1;西卡(Sica),G.L.等人(2003)“B7-4,B7家族的分子,负向地调节T细胞免疫性(B7-4,A Molecule Of The B7 Family,Negatively Regulates T Cell Immunity)”,免疫性(Immunity)18:849-861;臧(Zang),X.等人(2003)B7x:抑制T细胞活化的广泛表达的B7家族成员(B7x:A Widely Expressed B7 Family Member That Inhibits T Cell Activation)”,美国国家科学院院刊(Proc.Natl.Acad.Sci.(USA))100:10388-10392;普拉萨德(Prasad),D.V.等人(2003)“B7S1,负向地调节T细胞活化的新颖的B7家族成员(B7S1,A Novel B7 Family Member That Negatively Regulates T Cell Activation)”,免疫性(Immunity)18:863-873),B7-H6(柯林斯(Collins),M.等人(2005)“免疫调节配体的B7家族(The B7 Family Of Immune-Regulatory Ligands)”,基因组生物学(Genome Biol.)6:223.1-223.7)和B7-H5(弗莱尼克(Flajnik),M.F.等人(2012)“B7家族的演化:B7H6和Nkp30的共演化,新B7家族成员B7H7的鉴定,以及B7与MHC的历史关系的鉴定(Evolution Of The B7 Family:Co-Evolution Of B7H6 And Nkp30,Identification Of A New B7 Family Member,B7H7,And Of B7's Historical Relationship With The MHC)”,免疫遗传学(Immunogenetics)64:571-590)。B7基因家族在适应性免疫系统的调节中是至关重要的。大部分B7家族成员包含免疫球蛋白超家族(IgSF)的可变(V)-类型域以及恒定(C)-类型域两者。

[0008] B7配体表达在很多不同细胞类型(包括抗原呈递细胞(APC))的细胞表面上,并且它们与T细胞上的受体分子的相互作用提供了调节T细胞活化和耐受性的活化和/或抑制性信号(柯林斯(Collins),M.等人(2005)“免疫调节配体的B7家族(The B7 Family Of Immune-Regulatory Ligands)”,基因组生物学(Genome Biol.)6:223.1-223.7)。一些抑制性B7配体还表达在肿瘤细胞上,导致免疫应答的抑制(凯依尔(Keir),M.E.等人(2008)“在耐受性和免疫性中的PD-1及其配体(PD-1 And Its Ligands In Tolerance And Immunity)”,免疫学年鉴(Annu.Rev.Immunol.)26:677-704;邹(Zou),W.等人(2008)“肿瘤微环境中的抑制性B7家族分子(Inhibitory B7-Family Molecules In The Tumour Microenvironment)”,自然评论:免疫学(Nat.Rev.Immunol.)8:467-477)。因此,刺激或弱化B7配体及其受体的相互作用保持了对自身免疫性疾病、癌症和感染性疾病的治疗潜力(WO 2011/020024;弗莱尼克(Flajnik,M.F.)等人(2012)“B7家族的演化:B7H6和Nkp30的共演化,新B7家族成员B7H7的鉴定,以及B7与MHC的历史关系的鉴定(Evolution Of The B7

Family:Co-Evolution Of B7H6 And Nkp30,Identification Of A New B7 Family Member,B7H7,And Of B7's Historical Relationship With The MHC)",免疫遗传学(Immunogenetics)64:571-590)。

[0009] 尽管存在炎症疾病或自身免疫性疾病的治疗中的所有现有进展,但是仍对能够提供用于此类病症的治疗的增强的免疫疗法的组合物存在需要。本发明是针对此类组合物和它们的治疗炎症疾病、自身免疫性疾病、与特征在于高活性免疫系统的类似疾病和病症的用途。

发明概述

[0010] 提供了抗体和它们的抗原结合片段,和能够免疫特异性地结合B7-H5:CD28H通路的B7-H5配体的其他分子,以及此类分子在治疗和诊断自身免疫性疾病、移植排斥和其他炎症疾病中的用途。

[0011] 一个实施例提供了抗体或具有免疫特异性地结合哺乳动物B7-H5(并且具体地,人类B7-H5)的抗体的抗原结合片段的其他分子。例如,B7-H5可以排列在活细胞的表面上。

[0012] 示例性活细胞包括但不限于巨噬细胞或树突细胞。

[0013] 另一实施例提供了免疫特异性地结合B7-H5并且基本上能够阻滞B7-H5与CD28H的相互作用的分子。仍另一实施例提供了免疫特异性地结合B7-H5并且基本上不能够阻滞B7-H5与CD28H的相互作用的分子。

[0014] B7-H5结合分子可以具有包含六个CDR的抗原结合片段。这些CDR可以包括抗B7-H5抗体的CDR的至少一个共有CDR:2D3和18C3,其中所有剩余的CDR选自:

(A)抗B7-H5抗体2D3的三个轻链CDR和三个重链CDR;或

(B)抗B7-H5抗体18C3的三个轻链CDR和三个重链CDR。

[0015] 另一实施例提供了B7-H5结合分子,其中六个CDR是:

(A)抗B7-H5抗体2D3的三个轻链CDR和三个重链CDR;或

(B)抗B7-H5抗体18C3的三个轻链CDR和三个重链CDR。

[0016] 在一些实施例中,分子是包括来自两个或更多个不同B7-H5抗体的抗原结合片段(Fab)的嵌合抗体。

[0017] B7-H5结合分子可以是单克隆抗体、人类抗体、嵌合抗体或人源化抗体和/或是双特异性的、三特异性的或多特异性的抗体。

[0018] B7-H5结合分子可以被可检测地标记或包括轭合的毒素、药物、受体、酶、或受体配体。

[0019] 仍另一实施例提供了包含治疗有效量的或预防有效量的任何上述B7-H5结合分子和生理学上可接受的载体或赋形剂的药用组合物。

[0020] 在其他实施例中,基本上能够阻滞B7-H5与CD28H的相互作用的分子是CD28H融合蛋白、优选是CD28H-Ig融合蛋白。

[0021] 还提供了在疾病治疗中使用药用组合物的方法。

[0022] 用于诊断受试者中疾病的方法包括针对它们结合任何上述B7-H5结合分子的能力测定受试者的细胞,其中该方法提供了用于诊断受试者中疾病的存在的细胞学测定。

[0023] 披露的B7-H5结合分子可以用于自身免疫性疾病或移植排斥。

附图简要说明

[0024] 图1提供了B7-H5及其反受体CD28H的示意性描绘。

[0025] 图2,小图A-J示出B7-H5组成型地表达在巨噬细胞上并且在树突细胞上可诱导。

[0026] 图3,小图A-D示出CD28H表达在T细胞和自然杀伤(NK)细胞上,但并不表达在B细胞上。

[0027] 图4A-4B示出由克隆2D3和18C3产生的抗体结合由CHO转染子表达的人类B7-H5的能力。

[0028] 图5A-5C示出分离的抗体和对照Ig阻滞B7-H5:CD28H相互作用的能力。

[0029] 图6示出B7-H5Ig融合体刺激T细胞应答的能力。

[0030] 图7示出B7-H5Ig诱导以下细胞因子的表达的能力:IL-2、IFN-γ和IL-10。

[0031] 图8示出本发明的抗B7-H5抗体2D3能够结合B7-H5,以便阻滞B7-H5与其CD28H反受体相互作用的能力。

[0032] 图9示出抗B7-H5抗体2D3抑制异体T细胞应答的能力。

[0033] 图10A和图10B示出在植入人类PBMC的NSG小鼠中,在脾脏(图10A)和外周血(图10B)中,在活化的($CD45RO^+$)人类T细胞中,抗人类B7-H5抗体对$CD28H^+$细胞的百分比的影响。

[0034] 图11A和图11B示出在植入人类PBMC的NSG小鼠中,在脾脏(图11A)和外周血(图11B)中,在原初($CD45RO^-$)人类T细胞中,抗人类B7-H5抗体对$CD28H^+$细胞的百分比的影响。

[0035] 图12A-12C示出重组抗人类B7-H5嵌合抗体(2D3和18C3)的结合特性。图12A-12C示出已经从它们的鼠IgG同种型重组地转化为人类IgG4同种型的抗人类B7-H5抗体(2D3和18C3)结合由CHO细胞表达的人类B7-H5的能力。

[0036] 图13(小图A-C)示出重组抗人类B7-H5嵌合抗体(2D3和18C3)完全阻滞CD28H融合蛋白至CHO B7-H5转染子的结合的能力。用对照上清液(小图A)或重组嵌合体2D3(小图B)和18C3上清液(小图C)预孵育人类B7-H5 FL CHO转染子,并且随后用生物素酰化的CD28HhIg融合蛋白进行染色。

[0037] 图14A-14B示出抗人类B7-H5抗体(2D3和18C3)的表位识别位点。图14A和图14B示出2D3和18C3识别B7-H5的第一IgV结构域。图14C示出B7-H5 IgV结构域介导B7-H5与CD28H的相互作用。

[0038] 图15比较了抗体2D3和18C3结合如表达在CHO细胞的表面上的人类B7-H5的亲和力。

[0039] 图16A-16B示出内源B7-H5:CD28H相互作用对TT特异性记忆T细胞的召回的影响。图16A示出在存在阻滞抗体2D3(小图B)或对照Ig(小图A)的B7-H5:CD28H相互作用时,TT疫苗诱导的T细胞增殖。图16B示出与这种应答关联的表达的细胞因子(小图A)和BrdU-阳性细胞(小图B)的水平。

[0040] 图17A-17B示出通过2D3的内源B7-H5:CD28H相互作用的阻滞抑制了人源化NSG小鼠中CD8+(图17A,小图A-B)和CD4+(图17B,小图A-B)T细胞二者的异体增殖应答。

[0041] 图18示出在刺激后30min,TCR和B7-H5融合蛋白的同时交联诱导AKT磷酸化,同时TCR交联单独诱导最小的AKT磷酸化。重要地,B7-H5抗体2D3的包含阻止了AKT活化,表明B7-H5共刺激利用了AKT通路来促进T细胞应答。

[0042] 图19示出通过诱饵受体融合蛋白CD28HIg阻滞B7-H5-CD28H通路抑制了异体CD8T细胞针对B7-H5转染的624Mel黑色素瘤细胞系的细胞毒性杀伤活性,表明B7-H5-CD28H通路

促进CD8T细胞对靶细胞的细胞毒性杀伤活性。

[0043] 图20A-B示出在可溶性重组人类IL-2存在时，自然杀伤细胞(NK)上的CD16和B7-H5融合蛋白的同时交联诱导NK活化和脱粒(CD107a表面上调)，而单独CD16接合并不诱导显著的NK脱粒。观察到B7-H5融合蛋白剂量依赖性NK活化(图20A)。重要地，B7-H5抗体2D3的包含阻止了NK活化(图20B)，表明B7-H5共刺激NK活化。

发明详细说明：

[0044] 本发明涉及抗体和它们的抗原结合片段，并且涉及能够免疫特异性地结合B7-H5:CD28H通路的B7-H5配体的其他分子，并且涉及此类分子在治疗和诊断自身免疫性疾病、移植排斥和其他炎症疾病中的用途。

[0045] B7-H5:CD28H通路是涉及配体B7-H5及其反受体CD28H的细胞免疫通路(图1)。B7-H5表达在抗原呈递细胞上；它组成型地表达在巨噬细胞上并且在树突细胞上是可诱导的(图2，小图A-J)。CD28H特别表达于原初T细胞、NK细胞、和浆细胞样树突细胞(尤其是在脾脏、淋巴结和胸腺)上，并且它的表达在成熟的或活化的细胞上被下调。它并不表达在γδT细胞或B细胞上(图3)，但是表达在T_n、T_{CM}、T_{EM}、和T_{EMRA} T细胞亚群上。人类脐带血T细胞表达CD28H，而$CD4^+$、$CD8^+$和$CD4^+/CD8^+$胸腺细胞也是如此。B7-H5与CD28H反受体相互作用，以刺激免疫系统，由此促进增强的免疫应答。已经发现CD28H的下调在体内损害了活化的T细胞/记忆T细胞存活。因此，对于体内天然T细胞引发和活化的T细胞/记忆T细胞存活，B7-H5和CD28H之间的相互作用是重要的。还见到在长期抗原暴露的/疲惫的T细胞中，CD28H下调。CD28H组成型地表达在自然杀伤细胞(NK)上。在NK活化信号，例如CD16交联存在时，B7-H5-CD28H通路促进NK活化和脱粒。

[0046] 本发明部分地反映了对以下的识别：分子例如免疫特异性地结合B7-H5的抗体及其抗原结合片段(“抗B7-H5抗体”)，并且具体地是阻滞B7-H5:CD28H相互作用以便损害(即阻止或弱化)B7-H5结合CD28H的能力的抗B7-H5抗体或诱饵受体融合蛋白CD28HIg，能够损害B7-H5经由CD28H相互作用促进增强的免疫应答的能力，并且因此能够用作T细胞增殖和细胞因子产生以及HK活化的拮抗剂。此类分子能够介导免疫系统活化中的生理学下降，并且因此具有在治疗炎症疾病和自身免疫性疾病中的效用。具体地，此类抗体能够降低体内活化的T细胞和NK细胞的百分比。

A.B7-H5

[0047] 发现B7-H5氨基酸序列类似于先前发现的人类基因，HHLA2(人类内源性逆转录病毒-H长末端重复相关蛋白2)(HHLA2)；梅格(Mager)，D.L.等人(1999)“内源性逆转录病毒提供了针对两个新人类基因(HHLA2和HHLA3)的初级聚腺苷酸化信号(Endogenous Retroviruses Provide The Primary Polyadenylation Signal For Two New Human Genes(HHLA2And HHLA3)”，基因组学(Genomics)59:255-263)，HHLA2不具有已知功能(弗莱尼克(Flajnik)，M.F.等人(2012)“B7家族的演化：B7H6和Nkp30的共演化，新B7家族成员B7H7的鉴定，以及B7与MHC的历史关系的鉴定(Evolution Of The B7 Family:Co-Evolution Of B7H6 And Nkp30,Identification Of A New B7 Family Member,B7H7,And Of B7's Historical Relationship With The MHC)”，免疫遗传学(Immunogenetics)64:571-590)。B7-H5在此和其他地方还称为B7-H7。相应地，术语B7-H5和B7-H7是在此可互换地使用。

[0048] 已经发现B7-H5序列在鸡、负鼠、有蹄哺乳动物(例如马、猪)、鲑鱼、和鲨鱼中具有同系物。然而,迄今仅在啮齿动物(小鼠以及大鼠)中鉴定了拟基因。此类基因的氨基酸序列在所有物种中揭示了一个类似的域结构,具有针对Ig超家族域的典型残基的保守性。

[0049] 人类B7-H5多肽长度是414个氨基酸,并且已经报道,它包含以下:信号序列、具有3个免疫球蛋白样(Ig样)结构域的胞外结构域、跨膜结构域、和胞质结构域。具体而言,该人类B7-H5多肽已被报导包含Ig样V-类型1结构域、Ig样C-1类型结构域、以及Ig样V-类型2结构域。存在B7-H5的多个天然存在的变体(例如登录号Q9UM44-1(智人)、NP_009003(GI:5901964,智人)、以及AAD48396(GI:15726285,智人);参见WO 2011/020024)。

[0050] 术语"天然B7-H5"(也称为"天然B7-H7")是指任何天然存在的B7-H5氨基酸序列,包括不成熟的或前体的以及成熟的形式。代表性人类B7-H5的氨基酸序列,登录号Q9UM44-1,是(SEQ ID NO:1):

[0051]
MKAQTALSFF LILITSLSGS QGIFPLAFFI YVPMNEQIVI GRLDEDIILP
SSFERGSEVV **IHWKYQDSYK VHSYYKGSDH LESQDPRYAN RTSLFYNEIQ**
NGNASLFFRR VSLLDEGIYT CYVGTAIQVI TNKVVLKVGV FLTPVMKYEK
RNTNSFLICS VLSVYPRPII TWKMDNTPIS ENNMEETGSL DSFSINSPLN
ITGSNSSYEC TIENSLLKQT WTGRWTMKDG LHKMQSEHVS LSCQPVNDYF
SPNQDFKVTW SRMKSGTFSV LAYYLSSSQN TIINESRFSW NKELINQSDF
SMNLMDLNLS DSGEYLCNIS SDEYTLLTIH TVHVEPSQET ASHNKGLWIL
VPSAILAAFL LIWSVKCCRA QLEARRSRHP ADGAQQERCC VPPGERCPSA
PDNGEENVPL SGKV

[0052] 已经报道人类B7-H5包含以下:大致在SEQ ID NO:1的氨基酸残基1至22的信号序列、大致在SEQ ID NO:1的氨基酸残基61至131的Ig样V型1结构域(以上下划线示出)、大致在SEQ ID NO:1的氨基酸残基138至222的Ig样C-1型结构域、大致在SEQ ID NO:1的氨基酸残基235至328的Ig样V型2结构域、和大致在SEQ ID NO:1的氨基酸残基345至365的跨膜结构域。针对人类B7-H5多肽的预测的二聚体界面是SEQ ID NO:1的氨基酸残基141-144、156、158、160、162、193-196、198、200、201、224、和225。针对人类B7-H5多肽的预测的N-连接的糖基化位点是SEQ ID NO:1的氨基酸残基90、103、和318。人类B7-H5多肽的自然变异包括BOT、N344K、和S346R(UniProt Q9UM44)(参见WO 2011/020024,将其针对人类B7-H5的结构和序列的传授内容,通过引用以其全文结合在此)。

[0053] 编码人类B7-H5(SEQ ID NO:1)的DNA序列是(SEQ ID NO:2):

atgaaggcac agacagcact gtctttcttc ctcattctca taacatctct
gagtggatct caaggcatat tccctttggc tttcttcatt tatgttccta
tgaatgaaca aatcgtcatt ggaagacttg atgaagatat aattctccct
tcttcatttg agaggggatc cgaagtcgta atacactgga agtatcaaga
tagctataag gttcatagtt actacaaagg cagtgaccat ttggaaagcc
aagatcccag atatgcaaac aggacatccc ttttctataa tgagattcaa
aatgggaatg cgtcactatt tttcagaaga gtaagccttc tggacgaagg
aatttacacc tgctatgtag gaacagcaat tcaagtgatt acaaacaaag
tggtgctaaa ggtgggagtt tttctcacac ccgtgatgaa gtatgaaaag
aggaacacaa acagcttctt aatatgcagc gtgttaagtg tttatcctcg
tccaattatc acgtggaaaa tggacaacac acctatctct gaaaacaaca
tggaagaaac agggtctttg gattcttttt ctattaacag cccactgaat
attacaggat caaattcatc ttatgaatgt acaattgaaa attcactgct
gaagcaaaca tggacagggc gctggacgat gaaagatggc cttcataaaa
tgcaaagtga acacgtttca ctctcatgtc aacctgtaaa tgattatttt
tcaccaaacc aagacttcaa agttacttgg tccagaatga aaagtgggac
tttctctgtc ctggcttact atctgagctc ctcacaaaat acaattatca
atgaatcccg attctcatgg aacaaagagc tgataaacca gagtgacttc
tctatgaatt tgatggatct taatctttca gacagtgggg aatatttatg
caatatttct tcggatgaat atactttact taccatccac acagtgcatg
tagaaccgag ccaagaaaca gcttcccata acaaaggctt atggattttg
gtgccctctg cgattttggc agcttttctg ctgatttgga gcgtaaaatg
ttgcagagcc cagctagaag ccaggaggag cagacaccct gctgatggag
cccaacaaga aagatgttgt gtccctcctg gtgagcgctg tcccagtgca
cccgataatg gcgaagaaaa tgtgcctctt tcaggaaaag ta

[0054] 代表性人类B7-H5IgV结构域人类IgG4融合蛋白的氨基酸序列是(SEQ ID NO:3)(以黑体字示出B7-H5序列;下划线示出IgG4序列):

IFPLAFFIYV PMNEQIVIGR LDEDIILPSS FERGSEVVIH WKYQDSYKVH
SYYKGSDHLE SQDPRYANRT SLFYNEIQNG NASLFFRRVS LLDEGIYTCY
VGTAIQVITN KVVLKVGVFL TPVMKYEKES KYGPPCPPCP APEFLGGPSV
FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK
PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK
GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN
YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS
LSLSPG

[0055] 融合蛋白可以另外包括N末端前导序列(SEQ ID NO:1的此类残基1-22,即天然存在的B7-H5前导序列(SEQ ID NO:4)):

MKAQTALSFF LILITSLSGS QG

[0056] 编码天然存在的B7-H5前导序列的DNA序列是(SEQ ID NO:5):

atgaaggccc agaccgccct gtccttcttc ctgatcctga tcacctccct
gtccggcagc caggga

[0057] 虽然根据本发明,已经示出B7-H5序列融合至人类IgG4区域,但是此类序列可以可替代地融合至任何Ig同种型,或确实融合至任何其他蛋白。

[0058] 编码人类B7-H5IgV-hIgG4(SEQ ID NO:3)的DNA序列是(SEQ ID NO:6):

atcttccctc tggccttctt catctacgtg cccatgaacg agcagatcgt
gatcggccgg ctggacgagg atattatcct gccctccagc ttcgagcggg
gctccgaggt cgtgatccac tggaagtacc aggactccta caaggtgcac
tcctactaca agggctccga ccacctggaa tcccaggacc ccagatacgc
caaccggacc agcctgttct acaacgagat ccagaacggc aacgcctccc
tgttcttccg gcgagtgtcc ctgctggatg agggcatcta cacctgttac
gtgggcaccg ccatccaagt gatcaccaac aaggtggtgc tgaaagtggg
cgtgttcctg acccccgtga tgaagtacga gaaagagtct aagtacggcc
ctccctgccc ccctttgtcct gcccctgaat ttctgggcgg accctctgtg
ttcctgttcc ccccaaagcc caaggacacc ctgatgatct cccggacccc
cgaagtgacc tgcgtggtgg tggatgtgtc ccaggaagat cccgaggtgc

agttcaattg gtacgtggac ggcgtggaag tgcacaacgc caagaccaag
cccagagagg aacagttcaa ctccacctac cgggtggtgt ccgtgctgac
cgtgctgcac caggattggc tgaacggcaa agagtacaag tgcaaggtgt
ccaacaaggg cctgcccagc tccatcgaaa agaccatctc caaggccaag
ggccagcccc gggaacccca ggtgtacaca ctgcctccaa gccaggaaga
gatgaccaag aaccaggtgt ccctgacctg tctcgtgaag ggcttctacc
cctccgatat cgccgtggaa tgggagtcca acggccagcc tgagaacaac
tacaagacca ccccccctgt gctggactcc gacggctctt tcttcctgta
ctcccgcctg accgtggaca agtccagatg gcaggaaggc aacgtgttct
cctgctccgt gatgcacgag gccctgcaca accactacac ccagaagtcc
ctgtccctga gccccggc

[0059] 包括B7-H5的胞外结构域的代表性人类B7-H5人类IgG4P融合蛋白的氨基酸序列是(SEQ ID NO:24)(B7-H5ECD-hIgG4P,其中B7-H5 ECD aa1-340加有下划线,并且丝氨酸228至脯氨酸的突变加有双下划线)

IFPLAFFIYVPMNEQIVIGRLDEDIILPSSFERGSEVVIHWKYQDSYKVHSYYKGSDHLESQDPRYANRTSLF
YNEIQNGNASLFFRRVSLLDEGIYTCYVGTAIQVITNKVVLKVGVFLTPVMKYEKRNTNSFLICSVLSVYPRPIITW
KMDNTPISENNMEETGSLDSFSINSPLNITGSNSSYECTIENSLLKQTWTGRWTMKDGLHKMQSEHVSLSCQPVNDY
FSPNQDFKVTWSRMKSGTFSVLAYYLSSSQNTIINESRFSWNKELINQSDFSMNLMDLNLSDSGEYLCNISSDEYTL
LTIHTVHVEPSQETESKYGPPCPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVD
GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN
HYTQKSLSLSPG

[0060] 信号序列可以是天然信号序列(SEQ ID NO:29)

MKAQTALSFFLIL工TSLSGSQG

[0061] 融合蛋白可以是由核酸序列(SEQ ID NO:25)编码

atgaaggcccagaccgccctgtccttcttcctgatcctgatcacctccctgtccggcagccagggaatcttcc
ctctggccttcttcatctacgtgcccatgaacgagcagatcgtgatcggccggctggacgaggatattatcctgccc
tccagcttcgagcggggctccgaggtcgtgatccactggaagtaccaggactcctacaaggtgcactcctactacaa
gggctccgaccacctggaatcccaggaccccagatacgccaaccggaccagcctgttctacaacgagatccagaacg
gcaacgcctccctgttcttccggcgagtgtccctgctggatgagggcatctacacctgttacgtgggcaccgccatc
caagtgatcaccaacaaggtggtgctgaaagtgggcgtgttcctgacccccgtgatgaagtacgagaagcggaatac
caactctttcctgatctgctccgtgctgtccgtgtaccctcggcccatcatcacctggaagatggacaacaccccca
tctccgagaacaacatggaagagacaggctccctggactccttctccatcaactcccccctgaacattaccggctcc
aactcctcctacgagtgcaccatcgagaactccctgctgaagcagacctggaccggcagatggactatgaaggacgg

cctgcacaagatgcagtccgagcacgtgtccctgtcctgccagcccgtgaacgactacttcagccccaaccaggact
tcaaagtgacctggtcccggatgaagtccggcaccttcagcgtgctggcctactacctgtccagctcccagaacacc
atcatcaacgagtcccggttctcctggaacaaagagctgatcaaccagtccgacttctccatgaacctgatggacct
gaacctgtccgacagcggcgagtacctgtgcaacatctccagcgacgagtacaccctgctgaccatccacaccgtgc
acgtggaaccctcccaggaaaccgagtctaagtacggccctccctgcccaccttgtcccgcccctgaatttctgggc
ggaccctctgtgttcctgttccccccaaagcccaaggacaccctgatgatctcccggacccccgaagtgacatgcgt
ggtggtggatgtgtcccaggaagatcccgaggtgcagttcaattggtacgtggacggcgtggaagtgcacaacgcca
agaccaagcccagagaggaacagttcaactccacctaccgggtggtgtctgtgctgaccgtgctgcaccaggactgg
ctgaacggcaaagagtacaagtgcaaggtgtccaacaagggcctgcccagctccatcgaaaagaccatctccaaggc
caagggccagccccgggaaccccaggtgtacacactgcctccaagccaggaagagatgaccaagaaccaggtgtccc
tgacttgcctcgtgaagggcttctacccctccgatatcgccgtggaatgggagtccaacggccagcctgagaacaac
tacaagaccacccccccctgtgctggactccgacggctctttcttcctgtactcccgcctgaccgtggacaagtccag
atggcaggaaggcaacgtgttctcctgcagcgtgatgcacgaggccctgcacaaccactacacccagaagtccctga
gcctgtcccccggctga

[0062] 相比于被广泛表达的人类B7-H4,发现人类B7-H5展现更受限的表达(例如,表达于肠、肾、肺、上皮细胞和淋巴细胞中)。人类HHLA2发现于靠近B7.1以及B7.2的染色体3q13.33上。B7-H5组成型地表达于巨噬细胞上并且在树突细胞(DC)上是可诱导的。

B.CD28H

[0063] CD28H,在此和其他地方还称为H7CR,是针对B7-H5的反受体。相应地,术语CD28H和H7CR是在此可互换地使用的。如在此使用,术语“天然CD28H”(还称为“天然H7CR”)是指B7-H5的天然存在的反受体、或其变体。T细胞、NK细胞、和浆细胞样树突细胞表达CD28H。在文献/数据库中,人类CD28H多肽另外称为含跨膜结构域和免疫球蛋白结构域2(TMIGD2)(拉希米(Rahimi),N.等人(2012年3月14日电子出版)“鉴定IGPR-1为血管发生中涉及的新颖粘附分子(Identification Of IGPR-1As A Novel Adhesion Molecule Involved In Angiogenesis)”,分子生物学和细胞生物学(Molec.Biol.Cell.)23(9):1646-1656),但是CD28H的功能并未在先前解释。针对此类天然CD28H分子的氨基酸序列的登录号的非限制性实例包括:Q96BF3-1(智人)、Q96BF3-2(智人)、NP_653216.1(GI:21389429;智人)和NP_653216.2(GI:281306838;智人)。以下提供了天然CD28H分子的代表性氨基酸序列(Q96BF3-2),为SEQ ID NO:7:

MGSPGMVLGL LVQIWALQEA SSLSVQQGPN LLQVRQGSQA TLVCQVDQAT
AWERLRVKWT KDGAILCQPY ITNGSLSLGV CGPQGRLSWQ APSHLTLQLD
PVSLNHSGAY VCWAAVEIPE LEEAEGNITR LFVDPDDPTQ NRNRIASFPG
FLFVLLGVGS MGVAAIVWGA WFWGRRSCQQ RDSGNSPGNA FYSNVLYRPR
GAPKKSEDCS GEGKDQRGQS IYSTSFPQPA PRQPHLASRP CPSPRPCPSP
RPGHPVSMVR VSPRPSPTQQ PRPKGFPKVG EE

[0064] 编码人类CD28H(SEQ ID NO:7)的DNA序列是(SEQ ID NO:8):

```
atggggtccc cgggcatggt gctgggcctc ctggtgcaga tctgggccct
gcaagaagcc tcaagcctga gcgtgcagca ggggcccaac ttgctgcagg
tgaggcaggg cagtcaggcg accctggtct gccaggtgga ccaggccaca
gcctgggaac ggctccgtgt taagtggaca aaggatgggg ccatcctgtg
tcaaccgtac atcaccaacg gcagcctcag cctgggggtc tgcgggcccc
agggacggct ctcctggcag gcacccagcc atctcaccct gcagctggac
cctgtgagcc tcaaccacag cggggcgtac gtgtgctggg cggccgtaga
gattcctgag ttggaggagg ctgagggcaa cataacaagg ctctttgtgg
acccagatga ccccacacag aacagaaacc ggatcgcaag cttcccagga
ttcctcttcg tgctgctggg ggtgggaagc atgggtgtgg ctgcgatcgt
gtggggtgcc tggttctggg gccgccgcag ctgccagcaa agggactcag
gtaacagccc aggaaatgca ttctacagca acgtcctata ccggccccgg
ggggccccaa agaagagtga ggactgctct ggagagggga aggaccagag
gggccagagc atttattcaa cctccttccc gcaaccggcc ccccgccagc
cgcacctggc gtcaagaccc tgccccagcc cgagaccctg ccccagcccc
aggccccggcc accccgtctc tatggtcagg gtctctccta gaccaagccc
cacccagcag ccgaggccaa aagggttccc caaagtggga gaggag
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C.定义

[0065] 如此处使用的,如果这种结合展现一种抗体结合至其同源抗原的特异性以及亲和力的话,则称一种分子能够“免疫特异性地结合”一种第二分子。如果这种结合涉及免疫球蛋白分子的抗原识别位点的话,则称抗体能够“免疫特异性地结合”至抗原(并且特别是抗原B7-H5)的靶区域或构造(“表位”)。免疫特异性地结合到具体的抗原的抗体可以按更低的亲和力结合到其他抗原,前提是如果该其他抗原具有由如通过例如免疫测定、BIACORE®测定、或本领域中已知的其他测定确定的抗原识别位点所识别的一些序列或构造相似性的话,但不会结合至完全不相关的抗原。然而,优选地,抗体(及其抗原结合片段)将不与其他抗原发生交叉反应。抗体也可以按非免疫特异性的方式结合到其他分子,诸如结合到FcR受体,凭借该分子的不涉及该抗原识别位点的其他区域/域中的结合结构域,诸如Fc区。

[0066] 如在结合或展现的效果的背景下使用的术语“基本上”旨在表示所观察到的效果是生理学或治疗相关的。因此,例如,如果阻滞的程度是生理学或治疗相关的话(例如如果这种程度大于完全阻滞的60%,大于完全阻滞的70%,大于完全阻滞的75%,大于完全阻滞的80%,大于完全阻滞的85%,大于完全阻滞的90%,大于完全阻滞的95%,或大于完全阻滞的97%),一种分子能够基本上阻滞B7-H5的活性。类似地,如果此类免疫特异性以及表征是大于60%相同的,大于70%相同的,大于75%相同的,大于80%相同的,大于85%相同的,大于90%相同的,大于95%相同的,或大于97%相同的,则称一种分子与另一种分子具有基本上相同的免疫特异性和/或表征)。

[0067] 如此处使用的,术语“受试者”旨在表示一种哺乳动物,诸如非灵长类(例如,母牛、猪、马、猫、狗、大鼠等)以及灵长类(例如,猴以及人),最优选是人。术语“患者”旨在表示接受用于诊断、治疗或预防目的的本发明组合物的受试者。

[0068] 如此处使用的,术语“抗体”旨在表示具有“可变区”抗原识别位点的免疫球蛋白分子。术语“可变区”旨在将该免疫球蛋白的这种域与抗体广泛共享的域(例如抗体Fc域)区分。该可变区包括“高变区”,该高变区的残基负责抗原结合。高变区包括来自“互补决定区”或“CDR”的氨基酸残基(即典型地,在轻链可变结构域中,大致在残基24-34(L1)、50-56(L2)和89-97(L3)处,并且在重链可变结构域中,大致在残基27-35(H1)、50-65(H2)和95-102

(H3)处;卡巴特(Kabat)等人,具有免疫学兴趣的蛋白的序列(Sequences of Proteins of Immunological Interest),第5版,公共卫生署,美国国立卫生研究院,贝塞斯达,马里兰州,(1991))和/或来自“高变环”的那些残基(即在轻链可变结构域中,残基26-32(L1)、50-52(L2)和91-96(L3),和在重链可变结构域中残基26-32(H1)、53-55(H2)和96-101(H3);乔西亚(Chothia)和Lesk(莱斯克),1987,分子生物学杂志(J.Mol.Biol.)196:901-917)。“框架区”或“FR”残基是除了如在此定义的高变区残基以外的那些可变结构域残基。术语抗体包括单克隆抗体、多特异性抗体、人类抗体、人源化抗体、合成抗体、嵌合抗体、骆驼化抗体(参见例如谬德曼斯(Muyldermans)等人,2001,生物化学科学趋势(Trends Biochem.Sci.)26:230;纳托尔(Nuttall)等人,2000,当今药物生物技术(Cur.Pharm.Biotech.)1:253;赖克曼(Reichmann)和谬德曼斯(Muyldermans),1999,免疫学方法杂志(J.Immunol.Meth.),231:25;国际公开号WO 94/04678和WO 94/25591;美国专利号No.6,005,079)、单链Fv(scFv)(参见例如,参见单克隆当体的药物学中的普朗克塞(Pluckthun in The Pharmacology of Monoclonal Antibodies),卷113,罗森堡(Rosenburg)和摩尔(Moore)编辑,施普林格出版公司,纽约,269-315页(1994))、单链抗体、二硫化物连接的Fv(sdFv)、胞内抗体、和抗个体基因型(抗Id)抗体(包括披露的B7-H5抗体的抗Id和抗-抗Id抗体)。具体地,此类抗体包括任何类型的免疫球蛋白分子(例如IgG、IgE、IgM、IgD、IgA、以及IgY)、类别(例如IgG1、IgG2、IgG3、IgG4、IgA1、以及IgA2)或子类。

[0069] 如在此使用,术语抗体的“抗原结合片段”是指抗体中的包括抗体的互补决定区(“CDR”)和任选地含抗体的“可变区”抗原识别位点的框架残基的、并且展现出免疫特异性地结合抗原的能力的一个或多个部分。此类片段包括Fab',F(ab')2,Fv,单链(ScFv),及其突变体,天然存在的变体,以及包括该抗体的“可变区”抗原识别位点以及一种异源蛋白(例如,毒素、针对不同抗原的抗原识别位点、酶、受体或受体配体等)的融合蛋白。如此处使用的,术语“片段”是指一种肽或多肽,该肽或多肽包括至少5个连续的氨基酸残基、至少10个连续的氨基酸残基、至少15个连续的氨基酸残基、至少20个连续的氨基酸残基、至少25个连续的氨基酸残基、至少40个连续的氨基酸残基、至少50个连续的氨基酸残基、至少60个连续的氨基残基、至少70个连续的氨基酸残基、至少80个连续的氨基酸残基、至少90个连续的氨基酸残基、至少100个连续的氨基酸残基、至少125个连续的氨基酸残基、至少150个连续的氨基酸残基、至少175个连续的氨基酸残基、至少200个连续的氨基酸残基、或至少250个连续的氨基酸残基的氨基酸序列。

[0070] 抗人类B7-H5抗体的人类、嵌合或人源化衍生物对于在人体内使用是特别优选的,然而,可有利地采用鼠抗体或其他物种的抗体用于许多用途(例如,体外或原位检测测定,急性体内使用等)。在一个或多个非人类CDR中,人源化抗体可以包括氨基酸残基取代、缺失或添加。当与非衍生物人源化抗体相比时,人源化抗体衍生物可以具有基本上相同的结合、更强的结合或更弱的结合。在特定实施例中,该CDR的一、二、三、四、或五个氨基酸残基已被取代、缺失或添加(即,突变)。完全人类抗体对于人类受试者的治疗性治疗是特别令人希望的。

[0071] 人类抗体可以通过在本领域中已知的多种方法来制造,包括使用衍生自人类免疫球蛋白序列的抗体文库的上述的噬菌体展示方法(参见美国专利号4,444,887和4,716,111;以及国际公开号WO 98/46645、WO 98/50433、WO 98/24893、WO 98/16654、WO 96/

34096、WO 96/33735、和WO 91/10741)。人类抗体可以使用转基因小鼠来产生,这些转基因小鼠不能表达功能内源性免疫球蛋白,但是可以表达人类免疫球蛋白基因。例如,可以随机地或通过同源重组将人类重链和轻链免疫球蛋白基因复合物引入到小鼠胚胎干细胞之中。可替代地,除了人类重链和轻链基因之外,还可以将人类可变区、恒定区和多样区(diversity region)引入到小鼠胚胎干细胞之中。可以单独使小鼠重链和轻链免疫球蛋白基因无功能,或在引入人类免疫球蛋白基因座的同时通过同源重组来使这些免疫球蛋白基因无功能。具体地说,JH区的纯合缺失防止内源性抗体产生。修饰的胚胎干细胞扩增并且微注射到胚囊中以便产生嵌合小鼠。然后使嵌合小鼠繁殖以便产生表达人类抗体的纯合子代。使用常规方法学,用选择的抗原(例如,B7-H5多肽的全部或一部分)来对转基因小鼠免疫。针对抗原的单克隆抗体可以使用常规的杂交瘤技术从免疫的转基因小鼠获得(参见例如美国专利号5,916,771)。转基因小鼠所具有的人类免疫球蛋白转基因在B细胞分化过程中重排,并且随后进行类别转换和体细胞突变。因此,使用这种技术,可能产生治疗有用的IgG、IgA、IgM以及IgE抗体。为了综述用于产生人类抗体的此技术,参见伦博格(Lonberg)和胡萨尔(Huszar)(1995,免疫学国际综述(Int.Rev.Immunol.)13:65-93,将其通过引用以其全文结合在此)。为了详细讨论用于产生人类抗体和人类单克隆抗体的此技术和用于产生此类抗体的方案,参见例如国际公开号WO 98/24893、WO 96/34096、和WO 96/33735;和美国专利号5,413,923、5,625,126、5,633,425、5,569,825、5,661,016、5,545,806、5,814,318、以及5,939,598,将其通过引用以其全文结合在此。此外,公司诸如安根尼克斯(Abgenix)公司(弗里蒙特(Freemont),加利福尼亚州)以及梅达瑞克斯(Medarex)(普林斯顿(Princeton),新泽西州)能够参与使用类似于上述的技术针对选择的抗原提供人类抗体。

[0072] “嵌合抗体”是其中抗体的不同部分衍生自不同的免疫球蛋白分子的分子,如具有衍生自一种非人类抗体的可变区和人类免疫球蛋白恒定区的抗体。产生嵌合抗体的方法在本领域中是已知的。参见例如莫里森(Morrison),1985,科学(Science)229:1202;维(Oi)等人,1986,生物技术(BioTechniques)4:214;吉利斯(Gillies)等人,1989,免疫学方法杂志(J.Immunol.Methods)125:191-202;和美国专利号6,311,415、5,807,715、4,816,567、和4,816,397。可以使用本领域已知的多种多样的技术,例如CDR移植(EP 239,400;国际公开号WO 91/09967;和美国专利号5,225,539、5,530,101、以及5,585,089),镶合(veneering)或表面重修(EP 592,106、EP 519,596;巴丹(Padlan),1991,分子免疫学(Molecular Immunology)28(4/5):489-498;斯塔德尼卡(Studnicka)等人,1994,蛋白工程(Protein Engineering)7:805;以及罗古斯卡(Roguska)等人,1994,美国国家科学院院刊(Proc.Natl.Acad.Sci.USA)91:969),以及链改组(美国专利号5,565,332),来产生包括来自非人类物种的一个或多个CDPvS和来自人类免疫球蛋白分子的框架区的嵌合抗体。

[0073] 本发明具体地涉及“人源化抗体”(参见例如欧洲专利号EP 239,400、EP 592,106、和EP 519,596;国际公开号WO 91/09967和WO 93/17105;美国专利号5,225,539、5,530,101、5,565,332、5,585,089、5,766,886、和6,407,213;以及巴丹(Padlan),1991,分子免疫学(Molecular Immunology)28(4/5):489-498;斯塔德尼卡(Studnicka)等人,1994,蛋白工程(Protein Engineering)7(6):805-814;罗古斯卡(Roguska)等人,1994,美国国家科学院院刊(PNAS)91:969-973;谭(Tan)等人,2002,免疫学杂志(J.Immunol)169:1119-1125;卡尔达(Caldas)等人,2000,蛋白工程(Protein Eng.)13:353-360;摩里亚(Morea)等人,2000,

方法(Methods)20:267-79;巴卡(Baca)等人,1997,生物化学杂志(J.Biol.Chem.)272:10678-10684;罗古斯卡(Roguska)等人,1996,蛋白工程(Protein Eng.)9:895-904;库托(Couto)等人,1995,癌症研究(Cancer Res.)55(23增刊):5973s-5977s;库托(Couto)等人,1995,癌症研究(Cancer Res.)55:1717-22;桑德胡(Sandhu),1994,基因(Gene)150:409-10;佩德森(Pedersen)等人,1994,分子生物学杂志(J.Mol.Biol.)235:959-973;琼斯(Jones)等人,1986,自然(Nature)321:522-525;赖克曼(Reichmann)等人,1988,自然(Nature)332:323-329;以及普雷斯塔(Presta),1992,结构生物学新见(Curr.Op.Struct.Biol.)2:593-596)。如在此使用,术语"人源化抗体"是指包括人类框架区和来自非人类(通常是小鼠或大鼠)免疫球蛋白的一个或多个CDR的免疫球蛋白。提供CDR的非人类免疫球蛋白称为"供体",并且提供框架的人类免疫球蛋白称为"受体"。并不需要存在恒定区,但是如果它们存在,它们必须与人类免疫球蛋白恒定区基本上一致,即至少约85%-90%、优选约95%或更多地一致。因此,除了可能地CDR外,人源化免疫球蛋白的所有部分基本上与天然人类免疫球蛋白序列的相应部分相同。人源化抗体是包括人源化轻链的和人源化重链的免疫球蛋白的抗体。例如,人源化抗体将不涵盖典型的嵌合抗体,因为例如嵌合抗体的完整可变区是非人类的。有人说,通过"人源化(humanization)"的过程供体抗体被"人源化",因为预期所得到的人源化抗体结合到与提供该CDR的供体抗体针对的相同的抗原上。对于大部分,人源化抗体是人类免疫球蛋白(受体抗体),其中该受体的高变区残基被来自具有希望的特异性、亲和力、以及能力的非人类物种(供体抗体)诸如小鼠、大鼠、兔或非人类灵长动物的高变区残基替代。在一些情况下,人类免疫球蛋白的框架区(FR)残基被相应的非人类残基替代。此外,人源化抗体可以包括在受体抗体或供体抗体中未发现的残基。进行这些修饰以便使抗体性能进一步改进。通常,人源化抗体将基本上都包括至少一个、并且典型地两个可变结构域,其中所有或基本上所有的高可变区对应于非人类免疫球蛋白的那些,并且所有或基本上所有的FR是具有人类免疫球蛋白序列的那些。人源化抗体任选地还将包括免疫球蛋白恒定区(Fc)的至少一部分,典型地是免疫特异性地结合到FcRIIB多肽的、已经通过引入氨基酸残基取代、缺失或添加(即突变)改变的人类免疫球蛋白的那部分。

[0074] 编码优选的人类受体框架序列的DNA序列包括但不限于来自人类种系VH区段VH1-18和JH6以及人类种系VL区段VK-A26和JK4的FR区段。在特定实施例中,使用常规重组DNA技术将这些CDR中的一个或多个插入框架区内。框架区可以是天然存在的或共有的框架区、并且优选是人类框架区(参见例如乔西亚(Chothia)等人,1998,"免疫球蛋白可变结构域的序列中的结构确定(Structural Determinants In The Sequences Of Immunoglobulin Variable Domain)",分子生物学杂志(J.Mol.Biol.)278:457-479中的人类框架区列表)。

[0075] 人源化或嵌合的B7-H5抗体基本上包括至少一个、并且典型地两个可变结构域的全部,其中所有或基本上所有的CDR区对应于非人类免疫球蛋白(即,供体抗体)的那些,并且所有或基本上所有的框架区是具有人类免疫球蛋白共有序列的那些。优选地,B7-H5抗体还包括免疫球蛋白恒定区(Fc)的至少一部分,典型地是人类免疫球蛋白的那部分。可相对于所提出的B7-H5抗体的功能(具体而言可能需要的效应子功能)来选择该抗体的恒定结构域。在一些实施例中,B7-H5抗体的恒定结构域是(或包括)人类IgA、IgD、IgE、IgG或IgM结构域。在特定实施例中,当人源化B7-H5抗体是旨在用于治疗用途并且需要抗体效应子功能

(例如抗体依赖性细胞介导的细胞毒性(ADCC)和补体依赖性细胞毒性(CDC)活性)时,使用人类IgG恒定结构域,尤其是IgG1和IgG3同种型的人类IgG恒定结构域。在替代实施例中,当B7-H5抗体是旨在用于治疗目的并且不要求抗体效应子功能时,使用IgG2和IgG4同种型。本发明涵盖包括改变抗体效应子功能(例如美国专利申请公开号2005/0037000和2005/0064514中披露的那些)的一个或多个氨基酸修饰的Fc恒定结构域。

[0076] 在一些实施例中,B7-H5抗体包含轻链连同至少重链的可变结构域二者。在其他实施例中,B7-H5抗体可以进一步包括重链的CH1、绞链、CH2、CH3、和CH4区中的一个或多个。抗体可以选自任何类别的免疫球蛋白(包括IgM、IgG、IgD、IgA和IgE)以及任何同种型(包括IgG1、IgG2、IgG3和IgG4)。在一些实施例中,恒定结构域是补体固定恒定结构域,其中希望抗体展示细胞毒性活性,并且该类别典型地是IgG1。在其他实施例中,在这种细胞毒性活性不是令人希望的情况下,恒定结构域可以是IgG2类别的。B7-H5抗体可以包括来自多于一个类别或同种型的序列,并且选择具体的恒定结构域来优化希望的效应子功能是在本领域普通技术内的。

[0077] 人源化抗体的框架区和CDR区不需要精确地对应于亲本序列,例如,供体CDR或共有框架可以通过至少一个残基的取代、插入或缺失来进行诱变,以使得在那个位点处的CDR或框架残基不对应于共有抗体或供体抗体。然而,此类突变优选不是广泛的。通常,人源化抗体残基的至少75%将对应于亲本框架区(FR)和CDR序列的那些,更通常90%、并且最优选大于95%。可以使用本领域已知的多种多样的技术来产生人源化抗体,这些技术包括但不限于CDR移植(欧洲专利号EP 239,400;国际公开号WO 91/09967;和美国专利号5,225,539、5,530,101、和5,585,089),镶合或表面重修(欧洲专利号EP 592,106和EP 519,596;巴丹(Padlan),1991,分子免疫学(Molecular Immunology)28(4/5):489-498;斯塔德尼卡(Studnicka)等人,1994,蛋白工程(Protein Engineering)7(6):805-814;和罗古斯卡(Roguska)等人,1994,美国国家科学院院刊(Proc.Natl.Acad.Sci.)91:969-973),链改组(美国专利号5,565,332),和在例如以下中披露的技术:美国专利号6,407,213、5,766,886、5,585,089,国际公开号WO 9317105,谭(Tan)等人,2002,免疫学杂志(J.Immunol)169:1119-1125;卡尔达(Caldas)等人,2000,蛋白工程(Protein Eng.)13:353-360;摩里亚(Morea)等人,2000,方法(Methods)20:267-79;巴卡(Baca)等人,1997,生物化学杂志(J.Biol.Chem.)272:10678-84;罗古斯卡(Roguska)等人,1996,蛋白工程(Protein Eng.)9:895-904;库托(Couto)等人,1995,癌症研究(Cancer Res.)55(23增刊):5973s-5977s,库托(Couto)等人,1995,癌症研究(Cancer Res.)55:1717-22,桑德胡(Sandhu),1994,基因(Gene)150:409-10;佩德森(Pedersen)等人,1994,分子生物学杂志(J.Mol.Biol.)235:959-973;琼斯(Jones)等人,1986,自然(Nature)321:522-525;赖克曼(Reichmann)等人,1988,自然(Nature)332:323;以及普雷斯塔(Presta),1992,结构生物学新见(Curr.Op.Struct.Biol.)2:593-5966。通常,框架区中的框架残基将用来自CDR供体抗体的对应残基来取代以便改变、优选地改进抗原结合。这些框架取代是通过本领域熟知的方法鉴定的,例如通过对CDR和框架残基的相互作用建模以鉴定对抗原结合重要的框架残基,并且通过序列比较以鉴定在特定位置上的不寻常框架残基。参见例如奎因(Queen)等人,美国专利号5,585,089;美国公开号2004/0049014和2003/0229208;美国专利号6,350,861;6,180,370;5,693,762;5,693,761;5,585,089;以及5,530,101和赖克曼(Riechmann)等人,

1988,自然(Nature)332:323)。

[0078] 用于本发明的方法中的抗体可以是单特异性的。还感兴趣的是双特异性的抗体、三特异性的抗体或具有更大的多特异性的抗体,这些抗体展现对除B7-H5外的不同靶标(诸如免疫系统的其他分子)的特异性。例如,此类抗体可结合到B7-H5和如下的一种抗原两者上,该抗原对将该抗体靶标至具体的细胞类型或组织是重要的(例如,靶标至与正治疗的肿瘤的癌抗原关联的一种抗原)。在另一实施例中,这种多特异性抗体结合至替代的或补充的免疫调节通路中涉及的分子(受体或配体),例如CTLA4、TIM3、TIM4、OX40、CD40、GITR、4-1-BB、CD27/CD70、ICOS、B7-H4、LIGHT、PD-1或LAG3,以便减弱,进一步调节免疫调节作用。此外,该多特异性抗体可以结合至效应子分子,例如细胞因子(例如IL-7、IL-15、IL-12、IL-4TGF-β、IL-10、IL-17、IFNg、Flt3、BLys)和趋化因子(例如CCL21),这对于下调急性以及慢性免疫应答两者可能是特别相关的。

[0079] 本发明的抗体可通过本领域中已知的对于产生多肽有用的任何方法产生,这些方法是例如体外合成、重组DNA生产等。优选地,通过重组DNA技术来产生抗体。可以使用重组免疫球蛋白表达技术来产生B7-H5抗体。免疫球蛋白分子(包括人源化抗体)的重组产生描述于美国专利号4,816,397(博斯(Boss)等人)、美国专利号6,331,415和4,816,567(都属于加比利(Cabilly)等人)、英国专利GB 2,188,638(温特(Winter)等人)、和英国专利GB 2,209,757。免疫球蛋白重组表达技术(包括人源化免疫球蛋白)也可见于戈德尔(Goeddel)等人,酶学基因表达技术方法(Gene Expression Technology Methods in Enzymology)第185卷学术出版社(Academic Press)(1991),以及博勒巴克(Borreback),抗体工程(Antibody Engineering),W.H.弗里曼(Freeman)(1992)。涉及重组抗体的产生、设计以及表达的另外信息可以发现于梅福思(Mayforth),设计抗体(Designing Antibodies),学术出版社(Academic Press),圣地亚哥(1993)。

[0080] 用于产生重组嵌合B7-H5抗体的示例性工艺可以包括以下:a)通过常规分子生物学方法,构建编码并表达抗体重链的表达载体,其中鼠抗人类B7-H5单克隆抗体的CDR和可变区被融合到源自人类免疫球蛋白的Fc区,由此产生用于表达嵌合抗体重链的载体;b)通过常规分子生物学方法,构建编码并表达鼠抗人类B7-H5单克隆抗体的抗体轻链的表达载体,由此产生用于表达嵌合抗体轻链的载体;c)通过常规分子生物学方法将表达载体转移至宿主细胞,以产生用于表达嵌合抗体的经转染的宿主细胞;并且d)通过常规细胞培养技术,培养该转染细胞,以产生嵌合抗体。

[0081] 用于产生重组人源化B7-H5抗体的示例性工艺可以包括以下:a)通过常规分子生物学方法,构建编码和表达抗人类B7-H5重链的表达载体,其中要求保持供体抗体结合特异性的CDR和可变区框架的最小部分源自非人类免疫球蛋白,例如鼠抗人类B7-H5单克隆抗体,并且抗体的剩余部分源自人类免疫球蛋白,由此产生用于表达人源化抗体重链的载体;b)通过常规分子生物学方法,构建编码并表达抗体轻链的表达载体,其中要求保持供体抗体结合特异性的CDR和可变区框架的最小部分源自非人类免疫球蛋白,例如鼠抗人类B7-H5单克隆抗体,并且抗体的剩余部分源自人类免疫球蛋白,由此产生用于表达人源化抗体轻链的载体;c)通过常规分子生物学方法将这些表达载体转移至宿主细胞,以产生用于表达人源化抗体的经转染的宿主细胞;并且d)通过常规细胞培养技术,培养该转染细胞,以产生人源化抗体。

[0082] 关于任一示例性方法，可以用此类表达载体共转染宿主细胞，这些表达载体可以包含不同的选择性标记，但是除了重链的和轻链的编码序列，优选地是相同的。这一程序提供了重链多肽和轻链多肽的等同表达。可替代地，可以使用编码重链多肽和轻链多肽二者的单个载体。重链和轻链的编码序列可以包括cDNA或基因组DNA或二者。用于表达重组B7-H5抗体的宿主细胞可以是细菌细胞，例如大肠杆菌，或更优选地是真核细胞(例如中国仓鼠卵巢(CHO)细胞或HEK-293细胞)。对表达载体的选择取决于对宿主细胞的选择，并且可以如此选择以在所选择的宿主细胞中具有希望的表达以及调节特性。可以使用的其他细胞系包括但不限于CHO-K1、NSO、和PER.C6(Crucell公司，莱顿市，荷兰)。

[0083] 可以使用本领域普通技术人员熟知的技术，使用任何上述抗体来产生抗独特型抗体(参见例如格林斯潘(Greenspan)，N.S.等人(1989)“独特型：结构和免疫原性(Idiotypes:Structure And Immunogenicity)”，美国实验生物学会联合会会志(FASEB J.)7:437-444；和伊斯诺夫(isinoff,A.)(1991)“独特型：概念和应用(Idiotypes:Concepts And Applications)”，免疫学杂志(J.Immunol.)147(8):2429-2438)。

[0084] 如果希望的话，任何以上抗体的结合特性可通过对展现此类希望的特性的变体进行筛选而进一步改进。例如，可以使用本领域已知的不同噬菌体展示方法来产生此类抗体。在噬菌体展示方法中，将功能抗体结构域展示在噬菌体颗粒的表面上，这些颗粒携带对其进行编码的多核苷酸序列。在一个具体实施例中，此类噬菌体可用于展示从全范围或组合抗体文库(例如，人类或鼠类)表达的抗原结合结构域，例如Fab和Fv或二硫键稳定的Fv。表达结合所感兴趣的抗原的抗原结合域的噬菌体可以用抗原来选择或鉴别，例如，使用标记的抗原或结合或捕获于固体表面或珠粒上的抗原。在这些方法中使用的噬菌体典型地是丝状噬菌体，包括fd以及M13。这些抗原结合域被表达为一种重组地与噬菌体基因III或基因VIII蛋白融合的蛋白。可以用于制造本发明的免疫球蛋白或其片段的噬菌体展示方法的实例包括在以下中披露的那些，布林克曼(Brinkman)，U.等人(1995)“二硫化物稳定化的Fv片段的噬菌体展示(Phage Display Of Disulflde-Stabilized Fv Fragments)”，免疫学方法杂志(J.Immunol.Methods)182:41-50，1995；阿梅斯(Ames)，R.S.等人(1995)“将分离自组合噬菌体展示文库的鼠Fab转化至全长免疫球蛋白(Conversion Of Murine Fabs Isolated From A Combinatorial Phage Display Library To Full Length Immunoglobulins)”，免疫学方法杂志(J.Immunol.Methods)184:177-186；凯特伯拉夫(Kettleborough)，C.A.等人(1994)“使用来自这些抗体片段的全抗体的噬菌体-抗体文库和重构，从免疫小鼠分离肿瘤细胞特异性单链Fv(Isolation Of Tumor Cell-Specific Single-Chain Fv From Immunized Mice Using Phage-Antibody Libraries And The Re-Construction Of Whole Antibodies From These Antibody Fragments)”，欧洲免疫学杂志(Eur.J.Immunol)，24:952-958，1994；佩尔西克(Persic)，L.等人(1997)“在从噬菌体展示文库选择后抗体或其片段的真核表达的整合载体系统(An Integrated Vector System For The Eukaryotic Expression Of Antibodies Or Their Fragments After Selection From Phage Display Libraries)”，基因(Gene)，187:9-18；伯顿(Burton)，D.R.等人(1994)“来自组合文库的人类抗体(Human Antibodies From Combinatorial Libraries)”，免疫学进展(Adv.Immunol.)57:191-280；PCT公开案WO 92/001047；WO 90/02809；WO 91/10737；WO 92/01047；WO 92/18619；WO 93/11236；WO 95/15982；WO 95/

20401;和美国专利号5,698,426;5,223,409;5,403,484;5,580,717;5,427,908;5,750,753;5,821,047;5,571,698;5,427,908;5,516,637;5,780,225;5,658,727;5,733,743和5,969,108。

[0085] 如上文参考文献中所述,在噬菌体选择之后,来自噬菌体的抗体编码区可以被分离并且用于产生包括人源化抗体的全抗体或任何其他所希望的片段,并且表达在任何所希望的宿主之中,包括哺乳动物细胞、昆虫细胞、植物细胞、酵母、以及细菌,例如如下文详细描述的。例如,重组地产生Fab、Fab'以及F(ab')2片段的技术还可以使用本领域中已知的方法来加以采用(这些技术例如是披露于以下中的那些:PCT公开案WO 92/22324;马利纳克斯(Mullinax),R.L.等人(1992)"在一个克隆步骤中,异源二聚体Fab抗体蛋白的表达(Expression Of A Heterodimeric Fab Antibody Protein In One Cloning Step)",生物技术(BioTechniques),12(6):864-869;和沙怀(Sawai)等人(1995)"使用聚合酶链式反应和cDNA表达载体直接产生源自精子固定抗体的Fab片段(Direct Production Of The Fab Fragment Derived From The Sperm Immobilizing Antibody Using Polymerase Chain Reaction And cDNA Expression Vectors)",美国生殖免疫学杂志(Am.J.Reprod.Immunol.)34:26-34;和贝特尔(Better),M.等人(1988)"活性嵌合抗体片段的大肠杆菌分泌(Escherichia coli Secretion Of An Active Chimeric Antibody Fragment)",科学(Science)240:1041-1043)。可以用于产生单链Fv和抗体的技术的实例包括描述于以下中的那些:美国专利号4,946,778和5,258,498;休斯顿(Huston),J.S.等人(1991)"单链Fv类似物和融合蛋白的蛋白工程化(Protein Engineering Of Single-Chain Fv Analogs And Fusion Proteins)",酶学方法(Methods in Enzymology)203:46-88;舒(Shu),L.等人,"来自骨髓瘤细胞的单基因编码免疫球蛋白的分泌(Secretion Of A Single-Gene-Encoded Immunoglobulin From Myeloma Cells)",美国国家科学院院刊(Proc.Natl.Acad.Sci.(USA))90:7995-7999;和斯盖拉(Skerra).A.等人(1988)"大肠杆菌中功能免疫球蛋白Fv片段的组装(Assembly Of A Functional Immunoglobulin Fv Fragment In Escherichia coli)",科学(Science)240:1038-1040。

[0086] 噬菌体展示技术可以用于增加抗体对B7-H5的亲和力。此技术在获得可被用于披露的这些组合方法的高亲和力抗体中是有用的。被称为亲和力成熟的技术采用诱变或者CDR步移和重选择,使用此类受体或配体(或它们的细胞外域)或其抗原片段来鉴别当与初始抗体或亲本抗体比较时以更高亲和力结合到该抗原的抗体(参见,例如,格拉泽(Glaser),S.M.等人(1992)"在丝状噬菌体载体系统中通过基于密码子的诱变进行的抗体工程化(Antibody Engineering By Codon-Based Mutagenesis In A Filamentous Phage Vector System)",免疫学杂志(J.Immunol.)149:3903-3913)。诱变整个密码子而不是单个核苷酸产生了氨基酸突变的半随机谱。可构建由一组变体克隆所组成的文库,每一克隆都在单个CDR中相差单个氨基酸改变,并且这些克隆包含代表了每个CDR残基的每个可能氨基酸取代的变体。可以通过使固定的突变体与标记的抗原接触,来筛选具有针对抗原的增加的结合亲和力的突变体。本领域中已知的任何筛选方法(例如ELISA)可以用于鉴定具有对抗原的增加的亲合力的突变体抗体(参见例如吴(Wu),H.等人(1998)"Vitaxin,一种Alphav β3特异性人源化Mab的逐步体外亲和力成熟(Stepwise In Vitro Affinity Maturation Of Vitaxin,An Alphav Beta3-Specific Humanized Mab)",美国国家科学院院刊

(Proc.Natl.Acad.Sci.(USA))95(11):6037-6042;耶尔顿(Yelton),D.E.等人(1995)“通过基于密码子的诱变的BR96抗癌抗体的亲和力成熟(Affinity Maturation Of The BR96 Anti-Carcinoma Antibody By Codon-Based Mutagenesis)”,免疫学杂志(J.Immunol.)155:1994-2004)。可以可能地使用使轻链随机化的CDR步移(参见希尔(Schier)等人(1996)“通过在抗体结合位点的中心中的互补决定区的分子演化的抗C-Erbb-2单链Fv的皮摩尔亲和力的分离(Isolation Of Picomolar Affinity Anti-C-Erbb-2 Single-Chain Fv By Molecular Evolution Of The Complementarity Determining Regions In The Center Of The Antibody Binding Site)”,分子生物学杂志(J.Mol.Biol.)263:551-567)。

[0087] 因此,本发明考虑了使用随机诱变来鉴定改进的CDR。噬菌体展示技术可以可替代地用于增加(或降低)CDR亲和力。这一技术(称为亲和力成熟),采用了诱变或“CDR步移”和重选择,使用靶抗原或其抗原片段,以鉴别当与初始或亲本抗体比较时,以更高(或更低)亲和力结合到抗原的CDR的抗体(参见例如格拉泽(Glaser),S.M.等人(1992)“在丝状噬菌体载体系统中通过基于密码子的诱变的抗体工程化(Antibody Engineering By Codon-Based Mutagenesis In A Filamentous Phage Vector System)”,免疫学杂志(J.Immunol.)149:3903-3913)。诱变整个密码子而不是单个核苷酸产生了氨基酸突变的半随机谱。可构建由一组变体克隆所组成的文库,每一克隆都在单个CDR中相差单个氨基酸改变,并且这些克隆包含代表了每个CDR残基的每个可能氨基酸取代的变体。可以通过使固定的突变体与标记的抗原接触,来筛选具有针对抗原的增加的(或降低的)结合亲和力的突变体。本领域中已知的任何筛选方法(例如ELISA)可以用于鉴定具有对抗原的增加(或降低的)的亲合力的突变体抗体(参见吴(Wu),H.等人(1998)“Vitaxin,一种Alphavβ3特异性人源化Mab的逐步体外亲和力成熟(Stepwise In Vitro Affinity Maturation Of Vitaxin, An Alphav Beta3-Specific Humanized Mab)”,美国国家科学院院刊(Proc.Natl.Acad.Sci.(USA))95(11):6037-6042;耶尔顿(Yelton,D.E.)等人(1995)“通过基于密码子的诱变的BR96抗癌抗体的亲和力成熟(Affinity Maturation Of The BR96 Anti-Carcinoma Antibody By Codon-Based Mutagenesis)”,免疫学杂志(J.Immunol.)155:1994-2004)。可以可能地使用使轻链随机化的CDR步移(参见希尔(Schier)等人(1996)“通过在抗体结合位点的中心中的互补决定区的分子演化的抗C-Erbb-2单链Fv的皮摩尔亲和力的分离(Isolation Of Picomolar Affinity Anti-C-Erbb-2 Single-Chain Fv By Molecular Evolution Of The Complementarity Determining Regions In The Center Of The Antibody Binding Site)”,分子生物学杂志(J.Mol.Biol.)263:551-567)。

[0088] 用于完成此类亲和力成熟的方法描述于以下中,例如:克劳斯(Krause),J.C.等人(2011)“扭曲抗体结合位点构造的插入突变增强了人类抗体的功能(An Insertion Mutation That Distorts Antibody Binding Site Architecture Enhances Function Of A Human Antibody)”,MBio.2(1)pii:e00345-10.doi:10.1128/mBio.00345-10;宽(Kuan),C.T.等人(2010)“靶向恶性胶质瘤和黑色素瘤的亲和力成熟的抗糖蛋白NMB重组免疫毒素(Affinity-Matured Anti-Glycoprotein NMB Recombinant Immunotoxins Targeting Malignant Gliomas And Melanomas)”,国际癌症杂志(Int.J.Cancer)10.1002/ijc.25645;哈克尔(Hackel),B.J.等人(2010)“稳定性和CDR组成偏移丰富了结合物功能景观(Stability And CDR Composition Biases Enrich Binder Functionality

Landscapes)”,分子生物学杂志(J.Mol.Biol.)401(1):84-96;蒙哥马利(Montgomery),D.L.等人(2009)“针对HIV-1gp41的人类单克隆抗体的亲和力成熟和表征(Affinity Maturation And Characterization Of A Human Monoclonal Antibody Against HIV-1gp41)”,MAbs 1(5):462-474;古斯查那(Gustchina),E.等人(2009)“通过源自合成原初人类抗体文库并且针对Gp41的内部三聚卷曲螺旋的单克隆Fab的CDR-H2环的靶向多样化的亲和力成熟产生了一组具有改进的HIV-1中和效力和幅度的Fab(Affinity Maturation By Targeted Diversification Of The CDR-H2 Loop Of A Monoclonal Fab Derived From A Synthetic Naive Human Antibody Library And Directed Against The Internal Trimeric Coiled-Coil Of Gp41 Yields A Set Of Fabs With Improved HIV-1 Neutralization Potency And Breadth)”,病毒学(Virology)393(1):112-119;芬利(Finlay),W.J.等人(2009)“用于抗RAGE治疗的人源化大鼠抗体的亲和力成熟:全面诱变揭示在互补决定区内外同时存在的高水平的突变可塑性(Affinity Maturation Of A Humanized Rat Antibody For Anti-RAGE Therapy:Comprehensive Mutagenesis Reveals A High Level Of Mutational Plasticity Both Inside And Outside The Complementarity-Determining Regions)”,分子生物学杂志(J.Mol.Biol.)388(3):541-558;博斯特伦(Bostrom),J.等人(2009)“改进抗体结合亲和力和特异性用于治疗开发(Improving Antibody Binding Affinity And Specificity For Therapeutic Development)”,分子生物学方法(Methods Mol.Biol.),525:353-376;施泰德尔(Steidl),S.等人(2008)“通过靶向的CDR多样化的人类GM-CSF抗体的体外亲和力成熟(In Vitro Affinity Maturation Of Human GM-CSF Antibodies By Targeted CDR-Diversification)”,分子免疫学(Mol.Immunol.)46(1):135-144;和班德拉斯(Barderas),R.等人(2008)“通过计算机建模辅助的抗体的亲和力成熟(Affinity maturation of antibodies assisted by in silico modeling)”,美国国家科学院院刊(Proc.Natl.Acad.Sci.(USA))105(26):9029-9034。

[0089] 本发明具体地考虑了任何上述抗体及其抗原结合片段的“衍生物”的产生和用途。

[0090] 术语“衍生物”是指免疫特异性地结合到一种抗原的一种抗体或其抗原结合片段,但其相对于“亲本”(或野生型)分子包括一、二、三、四、五个或更多个氨基酸取代、添加、缺失或修饰。此类氨基酸取代或添加可引入天然存在(即,DNA-编码的)或非天然存在的氨基酸残基。术语“衍生物”包含例如,抗体1.3、4.5或7.8中任一个的嵌合或人源化变体,连同具有改变的CH1、铰链、CH2、CH3或CH4区域的变体,以形成例如具有展现增强或受损的效应子或结合特性的变体Fc区的抗体等。术语“衍生物”另外包含非氨基酸修饰,例如氨基酸可通过已知的保护/阻滞基团、蛋白质水解裂解而被糖基化(例如,具有改变的甘露糖、2-N-乙酰葡糖胺、半乳糖、果糖、葡萄糖、唾液酸、5-N-乙酰神经氨酸、5-二醇神经氨酸等内容物)、乙酰化、聚乙二醇化、磷酸化、酰胺化、衍生化,连接至细胞配体或其他蛋白等。在一些实施例中,改变的碳水化合物修饰调节了以下中的一个或多个:抗体的溶解、抗体的亚细胞转运和分泌的促进、抗体组装的促进、构象完整性、和抗体介导的效应子功能。在一个特定实施例中,相对于缺少该碳水化合物修饰的抗体,该改变的碳水化合物修饰增强了抗体介导的效应子功能。导致改变的抗体介导的效应子功能的碳水化合物修饰是本领域熟知的(例如参见希尔兹(Shields),R.L等人(2002)“人类IgGN连接的寡糖上海藻糖的缺乏改进了至人类

γRIII的结合和抗体依赖的细胞毒性(FcLack Of Fucose On Human IgG N-Linked Oligosaccharide Improves Binding To Human Fcgamma RIII And Antibody-Dependent Cellular Toxicity)",生物化学杂志(J.Biol.Chem.)277(30):26733-26740;戴维斯(Davies)J.等人(2001)"重组抗CD20CHO生产细胞系中的GnTIII的表达:具有改变的糖形的抗体的表达导致通过对FCγRIII的更高亲和力的ADCC中的增加(Expression Of GnTIII In A Recombinant Anti-CD20 CHO Production Cell Line:Expression Of Antibodies With Altered Glycoforms Leads To An Increase In ADCC Through Higher Affinity For FC Gamma RIII)",生物技术&生物工程(Biotechnology&Bioengineering)74(4):288-294)。改变碳水化合物内容物的方法是本领域普通技术人员已知的,参见例如华力克(Wallick),S.C.等人(1988)"针对α(1-6)右旋糖酐的单克隆抗体的VH残基的糖基化增加了其对抗原的亲和力(Glycosylation Of A VH Residue Of A Monoclonal Antibody Against Alpha(1-6)Dextran Increases Its Affinity For Antigen)",实验医学杂志(J.Exp.Med.)168(3):1099-1109;陶(Tao),M.H.等人(1989)"由人类IgG恒定区介导的结构和效应子功能中的糖基化合物的无糖基化的嵌合小鼠-人类IgG作用的研究(Studies Of Aglycosylated Chimeric Mouse-Human IgG.Role Of Carbohydrate In The Structure And Effector Functions Mediated By The Human IgG Constant Region)",免疫学杂志(J.Immunol.)143(8):2595-2601;劳特利奇(Routledge),E.G.等人(1995)"无糖基化对人源化治疗性CD3单克隆抗体的免疫原性的影响(The Effect Of Aglycosylation On The Immunogenicity Of A Humanized Therapeutic CD3 Monoclonal Antibody)",移植(Transplantation)60(8):847-53;艾略特(Elliott),S.等人(2003)"通过糖工程的治疗性蛋白体内活性的增强(Enhancement Of Therapeutic Protein In Vivo Activities Through Glycoengineering)",自然生物技术(Nature Biotechnol.)21:414-21;希尔兹(Shields),R.L等人(2002)"人类IgGN连接的寡糖上海藻糖的缺乏改进了至人类FcγRIII的结合和抗体依赖的细胞毒性(Lack Of Fucose On Human IgG N-Linked Oligosaccharide Improves Binding To Human Fcgamma RIII And Antibody-Dependent Cellular Toxicity)",生物化学杂志(J.Biol.Chem.)277(30):26733-26740)。

[0091] 在一些实施例中,人源化抗体是衍生物。这种人源化抗体包括在一个或多个非人类CDR中的氨基酸残基取代、缺失或添加。当与非衍生物人源化抗体相比时,人源化抗体衍生物可以具有基本上相同的结合、更好的结合或更差的结合。在特定实施例中,该CDR的一、二、三、四、或五个氨基酸残基已被取代、缺失或添加(即,突变)。

[0092] 一种衍生物抗体或抗体片段可使用本领域的普通技术人员已知的技术通过化学修饰进行修饰,这些技术包括但不限于,特异性的化学裂解、乙酰化、配制、衣霉素的代谢合成等。在一个实施例中,抗体衍生物将具有与亲本抗体类似的或相同的功能。在另一个实施例中,抗体衍生物将展现相对于亲本抗体改变的活性。例如,相比于亲本抗体,衍生物抗体(或其片段)可更紧密地结合到其表位或对蛋白水解更加耐受。

[0093] 衍生化抗体可用于改变哺乳动物(优选人类)中的亲本抗体的半衰期(例如,血清半衰期)。优选地,此类改变将导致超过15天、优选超过20天、超过25天、超过30天、超过35天、超过40天、超过45天、超过2个月、超过3个月、超过4个月、或超过5个月的半衰期。本发明的人源化抗体或其片段在哺乳动物(优选人类)中的增加的半衰期导致所述抗体或抗体片

段在该哺乳动物中的更高血清滴度，并且因此降低了给予所述抗体或抗体片段的频率，和/或降低了待给予的所述抗体或抗体片段的浓度。可以通过本领域普通技术人员已知的技术来产生具有增加的体内半衰期的抗体或其片段。例如，具有增加的体内半衰期的抗体或其片段可以通过修饰(例如，取代、缺失或添加)多个鉴别为涉及到Fc域与FcRn受体之间的相互作用中的氨基酸残基来产生。人源化B7-H5抗体可被工程化以增加生物半衰期(参见，例如美国专利号6,277,375)。例如，人源化B7-H5抗体可在Fc-铰链域中被工程化以具有增加的体内或血清半衰期。

[0094] 具有增加的体内半衰期的抗体或其片段可以通过将多聚体分子诸如高分子量聚乙二醇(PEG)附接至所述抗体或抗体片段来产生。PEG可以在存在或不存在多官能连接子的情况下，通过PEG与所述抗体或抗体片段的N末端或C末端的位点特异性轭合或经由赖氨酸残基上存在的ε-氨基基团附接到所述抗体或抗体片段。将使用导致生物活性最少损失的线性或分支的聚合物衍生化。可通过SDS-PAGE和质谱密切监测轭合的程度，以确保PEG分子与抗体的正确轭合。可以通过例如尺寸排除或离子交换色谱来从抗体PEG轭合物中分离未反应的PEG。

[0095] 还可以通过由戴维斯(Davis)等人(参见美国专利号4,179,337)描述的方法和偶联剂来修饰B7-H5抗体，以便提供可以被注射进哺乳动物循环系统而基本上不具有免疫原性应答的组合物。

[0096] 一个实施例涵盖人源化B7-H5抗体的框架残基的修饰。框架区中的框架残基可以用来自CDR供体抗体的相应残基来取代以便改变，优选地改进抗原结合。这些框架取代是通过本领域熟知的方法鉴定的，例如通过对CDR和框架残基的相互作用建模以鉴定对抗原结合重要的框架残基，并且通过序列比较以鉴定在特定位置上的不寻常框架残基。(参见美国专利号5,585,089；和赖克曼(Riechmann)L.等人(1988)“将用于治疗的人类抗体再成形(Reshaping Human Antibodies For Therapy)”，自然(Nature)332:323-327)。

[0097] 又另一实施例涵盖重组地融合至或化学轭合(包括共价轭合和非共价轭合二者)至异源分子(即无关分子)的抗人类B7-H5抗体(并且更优选人源化抗体)及其抗原结合片段。融合并非必须是直接的，而可以通过接头序列来发生。

[0098] 在一个实施例中，此类异源分子是具有至少10个、至少20个、至少30个、至少40个、至少50个、至少60个、至少70个、至少80个、至少90个或至少100个氨基酸的多肽。此类异源分子可以可替代地是酶、激素、细胞表面受体、药物部分，诸如：毒素(例如相思豆毒素、蓖麻毒素A、假单胞菌外毒素(即PE-40)、白喉毒素、蓖麻毒素、白树毒素、或美洲商陆抗病毒蛋白)，蛋白质(例如肿瘤坏死因子、干扰素(例如α-干扰素、β-干扰素)、神经生长因子、血小板衍生的生长因子、组织纤溶酶原激活剂或凋亡剂(例如肿瘤坏死因子-α、肿瘤坏死因子-β))，生物反应调节剂(例如像淋巴因子(例如白介素-1(“IL-1”)、白介素-2(“IL-2”)、白介素-6(“IL-6”))、粒细胞巨噬细胞集落刺激因子(“GM-CSF”)、粒细胞集落刺激因子(“G-CSF”)、或巨噬细胞集落刺激因子(“M-CSF”))、或生长因子(例如生长激素(“GH”)))，细胞毒素(例如细胞抑制剂或杀细胞剂，如紫杉醇、细胞松弛素B、短杆菌肽D、溴化乙锭、吐根碱、丝裂霉素、依托泊苷、替尼泊苷(tenoposide)、长春新碱、长春碱、秋水仙碱、阿霉素、道诺霉素、二羟基炭疽菌素二酮、米托蒽醌、光辉霉素、放线菌素D、1-去氢睾酮、糖皮质激素、普鲁卡因、丁卡因、利多卡因、普萘洛尔、和嘌呤霉素及其类似物或同系物)，抗代谢物(例如甲氨

蝶呤、6-巯基嘌呤、6-硫鸟嘌呤、阿糖胞苷、5-氟尿嘧啶达卡巴嗪(5-fluorouracil decarbazine)),烷基化剂(例如氮芥、苯丁酸氮芥、美法仑、BiCNU®(卡莫司汀;BSNU)和洛莫司汀(CCNU)、环磷酰胺(cyclothosphamide)、白消安、二溴甘露醇、链脲霉素、丝裂霉素C、和顺二氯二氨铂(II)(DDP)顺铂),蒽环类(例如道诺霉素(原名正定霉素)和阿霉素),抗体(例如更生霉素(原名放线菌素)、博来霉素、光辉霉素、和安曲霉素(AMC)),或抗有丝分裂剂(例如长春新碱和长春碱)。

[0099] 用于将此类治疗部分轭合至抗体的技术是熟知的;参见例如阿尔农(Arnon)等人,"用于癌症治疗中的药物的免疫靶向的单克隆抗体(Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy)"单克隆抗体和癌症治疗(MONOCLONAL ANTIBODIES AND CANCER THERAPY),赖斯费尔德(Reisfeld)等人(编辑),1985,243-56页,艾伦R.利斯有限公司(Alan R.Liss,Inc.);赫尔斯特伦(Hellstrom)等人,"用于药物递送的抗体(Antibodies For Drug Delivery)",受控药物递送(CONTROLLED DRUG DELIVERY),(第2版),鲁滨逊(Robinson)等人(编辑),1987,623-53页,马塞尔德克有限公司(Marcel Dekker,Inc.);索普(Thorpe),"癌症治疗中细胞毒性剂的抗体载体:综述(Antibody Carriers Of Cytotoxic Agents In Cancer Therapy:A Review)",单克隆抗体'84:生物学的和临床的应用(MONOCLONAL ANTIBODIES'84:BIOLOGICAL AND CLINICAL APPLICATIONS),平查(Pinchera)等人(编辑),1985,475-506页);"癌症治疗中放射标记抗体的治疗用途的分析、结果和未来展望(Analysis,Results,And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy)",用于癌症检测和治疗的单克隆抗体(MONOCLONAL ANTIBODIES FOR CANCER DETECTION AND THERAPY),鲍德温(Baldwin)等人(编辑),1985,第303-16页,学术出版社(Academic Press);和索普(Thorpe)等人(1982)"抗体毒素轭合物的制备和细胞毒性特性(The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates)",免疫学综述(Immunol.Rev.)62:119-158。

[0100] 在一个实施例中,B7-H5抗体或B7-H5融合分子包括Fc部分。此类分子的Fc部分可按照同种型或子类而不同,可以是嵌合体或杂合体,和/或可经过修饰以便例如改进效应子功能、控制半衰期、组织可接近性、增进生物物理特性例如稳定性,并且改进产生效率(并且成本更小)。在披露的融合蛋白的构造和用于制造它们的方法中有用的很多修饰是本领域已知的,参见例如米勒(Mueller),J.P.等人(1997)"具有嵌合IgG2/G4恒定区的人源化猪VCAM特异性单克隆抗体阻滞人类白细胞结合猪内皮细胞(Humanized Porcine VCAM-Speciflc Monoclonal Antibodies With Chimeric IgG2/G4Constant Regions Block Human Leukocyte Binding To Porcine Endothelial Cells)",分子免疫学(Mol.Immun.)34(6):441-452,斯旺(Swann),P.G.(2008)"对治疗的单克隆抗体的开发的考虑(Considerations For The Development Of Therapeutic Monoclonal Antibodies)",免疫学新见(Curr.Opin.Immun.)20:493-499(2008),和普雷斯塔(Presta),L.G.(2008)"治疗性抗体的分子工程化和设计(Molecular Engineering And Design Of Therapeutic Antibodies)",免疫学新见(Curr.Opin.Immun.)20:460-470。在一些实施例中,Fc区是天然IgG1、IgG2、或IgG4Fc区。在一些实施例中,Fc区是杂合体,例如由IgG2/IgG4Fc恒定区组成的嵌合体。对Fc区的修饰包括但不限于修饰IgG4以阻止结合Fcγ受体和补体,修饰IgG1以

改进与一种或多种Fcγ受体的结合，修饰IgG1以最小化效应子功能(氨基酸改变)，IgG1具有改变的聚糖/不具有聚糖(典型地通过改变表达宿主)，以及IgG1具有与FcRn的改变的pH依赖性结合，以及IgG4在绞链区的氨基酸残基#228处的丝氨酸改变为脯氨酸(S228P)以增强稳定性。Fc区可以包括整个绞链区，或不足整个铰链区。

[0101] 用利妥昔单抗(针对CD20的嵌合小鼠/人类IgG1单克隆抗体)治疗患者的非霍奇金氏淋巴瘤或瓦尔登斯特伦氏巨球蛋白血症的治疗结果与Fcγ受体的等位变体的个体表达相关，这些Fcγ受体对人类IgG1的Fc结构域具有不同的内在亲和力。具体而言，具有低亲和力激活型Fc受体CD16A(FcγRIIIA)的高亲和力等位基因的患者显示更高的反应率，并且在非霍奇金氏淋巴瘤的情况下显示改进的无进展生存期。在另一个实施例中，Fc域可含有一个或多个氨基酸插入、缺失或取代，这些插入、缺失或取代减少与低亲和力抑制性Fc受体CD32B(FcγRIIB)的结合并且保持野生型水平的与低亲和力活化Fc受体CD16A(FcγRIIIA)的结合或增强与之的结合。

[0102] 另一实施例包括IgG_{2-4}杂合体和IgG4突变体，它们具有降低的与FcR的结合，这增加了它们的半衰期。代表性IgG_{2-4}杂合体和IgG4突变体描述于安盖尔(Angal)，S.等人(1993)“单个氨基酸取代消除了嵌合的小鼠/人(Igg4)抗体的异质性(A Single Amino Acid Substitution Abolishes The Heterogeneity Of Chimeric Mouse/Human(Igg4) Antibody)”，分子免疫学(Molec.Immunol.)30(1):105-108；穆勒(Mueller)，J.P.等人(1997)“具有嵌合的Igg2/G4恒定区的人源化猪VCAM特异性单克隆抗体阻滞人白细胞结合到猪内皮细胞(Humanized Porcine VCAM-Specific Monoclonal Antibodies With Chimeric IgG2/G4Constant Regions Block Human Leukocyte Binding To Porcine Endothelial Cells)”，分子免疫学(Mol.Immun.)34(6):441-452；以及美国专利号6,982,323。在一些实施例中，该IgG_1和/或IgG_2结构域是缺失的，例如，安盖尔(Angal)，s.等人描述了丝氨酸241替换为脯氨酸的IgG_1和IgG_2。

[0103] 在衍生化的抗体中的取代、添加或缺失可在该抗体的Fc区并且可由此起作用以修饰该抗体至一个或多个Fc R的结合亲和力。用于修饰抗体(具有修饰的至一个或多个Fc R的结合)的方法在本领域中是已知的，参见例如PCT公开号WO 04/029207、WO 04/029092、WO 04/028564、WO 99/58572、WO 99/51642、WO 98/23289、WO 89/07142、WO 88/07089、以及美国专利号5,843,597以及5,642,821。在一个具体实施例中，对Fc区的修饰得到如下的一种抗体，该抗体具有改变的抗体介导的效应子功能、改变的与其他Fc受体(例如，Fc激活受体)的结合、改变的抗体依赖性细胞介导的细胞毒性(ADCC)活性、改变的C1q结合活性、改变的补体依赖性细胞毒性活性(CDC)、吞噬细胞活性、或其任何组合。

[0104] 在一些实施例中，本发明涵盖Fc区将已经被修饰的抗体，这样使得该分子将展示改变的Fc受体(FcR)结合活性，例如，展现降低的朝向活化受体(例如FcγRIIA或FcγRIIIA)的活性，或增加的朝向抑制性受体(例如FcγRIIB)的活性。优选地，此类抗体将展现降低的抗体依赖性细胞介导的细胞毒性(ADCC)或补体依赖性细胞毒性(CDC)活性(相对于野生型Fc受体)。

[0105] 影响Fc介导的效应子功能的修饰是本领域熟知的(参见美国专利号6,194,551和WO 00/42072；斯塔文哈根(Stavenhagen)，J.B.等人(2007)“治疗的抗体的Fc优化增强了它们经由低亲和力活化Fcγ受体在体外杀死肿瘤细胞并且在体内控制肿瘤扩张的能力(Fc

Optimization Of Therapeutic Antibodies Enhances Their Ability To Kill Tumor Cells In Vitro And Controls Tumor Expansion In Vivo Via Low-Affinity Activating Fcgamma Receptors)",癌症研究(Cancer Res.)57(18):8882-8890;希尔兹(Shields),R.L等人(2001)"人类IgG1上针对FcγRI、FcγRII、FcγRIII、和FcRn的结合位点的高分辨作图和具有至FcγR的改进的结合的IgG1变体的设计(High Resolution Mapping of the Binding Site on Human IgG1 for FcγRI,FcγRII,FcγRIII,and FcRn and Design of IgG1Variants with Improved Binding to the FcγR)",生物化学杂志(J.Biol.Chem.)276(9):6591-6604)。具有至FcγRIIA或FcγRIIIA的降低结合但是具有至FcγRIIB的不变的或增强的结合的人类IgG1Fc结构域的示例性变体包括S239A、H268A、S267G、E269A、E293A、E293D、Y296F、R301A、V303A、A327G、K322A、E333A、K334A、K338A、A339A、D376A。

[0106] 在一些实施例中,本发明涵盖Fc区将已经被缺失的抗体(例如Fab或$F(ab)_2$等)。

[0107] 本发明的任何分子可以融合至标记序列,例如肽,以协助纯化。在优选的实施例中,标记氨基酸序列是一种六-组氨酸肽,即血球凝集素"HA"标签,其对应于源自于流感血球凝集素蛋白的表位(威尔逊(Wilson),I.A.等人(1984)"抗原决定簇在蛋白中的结构(The Structure Of An Antigenic Determinant In A Protein)",细胞,37:767-778)并且"flag"标签(克纳皮克(Knappik),A.等人(1994)"基于FLAG肽的改进的亲和力标签用于重组抗体片段的检测以及纯化(An Improved Affinity Tag Based On The FLAG Peptide For The Detection And Purification Of Recombinant Antibody Fragments)",生物技术17(4):754-761)。

[0108] 本发明还涵盖轭合至一种诊断或治疗剂的抗体或其抗原结合片段或任何其他希望血清半衰期增加的分子。这些抗体可诊断地使用(体内、原位或体外)以例如作为一个临床测试程序的部分监测一种疾病、失调或感染的发展或进展,例如以确定给定治疗方案的效力。通过使抗体偶联到可检测物质上可以促进检测。可检测物质的实例包括各种酶、辅基、荧光物质、发光物质、生物发光物质、放射性材料、正电子发射金属以及非放射性顺磁金属离子。该可检测物质可被直接偶联或轭合至该抗体,或使用本领域中已知的技术间接通过中间体(诸如,例如本领域中已知的接头)进行。参见例如美国专利号4,741,900关于可以轭合至根据本发明的抗体用作诊断剂的金属离子。可通过将抗体与可检测物质偶联完成这种诊断和检测,所述可检测物质包括但不限于各种酶,酶包括但不限于辣根过氧化物酶、碱性磷酸酶、β-半乳糖苷酶、或乙酰胆碱酯酶;辅基复合体,诸如但不限于抗生蛋白链菌素/生物素以及抗生物素蛋白/生物素;荧光材料,诸如但不限于伞形酮、荧光素、异硫氰酸荧光素、若丹明、二氯三唤胺荧光素、丹磺酰氯或藻红蛋白;发光材料,诸如但不限于发光胺;生物发光材料,诸如但不限于荧光素酶、荧光素、以及水母发光蛋白;放射性材料,诸如但不限于铋(^{213}Bi),碳(^{14}C),铬(^{51}Cr),钴(^{57}Co),氟(^{18}F),钆(^{153}Gd,^{159}Gd),镓(^{68}Ga,^{67}Ga),锗(^{68}Ge),钬(^{166}Ho),铟(^{115}In,^{113}In,^{112}In,^{111}In),碘(^{131}I,^{125}I,^{123}I,^{121}I),镧(^{140}La),镥(^{177}Lu),锰(^{54}Mn),钼(^{99}Mo),钯(^{103}Pd),磷(^{32}P),镨(^{142}Pr),钷(^{149}Pm),铼(^{186}Re,^{188}Re),铑(^{105}Rh),钌(^{97}Ru),钐(^{153}Sm),钪(^{47}Sc),硒(^{75}Se),锶(^{85}Sr),硫(^{35}S),锝(^{99}Tc),铊(^{201}Ti),锡(^{113}Sn,^{117}Sn),氚(^{3}H),氙(^{133}Xe),镱(^{169}Yb,^{175}Yb),钇(^{90}Y),锌(^{65}Zn);使用不同正电子发射断层扫描以及非放射性顺磁金属离子的正电子发射金属。

[0109] 本发明的这些分子可以轭合至第二抗体，以形成抗体杂轭合物，如由西格尔(Segal)在美国专利号4,676,980中所述。此类杂轭合物抗体可以另外结合到半抗原(例如荧光素、等)，或结合到细胞标记物(例如PD-1、4-1-BB、B7-H4、B7-H5、CD4、CD8、CD14、CD25、CD27、CD40、CD68、CD163、CTLA4、GITR、LAG-3、OX40、TIM3、TIM4、TLR2、LIGHT、等)，或结合到细胞因子(例如IL-7、IL-15、IL-12、IL-4TGF-β、IL-10、IL-17、IFNg、Flt3、BLys)或趋化因子(例如CCL21)，等。

[0110] 本发明的这些分子可被附接至固相支撑体，其对于靶标抗原或能够结合到靶标抗原的其他分子的免疫测定或纯化是特别有用的，该靶标抗原已通过结合到本发明的一种抗体或抗原结合片段而固定到支撑体上。此类固体支撑体包括但不限于玻璃、纤维素、聚丙烯酰胺、尼龙、聚苯乙烯、聚氯乙烯或聚丙烯。

[0111] 本发明另外包括编码任何此类抗体、融合蛋白或片段的核酸分子(DNA或RNA)，连同能够在细胞系中传递或复制此类核酸分子并且表达此类抗体、融合蛋白或片段的载体分子(例如质粒)。这些核酸可以是单链的、双链的，可包含单链以及双链部分两者。

D.本发明的优选调节剂组合物

[0112] 如此处使用的术语“调节”涉及改变效果或结果的能力。具体地，本发明涉及多肽(这些多肽包括免疫特异性地结合人类B7-H5的抗人类B7-H5抗体或任何其抗原结合片段)或生理特异性地结合B7-H5的分子(这些分子能够调节B7-H5及其同源配体之间的结合和/或能够调节由于B7-H5同源反受体结合而发生的信号传导)。另外，多特异性抗B7-H5抗体、抗B7-H5抗原结合片段及其代表性融合产物(它们具有增加的结合CD28H或其他细胞配体或受体的能力)在协助将表达此类配体或受体的细胞共定位至表达CD28H的细胞中具有特别的效用。

[0113] 本发明涉及抗体、或其片段、或包括此类抗体或片段的融合分子，它们免疫特异性地结合至B7-H5并且能够在体外、或在接受者受试者或患者中基本上阻滞B7-H5与CD28H的相互作用。如在此使用，如通过在此披露的任何测定测量的，“能够基本上阻滞B7-H5与CD28H的相互作用”的分子是指提供此类分子将B7-H5-CD28H相互作用衰减了多于50％、更优选多于60％、多于70％、多于80％、多于90％、多于95％、多于99％，或最优选完全衰减此类相互作用。此类抗体、片段以及融合分子在衰减B7-H5-CD28H相互作用的生物效应上具有特别的效用。

[0114] 本发明因此具体地涉及此类实施例的分子，这些实施例涉及人源化抗体和片段或人类抗体和片段。

[0115] 最优选地，当以内源浓度表达并且排列于受试者的细胞的表面时，此类分子将具有能够结合到B7-H5的足够亲和力以及亲合力。术语“内源浓度”是指如下的水平，在此水平一种分子在正常的、癌症或病原体感染的细胞中是天然表达的(即，在不存在表达载体或重组启动子的情况下)。

(1)优选的抗人类B7-H5抗体及其CDR

[0116] 根据本发明，此类分子可以通过以下方式产生：筛选杂交瘤系中产生免疫特异性针对人类B7-H5的抗体的那些，并且然后在此类系中任选地筛选展现调节活性(例如中和活性、激动活性、改变的信号传导活性、等)的那些。本发明具体地提供了仓鼠抗人类B7-H5克隆：2D3和18C3。抗体2D3和18C3能够结合至人类B7-H5且基本上能够阻滞B7-H5与CD28H的相

互作用。在治疗炎症疾病和自身免疫性疾病中，基本上能够阻滞B7-H5与CD28H的相互作用的抗体具有特别用途。在诊断学中，基本上不能阻滞B7-H5与CD28H的相互作用的抗体具有特别用途，因为此类抗体可以用于检测B7-H5:CD28H相互作用的存在、位置和流行率。

[0117] 对由能够阻滞B7-H5与CD28H的相互作用的抗人类B7-H5克隆表达的抗体进行测序，以揭示它们的可变结构域。这些可变域的CDR序列以加粗和加下划线示出：

抗人类B7-H5克隆2D3

重链可变区(SEQ ID NO:9)：

QVQLQQSGAE LVKPGASVKL SCKAS**GYTFT** **SHD**INWVRQR PELGLEWIGW
IFPGDGSTKF NEKFKGKATL TTDKSSSTAY IQLSRLTSED SAVYFC**ARNS**
FYSMDYWGQG TSVTVSS

编码重链可变区的多核苷酸(SEQ ID NO:10)：

caggttcaac tgcaacagtc tggagctgaa ctggtaaagc ctggggcttc
agtgaagttg tcctgcaagg cttctggcta caccttcaca agccatgata
taaactgggt gaggcagagg cctgaactgg gacttgagtg gattggatgg
atttttcctg gggatggtag tactaagttc aatgagaagt tcaagggcaa
ggccacactg actacagaca aatcctccag cacagcctac atacagctca
gcaggctgac gtctgaggac tctgctgtct atttctgtgc aagaaactcc
ttctactcta tggactattg gggtcaagga acctcagtca ccgtctcctc
a

轻链可变区(SEQ ID NO:11)：

DIVMSQSPSS LAVSAGEKVT MSCKSS**QSLL** **NSRTRKNQ**LA WYQQKPGQSP
KLLIY**WAF**IR ESGVPDRFTG SGSGTDFTLT ISSVQAEDLA VYYC**KQSYNL**
RTFGGGTKLE IK

编码轻链可变区的多核苷酸(SEQ ID NO:12)：

gacattgtga tgtcacagtc tccatcctcc ctggctgtgt cagcaggaga
gaaggtcact atgagctgca aatccagtca gagtctgctc aacagtagaa
cccgaaagaa ccagttggct tggtaccagc agaaaccagg acagtctcct
aaattactga tctactgggc attcattagg gaatctgggg tccctgatcg
cttcacaggc agtggatctg ggacagattt cactctcacc atcagcagtg
tgcaggctga agacctggca gtttattact gcaagcaatc ttataatctt
cggacgttcg gtggaggcac caagctggaa atcaaac

抗人类B7-H5克隆18C3

重链可变区(SEQ ID NO:13)：

QVQLQQSGAE LVKPGASVKL SCKAS**GYTFT** **SHD**INWVRQR PEQGLEWIGW
IFPGDGSTKF NEKFKGKATL TTDKSSSTAY IQLSRLTSED SAVYFC**ARNS**
FYSMDYWGQG TSVTVSS

编码重链可变区的多核苷酸(SEQ ID NO:14)：

caggttcaac tgcagcagtc tggagctgaa ctggtaaagc ctggggcttc
agtgaagttg tcctgcaagg cttctggcta caccttcaca agccatgata
taaactgggt gaggcagagg cctgaacagg gacttgagtg gattggatgg
atttttcctg gggatggtag tactaagttc aatgagaagt tcaagggcaa
ggccacactg actacagaca aatcctccag cacagcctac atacagctca
gcaggctgac atctgaggac tctgctgtct atttctgtgc aagaaactcc
ttctattcta tggactactg gggtcaagga acctcagtca ccgtctcctc
a

轻链可变区(SEQ ID NO:15)：

DIVMSQSPSS LAVSAGEKVT MSCKSSQSLL NSRTRKNQLA WYQQKPGQSP
KLLIYWASTR ESGVPDRFTG SGSGTDFTLT ISSVQAEDLA VYYCKQSYNL
RTFGGGTKLE IK

编码轻链可变区的多核苷酸(SEQ ID NO:28):

gacattgtga tgtcacagtc tccatcctcc ctggctgtgt cagcaggaga
gaaggtcact atgagctgca aatccagtca gagtctgctc aacagtagaa
cccgaaagaa ccagttggct tggtaccagc agaaaccagg gcagtctcct
aaactgctga tctactgggc atccactagg gaatctgggg tccctgatcg
cttcacaggc agtggatctg ggacagattt cactctcacc atcagcagtg
tgcaggctga agacctggca gtttattact gcaagcaatc ttataatctt
cggacgttcg gtggaggcac caagctggaa atcaaac

(2)抗人类B7-H3的共有CDR

阻滞B7-H5:CD28H相互作用的本发明的抗体

[0118] 对所鉴定的抗体的CDR进行分析,以鉴定共有CDR序列以及可能的会提供类似结合属性的变体CDR序列。使用Blosum62.iij分析根据表1计算此类变体CDR。表1呈现出Blosum62.iij取代得分。得分越高取代越保守,并且因此更加可能地,该取代将不会影响功能。

表1

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	+4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	+1	0	-3	-2	0
R	-1	-5	0	-2	-3	+1	0	-2	0	-3	-2	+2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	+6	+1	-3	0	0	0	+1	-3	-3	0	-2	-3	-2	+1	0	-4	-2	-3
D	-2	-2	+1	+6	-3	0	+2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	+9	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	+1	0	0	-3	+5	+2	-2	0	-3	-2	+1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	+2	-4	+2	+5	-2	0	-3	-3	+1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	+6	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	+1	-1	-3	0	0	-2	+8	-3	-3	-1	-2	-1	-2	-1	-2	-2	+2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	+4	+2	-3	+1	0	-3	-2	-1	-3	-1	+3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	+2	+4	-2	+2	0	-3	-2	-1	-2	-1	+1
K	-1	+2	0	-1	-3	+1	+1	-2	-1	-3	-2	+5	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	+1	+2	-1	+5	0	-2	-1	-1	-1	-1	+1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	+6	-4	-2	-2	+1	+3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	+7	-1	-1	-4	-3	-2
S	+1	-1	+1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	+4	+1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	+1	+5	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	+1	-4	-3	-2	+11	+2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	+2	-1	-1	-2	-1	+3	-3	-2	-2	+2	+7	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	+3	+1	-2	+1	-1	-2	-2	0	-3	-1	+4

[0119] 本发明允许具有1、2、3、4、5或6个变体CDR的新颖抗体和抗原结合片段的形成。因为本发明的方法已经鉴定了相当数量的不同CDR,所以本发明允许在具体鉴定的CDR中任何变体中可能要求的CDR残基的识别。在表2和表3的黑体字中示出了此类残基。对于那些被发现在比较的CDR中变化的残基,表1的取代得分提供了用于确定被允许的取代的身份的手段。例如,如果具体CDR的具体残基被发现变化为R或S,那么由于R和S具有-1的取代得分,R或S的具有-1或更大的取代得分的任何取代很可能作为观察变体(R或S)(或比R或S更多可

能性)以建立具有与该具体的CDR的结合属性充分地类似的结合属性的变体CDR,以允许该变体CDR的替代使用,以形成一种功能性抗B7-H5抗体或抗原结合片段。对于每个位置,具有更高取代得分的残基的选择的优选性超过了具有更低取代得分的残基的选择。

[0120] 表2呈现了抗B7-H5抗体的重链CDR的分析并且提供了本发明的观察的和优选的变体轻链("LC")抗B7-H5CDR的共有序列。

表 2：抗 B7-H5 重链 CDR

重链 CDR1										
抗体	**序列**									**SEQ ID NO**
2D3	G	Y	T	F	T	S	H	D		**16**
18C3	G	Y	T	F	T	S	H	D		**16**
HC CDR1 共有序列：	**G**	**Y**	**T**	**F**	**T**	**S**	**H**	**D**		**16**
重链 CDR2										
抗体	**序列**									**SEQ ID NO**
2D3	I	F	P	G	D	G	S	T		**17**
18C3	I	F	P	G	D	G	S	T		**17**
HC CDR2 共有序列：	**I**	**F**	**P**	**G**	**D**	**G**	**S**	**T**		**17**
重链 CDR3										
抗体	**序列**									**SEQ ID NO**
2D3	A	R	N	S	F	Y	S	M	D	Y	**18**
18C3	A	R	N	S	F	Y	S	M	D	Y	**18**
HC CDR3 共有序列：	**A**	**R**	**N**	**S**	**F**	**Y**	**S**	**M**	**D**	**Y**	**18**

[0121] 表3呈现了抗B7-H5抗体的轻链CDR的分析并且提供了本发明的观察的和优选的变体抗B7-H5重链("HC")CDR的共有序列。

表 3：抗 B7-H5 轻链 CDR

轻链 CDR1													
抗体	**序列**												**SEQ ID NO**
2D3	Q	S	L	L	N	S	R	T	R	K	N	Q	**19**
18C3	Q	S	L	L	N	S	R	T	R	K	N	Q	**19**
HC CDR1 共有序列	**Q**	**S**	**L**	**L**	**N**	**S**	**R**	**T**	**R**	**K**	**N**	**Q**	**19**
轻链 CDR2													
抗体	**序列**												**SEQ ID NO**
2D3	W	A	F										**20**
18C3	W	A	S										**21**
LC CDR2 共有序列	**W**	**A**	**X_1**										**22**
X_1 是 F 或 S 或具有相等的或更大的取代得分（即，≥-2）的取代：A、C、H、I、L、M、F、S、T、W、Y、或 V													
轻链 CDR3													
抗体	**序列**												**SEQ ID NO**
2D3	K	Q	S	Y	N	L	R	T					**23**
18C3	K	Q	S	Y	N	L	R	T					**23**
LC CDR3 共有序列	**K**	**Q**	**S**	**Y**	**N**	**L**	**R**	**T**					**23**

[0122] 因此，除了具有抗B7-H5抗体2D3和13C3的CDR的抗体及其抗原结合片段，本发明另外提供了拥有具有上述轻链和/或重链共有序列的CDR的抗体及其抗原结合片段。

[0123] 本发明涵盖包括与通过任何以上克隆产生的仓鼠单克隆抗体的可变重链和/或轻链的氨基酸序列至少45％、至少50％、至少55％、至少60％、至少65％、至少70％、至少75％、至少80％、至少85％、至少90％、至少95％、或至少99％一致的可变重链和/或可变轻链的氨基酸序列的、并且展现免疫特异性地结合至B7-H5的抗体或其片段。本发明进一步涵盖包括与以上列出的克隆的CDR的氨基酸序列至少45％、至少50％、至少55％、至少60％、至少65％、至少70％、至少75％、至少80％、至少85％、至少90％、至少95％、或至少99％一致的CDR的、并且展现免疫特异性地结合至B7-H5的抗体或其片段。

两种氨基酸序列的百分比一致性的确定可以由BLAST蛋白比较来确定。

[0124] 在一个优选实施例中，抗体是包括一个、两个或三个轻链CDR和一个、两个或三个重链CDR(最优选是三个轻链CDR和三个重链CDR)的免疫球蛋白分子(例如抗体、双抗体、融合蛋白、等)，其中轻链CDR包括：

(1)抗人类B7-H5抗体2D3/18C3的轻链CDR1；

(2)抗人类B7-H5抗体2D3或抗人类B7-H5抗体18C3的轻链CDR2或此类抗体的CDR2的共有序列；以及

(3)抗人类B7-H5抗体2D3/18C3的轻链CDR3；

[0125] 在一个替代优选实施例中，免疫球蛋白分子包括一个、两个或三个轻链CDR和一个、两个或三个重链CDR(最优选是三个轻链CDR和三个重链CDR)，其中重链CDR包括：

(1)抗人类B7-H5抗体2D3/18C3的重链CDR1；

(2)抗人类B7-H5抗体2D3/18C3的重链CDR2；以及

(2)抗人类B7-H5抗体2D3/18C3的重链CDR3；

[0126] 在一个具体实施例中，本发明的抗体或其抗原结合片段将包括上述优选抗体中的一个、两个、三个、四个、五个、或更优选全部6个CDR，并且将展现结合至人类B7-H5的能力。

(3)CD28H融合蛋白

[0127] 已经发现，CD28融合蛋白可以是B7-H5和CD28H之间信号传导的拮抗剂。因此，还披露了CD28H融合蛋白及使用它以下调免疫系统的方法。因此，受体融合多肽典型地阻滞配体结合跨膜CD28H的能力，并且诱导或维持B7-H5:CD28H介导的信号传导。

[0128] 在此披露的CD28H融合多肽具有第一融合伴侣，该第一融合伴侣包括(i)直接融合至第二多肽的，或(ii)任选地融合至接头肽序列(该接头肽序列融合至第二多肽)的CD28H多肽的全部或一部分。此类融合蛋白可以形成二聚体或多聚体。肽/多肽接头结构域可以是分开的结构域，或可替代地，可以被包含在融合蛋白的其他结构域(CD28H多肽或第二多肽)之一内。类似地，起作用以便使融合蛋白二聚化或多聚化的这个结构域可为单独的结构域，或可替代地可包含于融合蛋白的一个其他结构域(CD28H多肽、第二多肽或肽/多肽接头结构域)中。在一个实施例中，二聚化/多聚化结构域和肽/多肽接头结构域是相同的。

[0129] 在此披露的融合蛋白具有式I:N-R_1-R_2-R_3-C，其中“N”表示融合蛋白的N末端，“C”表示融合蛋白的C末端。在优选实施例中，“R_1”是CD28H多肽，“R_2”是任选的肽/多肽接头结构域，并且“R_3”是第二多肽。可替代地，R_3可以是CD28H多肽并且R_1可以是第二多肽。

[0130] a.第一融合伴侣

[0131] 受体融合蛋白可以包括全长CD28H多肽，或可以包含全长CD28H的片段或变体。在优选实施例中，第一融合伴侣是CD28H的可溶片段，例如CD28H的胞外结构域的一部分或全部，或其变体。可以使用任何哺乳动物序列CD28H。作为实例，在以上序列中提供了人类序列，连同其已知亚型或变体。在一些实施例中，其他哺乳动物序列，例如小鼠序列，是本领域中已知的并且可以使用。在披露的融合蛋白中有用的人类CD28H多肽可以具有与SEQ ID NO:7具有至少60％、65％、70％、75％、80％、85％、90％、95％、99％、或100％序列一致性的氨基酸序列，或优选具有与SEQ ID NO:7的胞外结构域具有60％、65％、70％、75％、80％、85％、90％、95％、99％、或100％序列一致性的氨基酸序列。在披露的融合蛋白中有用的人类CD28H多肽可以由与SEQ ID NO:8具有至少60％、65％、70％、75％、80％、85％、90％、95％、99％、或100％序列一致性的核酸序列编码，或优选由与SEQ ID NO:8的核酸序列编码的胞外结构域具有60％、65％、70％、75％、80％、85％、90％、95％、99％、或100％序列一致性的核酸序列编码。

[0132] b.第二融合伴侣

[0133] CD28H多肽可以融合至第二多肽。第二多肽的存在可以改变融合多肽的溶解性、稳定性、亲和力和/或化合价(valency)。如在此使用,“化合价”是指每个分子可供使用的结合位点的数目。在一个实施例中,第二多肽是来自不同来源或不同蛋白的多肽。

[0134] 在一个实施例中,第二多肽包含免疫球蛋白重链恒定区的一个或多个结构域,该免疫球蛋白重链恒定区优选具有与人类免疫球蛋白Cγ1链的铰链、C_H2和C_H3区对应的、或与鼠免疫球蛋白Cγ2a链的铰链、C_H2和C_H3区对应的氨基酸序列。

[0135] 在一个优选的二聚体融合蛋白中,二聚体由两个Ig重链的铰链区中的Cys残基的共价键结产生,这些Cys残基是二聚化的正常Ig重链中以二硫键连接的相同Cys残基。此类蛋白可以称为CD28H-Ig。

[0136] 在一个实施例中,免疫球蛋白恒定域可含有增强CD28H多肽、其融合蛋白或片段的与具体细胞类型的结合、增加其生物利用度或增加其稳定性的一个或多个氨基酸插入、缺失或取代。适合的氨基酸取代包括如上所述的保守以及非保守取代。

[0137] 在另一个实施例中,第二多肽可具有轭合结构域,通过该轭合结构域另外的分子可结合至这些CD28H融合蛋白。在一个这样的实施例中,轭合分子能够将融合蛋白靶向至具体的器官或组织。在另一个这样的实施例中,轭合分子是可以增强或增加CD28H融合蛋白的效果的另一免疫调节剂。在另一个实施例中,轭合分子是聚乙二醇(PEG)。

[0138] 融合蛋白的Fc部分可按照同种型或子类而不同,可以是嵌合体或杂合体,和/或可经过修饰以便例如改进效应子功能、控制半衰期、组织可接近性、增进生物物理特性例如稳定性,并且改进产生效率(并且成本更小)。在构建所披露的融合蛋白中有用的许多修饰以及制备它们的方法在本领域是已知的,参见例如穆勒(Mueller)等人,分子免疫学(Mol.Immun.),34(6):441-452(1997),斯旺(Swann)等人,免疫学当前观点(Cur.Opin.Immun.),20:493-499(2008),以及普雷斯塔(Presta),免疫学当前观点(Cur.Opin.Immun.)20:460-470(2008)。在一些实施例中,Fc区是天然IgG1、IgG2、或IgG4Fc区。在一些实施例中,Fc区是杂合体,例如由IgG2/IgG4Fc恒定区组成的嵌合体。该Fc区的修饰包括但不局限于:IgG4被修饰以阻止与Fcγ受体和补体的结合,IgG1被修饰以改进与一种或多种Fcγ受体的结合,IgG1被修饰以最小化效应因子功能(氨基酸变化),IgG1具有改变的/不具有聚糖(典型地通过改变表达宿主),以及IgG1具有与FcRn的改变的pH-依赖性结合。Fc区可以包括整个绞链区,或不足整个铰链区。

[0139] 在用利妥昔单抗(一种针对CD20的嵌合小鼠/人类IgG1单克隆抗体)治疗患者的非霍奇金氏淋巴瘤或瓦尔登斯特伦氏巨球蛋白血症的治疗成果与个体的Fcγ受体的等位基因变体的表达相关,这些变体对于人类IgG1的Fc域具有不同的内在亲和力。具体而言,具有低亲和力激活型Fc受体CD16A(FcγRIIIA)的高亲和力等位基因的患者显示更高的反应率,并且在非霍奇金氏淋巴瘤的情况下显示改进的无进展生存期。在另一个实施例中,Fc域可含有一个或多个氨基酸插入、缺失或取代,这些插入、缺失或取代减少与低亲和力抑制性Fc受体CD32B(FcγRIIB)的结合并且保持野生型水平的与低亲和力活化Fc受体CD16A(FcγRIIIA)的结合或增强与之的结合。

[0140] 另一个实施例包括IgG2-4杂合体和IgG4突变体,它们具有降低的与FcR的结合,这增加了它们的半衰期。代表性IG2-4杂合体和IgG4突变体描述于安盖尔(Angal),S.等人,分子免疫学(Molecular Immunology),30(1):105-108(1993);穆勒(Mueller),J.等人,分子

免疫学,34(6):441-452(1997);以及王(Wang)等人的美国专利号6,982,323中。在一些实施例中,该IgG1和/或IgG2结构域是缺失的,例如,安盖尔等人描述了丝氨酸241替换为脯氨酸的IgG1和IgG2。

[0141] 在一个优选实施例中,Fc结构域包含增强与CD16A的结合的氨基酸插入、缺失或取代。在人类IgG1的Fc结构域内增加与CD16A的结合并且减少与CD32B的结合的大量取代在本领域是已知的并且描述于斯塔文哈根(Stavenhagen)等人,癌症研究(Cancer Res.),57(18):8882-90(2007)中。具有降低的与CD32B的结合和/或增加的与CD16A的结合的人类IgG1Fc结构域的示例性变体包含F243L、R929P、Y300L、V305I或P296L取代。这些氨基酸取代可按任何组合存在于人类IgG1Fc结构域中。在一个实施例中,该人类IgG1Fc结构域变体含有F243L、R929P以及Y300L取代。在另一个实施例中,该人类IgG1Fc结构域变体含有F243L、R929P、Y300L、V305I以及P296L取代。在另一个实施例中,人类IgG1Fc结构域变体包含N297Q取代,因为这个突变消除了FcR结合。

[0142] c.肽的或多肽接头结构域

[0143] 披露的CD28H融合蛋白任选地包含将CD28H多肽与第二多肽分开的肽或多肽接头结构域。

[0144] 1.抗体的铰链区

[0145] 在一个实施例中,接头结构域包含免疫球蛋白的铰链区。在一个优选实施例中,铰链区是源自人类免疫球蛋白。可以作为铰链来源的适合的人类免疫球蛋白包括IgG、IgD和IgA。在一个优选实施例中,铰链区是源自人类IgG。免疫球蛋白铰链区和其他结构域的氨基酸序列是本领域熟知的。

[0146] 2.其他肽或多肽接头结构域

[0147] 其他适合的肽/多肽接头结构域包含天然存在或非天然存在的肽或多肽。肽接头序列长度是至少2个氨基酸。优选地,肽或多肽结构域是柔性肽或多肽。“柔性接头”在此是指包含由一个或多个肽键连接的两个或更多个氨基酸残基的肽或多肽,这为由此连接的两个多肽提供了比在该柔性接头不存在下这两个被连接的多肽将具有的转动自由度增加的转动自由度。这种转动自由度允许由该柔性接头连接的两个或更多个抗原结合位点各自更有效地接近一个或多个靶抗原。示例性柔性肽/多肽包括但不限于氨基酸序列Gly-Ser、Gly-Ser-Gly-Ser(SEQ ID NO:16)、Ala-Ser、Gly-Gly-Gly-Ser(SEQ ID NO:17)、$(Gly_4-Ser)_3$(SEQ ID NO:18)和$(Gly_4-Ser)_4$(SEQ ID NO:19)。另外的柔性肽/多肽序列是本领域熟知的。

[0148] 在一个实施例中,第一融合伴侣是CD28H的片段。在一个优选实施例中,融合蛋白包括融合至Ig Fc区的CD28H的胞外结构域、或其片段。如先前所述(查波瓦尔(Chapoval)等人,分子医学方法(Methods Mol.Med.),45:247-255(2000)),可以通过将神经毡蛋白或丛蛋白的编码区或其片段融合至人类IgG1或小鼠IgG2a的Fc区、或其他适合的Ig结构域,来制备重组CD28H-Ig融合蛋白。

d.示例性融合蛋白

代表性人类CD28H-Ig融合蛋白的氨基酸序列是(SEQ ID NO:20),CD28H-hIgG4(CD28H ECD aa23-140,轻链κ信号肽:CD28H序列以黑体字示出;突变的人类IgG4Fc序列加下划线示出,丝氨酸228至脯氨酸的突变加双下划线):

LSVQQGPNLLQVRQGSQATLVCQVDQATAWERLRVKWTKDGAILCQPYITNGSLSLGVCGPQ
GRLSWQAPSHLTLQLDPVSLNHSGAYVCWAAVEIPELEEAEGNITRLFVDPDDPTQESKYGPPCP
CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPGK

融合蛋白可以另外包括N末端前导序列(轻链κ前导序列)(SEQ ID NO:21的此类残基1-20),

MSVPTQVLGLLLLWLTDARC

或天然存在的CD28H前导序列,例如(SEQ ID NO:22):

MGSPGMVLGLLVQIWALQEASS

虽然根据本发明,已经示出CD28H序列融合至人类IgG4区域,但是此类序列可以可替代地融合至任何Ig同种型,或确实融合至任何其他蛋白。

编码包括SEQ ID NO:21的信号序列的SEQ ID NO:20的人类CD28H-Ig的DNA序列是(SEQ ID NO:23):

ATGTCCGTGCCCACCCAGGTGCTGGGATTGCTGCTGCTGTGGCTGACCGACGCCAGATGCCTGTCTGTGCAGC
AGGGCCCTAACCTGCTGCAAGTGCGGCAGGGCTCTCAGGCTACACTCGTGTGTCAGGTGGACCAGGCCACCGCCTGG
GAGAGACTGAGAGTGAAGTGGACCAAGGACGGCGCCATCCTGTGCCAGCCCTACATCACCAACGGCTCCCTGTCCCT
GGGCGTGTGTGGACCTCAGGGCAGACTGTCTTGGCAGGCCCCTTCTCACCTGACCCTGCAGCTGGACCCTGTGTCCC
TGAATCACTCCGGCGCCTACGTGTGTTGGGCCGCTGTGGAAATCCCCGAGCTGGAAGAGGCCGAGGGCAACATCACC
CGGCTGTTCGTGGACCCTGACGACCCTACCCAGGAATCTAAGTACGGCCCTCCCTGCCCTCCTTGCCCAGCCCCTGA
ATTTCTGGGCGGACCCTCCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAG
TGACCTGCGTGGTGGTGGATGTGTCCCAGGAAGATCCCGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAAGTG
CACAACGCCAAGACCAAGCCCAGAGAGGAACAGTTCAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCA
CCAGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGGCCTGCCCAGCTCCATCGAAAAGACCA
TCTCCAAGGCCAAGGGCCAGCCCCGGGAACCCCAGGTGTACACACTGCCTCCAAGCCAGGAAGAGATGACCAAGAAC
CAGGTGTCACTGACCTGTCTCGTGAAGGGCTTCTACCCCTCCGATATCGCCGTGGAATGGGAGTCCAACGGCCAGCC
TGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCCTTCTTCCTGTACTCCCGCCTGACCGTGG
ACAAGTCCAGATGGCAGGAAGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAG
AAGTCCCTGAGCCTGTCCCCCGGCAAGTGA

E.本发明的优选组合物的治疗的和预防的用途

[0149] 如此处使用的,术语“治疗(treat、treating、treatment)”以及“治疗用途”是指消除、减少或改善将从增加或降低的免疫应答受益的一种疾病或失调的一个或多个症状。如此处使用的,“治疗有效量”是指一种治疗剂足够介导改变的免疫应答并且更优选临床上相关的改变的免疫应答、足够介导一种疾病或病症的症状的减少或改善的量。如果一种效果的幅度足以影响接受者受试者的健康或预后的话,则该效果是临床上相关的。治疗有效量可以指足以减少或最小化疾病进展,例如延迟或最小化自身免疫应答或炎症反应或移植排斥的治疗剂的量。治疗有效量还指在疾病的治疗或管理中,提供治疗益处的治疗剂的量。进一步地,针对治疗剂、B7-H5抗体或B7-H5融合蛋白或CD28H融合蛋白的治疗有效量意指治疗剂单独或与其他疗法组合在疾病的治疗或管理中提供治疗益处的量,该治疗益处是例如,

足以增强治疗性抗体的治疗效力，足以治疗或管理疾病。

[0150] 如此处使用的，术语“预防剂”是指可在检测到失调或疾病的任何症状之前在预防这种失调或疾病中使用的药剂。“预防有效”量是足够介导这种保护的预防剂的量。预防有效量还指在疾病的预防中，提供预防益处的预防剂的量。另外，关于预防剂的预防有效量是指预防剂单独或与其他药剂相组合在疾病的预防中提供预防益处的量。

[0151] 在此提供的给药剂量和频率是涵盖在术语治疗有效和预防有效内的。典型地，将取决于给予的具体治疗剂或预防剂，癌症的严重性和类型，给药途径，连同患者的年龄、体重、反应和既往病史，根据特异性针对每一患者的因素，来进一步改变剂量和频率。适宜的方案可以由本领域的普通技术人员通过考虑到此类因素并且通过遵循例如文献中所报道的以及在医师手册(Physician's Desk Reference)(第56版，2002)中所推荐的剂量进行选择。

[0152] 本发明涉及多个分子，例如抗B7-H5抗体(和结合至B7-H5的此类抗体的片段)或CD28H Ig，这些分子通过结合至B7-H5和/或通过结合至CD28H而拮抗(即衰减或损害)B7-H5功能，并且因此衰减或损害T细胞增殖和/或细胞因子产生。将此类分子给予受试者下调了该受试者的免疫系统。

[0153] 在治疗炎症疾病和自身免疫性疾病和移植排斥中，免疫系统的这种下调是令人希望的。可通过给予本发明的这些抗体治疗的自身免疫失调的实例包括但不限于斑形脱发、强直性脊柱炎、抗磷脂综合征、自身免疫性阿狄森氏病、肾上腺的自身免疫性疾病、自身免疫性溶血性贫血、自身免疫性肝炎、自身免疫性卵巢炎以及睾丸炎、自身免疫性血小板减少症、白塞氏病、大疱性类天疱疮、心肌病、乳糜泻性皮炎(celiac sprue-dermatitis)、慢性疲劳免疫功能紊乱综合征(CFIDS)、慢性发炎性脱髓鞘性多发性神经病、丘-施二氏综合征(Churg-Strauss syndrome)、瘢痕性类天疱疮、CREST综合征、冷凝集素病、克罗恩氏疾病、盘状狼疮、原发性混合型冷球蛋白血症、纤维肌痛-纤维性肌炎、肾小球肾炎、格拉夫疾病、格林-巴利综合征(guillain-barre)、桥本氏甲状腺炎(hashimoto's thyroiditis)、特发性肺纤维化、特发性血小板减少性紫癜(ITP)、IgA神经病、幼年型关节炎、扁平苔癣、红斑狼疮、美尼尔氏病、混合结缔组织病、多发性硬化症、视神经脊髓炎(NMO)、1型或免疫介导的糖尿病、重症肌无力、寻常天疱疮、恶性贫血、结节性多动脉炎、多发性软骨炎、多腺体综合征、风湿性多肌痛、多发性肌炎以及皮肤肌炎、原发性无丙种球蛋白血症、原发性胆汁性肝硬化、牛皮癣、牛皮癣性关节炎、雷诺氏现象(Raynaud'sphenomenon)、赖特氏综合征(Reiter's syndrome)、类风湿性关节炎、类肉瘤病、硬皮病、修格连氏综合征(Sj gren's syndrome)、僵人综合征、全身性红斑狼疮、红斑狼疮、高安氏动脉炎(Takayasu arteritis)、颞动脉炎/巨细胞性动脉炎、溃疡性结肠炎、葡萄膜炎、血管炎如疱疹样皮炎血管炎、白癜风以及韦格纳氏肉芽肿(Wegener's granulomatosis)。

[0154] 可以用披露的抗B7-H5抗体及其抗原结合片段和CD28H融合蛋白，具体地CD28H-Ig融合蛋白预防、治疗或管理的炎症疾病的实例包括但不限于哮喘、脑炎、发炎性肠病、慢性阻塞性肺病(COPD)、过敏性失调、败血性休克、肺纤维化、未分化的脊椎关节病(undifferentitated spondyloarthropathy)、未分化的关节病、关节炎、炎性骨质溶解以及慢性病毒性或细菌性感染所致的慢性炎症。

[0155] 可以采用抗B7-H5抗体来产生B7-H5的抗独特型肽或抗体(沃尔曼(Wallmann)，J.

等人(2010)“过敏症中的抗Id:古典概念的时效性(Anti-Ids in Allergy:Timeliness of a Classic Concept)”,世界过敏症组织杂志(World Allergy Organiz.J)3(6):195-201;纳迪(Nardi),M.等人(2000)“在HIV-1-ITP患者中,针对血小板抗GpIII的抗独特型抗体有助于调节血小板减少(Antiidiotype Antibody Against Platelet Anti-GpIIIa Contributes To The Regulation Of Thrombocytopenia In HIV-1-ITP Patients)”,实验医学杂志(J.Exp.Med.)191(12):2093-2100)或模拟物(臧(Zang),Y.C.等人(2003)“在髓鞘碱性蛋白反应性T细胞中,对应于常见CDR3序列基序的TCR肽诱导的人类抗独特型T细胞(Human Anti-Idiotypic T Cells Induced By TCR Peptides Corresponding To A Common CDR3 Sequence Motif In Myelin Basic Protein-Reactive T Cells)”,国际免疫学(Int.Immunol.)15(9):1073-1080;洛亚诺(Loiarro),M.等人(2010年4月8日电子出版)“人类疾病中的靶向的TLR/IL-1R信号传导(Targeting TLR/IL-1R Signalling In Human Diseases)”,炎症介导物(Mediators Inflamm.)2010:674363)。此类分子用作B7-H5的替代物,并且因此向受试者给予它们,通过接合CD28H反受体并且防止它结合内源B7-H5受体而下调该受试者的免疫系统。在移植物抗宿主疾病、炎症疾病、和自身免疫性疾病的治疗中,此类分子具有效用。

[0156] 因此,在移植物抗宿主疾病、炎症疾病和自身免疫性疾病的治疗中,本发明的抗体和抗原结合片段和融合蛋白具有效用。

[0157] 类似地,增强此类抗体和此类受体/配体之间的结合的激动剂抗体,以及其他受体激动剂(例如B7-H5融合蛋白)具有作为B7-H5信号传导的激动剂的效用,并且因此在癌症和感染性疾病的治疗中具有效用。相应地,本发明还涉及CD28H激动剂分子,例如B7-H5-Ig融合蛋白,这些分子通过结合至CD28H,激动CD28H功能(即信号传导)和/或通过结合至B7-H5,并且因此增加或刺激T细胞增殖和/或细胞因子产生。将此类分子给予受试者上调受试者的免疫系统,并且可以用于治疗患有癌症或感染性疾病的受试者。因为在T细胞活化中涉及B7-H5通路,所以当结合疫苗时,此类分子是特别有用的。

[0158] 在此所提供的CD28H激动剂在体内和离体中作为免疫应答刺激治疗剂通常是有用的。例如,对于通过给予受试者一个量的CD28H激动剂,刺激或增强宿主中的免疫应答,以便治疗癌症,CD28H激动剂是有用的。可以用提供的组合物和方法治疗的癌症的类型包括但不限于以下项:膀胱癌、脑癌、乳癌、宫颈癌、大肠癌、食道癌、肾癌、肝癌、肺癌、鼻咽癌、胰腺癌、前列腺癌、皮肤癌、胃癌、子宫癌、卵巢癌、睾丸癌和血液癌。

[0159] 根据肿瘤起源的组织的胚胎起源,在此将可以治疗的恶性肿瘤分类。癌瘤(carcinomas)是起源自内胚层组织或外胚层组织,例如皮肤或内脏器官和腺体的上皮层的肿瘤。肉瘤,它不太经常出现,是源自中胚层结缔组织,例如骨骼、脂肪、和软骨。白血病和淋巴瘤是骨髓的造血细胞的恶性肿瘤。白血病作为单细胞增殖,而淋巴瘤倾向于作为肿瘤团块生长。恶性肿瘤可以在身体的多个器官或组织上显现,以建立癌症。

[0160] 可以给予CD28H激动剂,用于治疗局部的或全身的病毒感染,包括但不限于免疫缺陷(例如HIV)、乳头状瘤(例如HPV)、疱疹(例如HSV)、脑炎、流感(例如人类流感病毒A)、以及普通感冒(例如人类鼻病毒)病毒感染。可以局部地给予包括CD28H激动剂组合物的药物配制品,以治疗病毒性皮肤病,例如疱疹损伤或带状疱疹、或生殖器疣。还可以给予CD28H激动剂组合物的药物配制品,以治疗全身性病毒疾病,包括但不限于AIDS、流感、普通感冒、或脑

炎。

[0161] 可以治疗的代表性感染包括但不限于由微生物引起的感染，这些微生物包括但不限于放线菌属，鱼腥藻属，芽胞杆菌属，拟杆菌属，蛭弧菌属，博代氏杆菌属，疏螺旋体属，弯曲菌属，柄杆菌属，衣原体，绿菌属，着色菌属，梭菌属，棒杆菌属，噬细胞菌属，异常球菌属，埃希氏杆菌属，弗朗西斯氏菌属，盐杆菌属，螺杆菌属，嗜血杆菌属，B型流感嗜血杆菌(HIB)，组织胞浆菌属，生丝微菌属，军团菌属，利什曼原虫属，钩端螺旋体，李斯特菌属，脑膜炎球菌A、B和C，甲烷杆菌属，微球菌属，分支杆菌属，支原体属，粘球菌属，奈瑟氏菌属，硝化菌属，颤藻属，原绿藻，变形杆菌属，假单胞菌属，红螺菌属，立克次氏体，沙门氏菌属，志贺氏菌属，螺菌属，螺旋体属，葡萄球菌属，链球菌属，链霉菌属，硫化叶菌属，热原体属，硫杆菌属，和密螺旋体属，弧菌属，耶尔森菌属，新型隐球菌，荚膜组织胞浆菌，白色念珠菌，热带假丝酵母，星状诺卡氏菌，立氏立克次氏体，斑疹伤寒立克次氏体，肺炎支原体，鹦鹉热衣原体，沙眼衣原体，恶性疟原虫，间日疟原虫，布氏锥虫，痢疾变形虫，鼠弓形体，阴道毛滴虫以及曼氏血吸虫。

[0162] 可以单独地或者结合任何其他适合的治疗，来给予披露的CD28H激动剂或编码它的核酸。在一个实施例中，可以结合疫苗组合物，或作为疫苗组合物的组分，来给予CD28H激动剂。疫苗组合物的适合的组分，抗原和/或佐剂。可以在给予疫苗之前、同时、或之后来给予披露的CD28H激动剂。在一个实施例中，在给予疫苗的同时来给予CD28H激动剂组合物。

[0163] 可以结合预防疫苗、或治疗疫苗来给予披露的CD28H激动剂组合物，这些疫苗可以用于启动或增强受试者对预先存在的抗原，例如患有癌症的受试者中的肿瘤抗原的免疫应答。

[0164] 根据本领域熟知的原理，预防的、治疗的、或脱敏的免疫应答的希望的结果可以根据疾病来变化。类似地，针对癌症、过敏原或感染性病原体的免疫应答可以完全地治疗疾病，可以减轻症状，或可以是针对疾病的整体治疗性干预的一个方面。例如，针对癌症的免疫应答的刺激可以偶联外科的、化学疗法的、放射的、激素的和其他免疫的方法来影响治疗。

F.给予方法

[0165] 不同递送系统是已知的并且可以用于给予治疗的或预防的组合物，例如，封装在脂质体、微粒、微胶囊中，能够表达抗体或融合蛋白的重组细胞，受体介导的内吞作用(参见(例如)吴(Wu)和吴(Wu)，1987，生物化学杂志(J.Biol.Chem.)262:4429-4432)，将核酸构建为逆转录病毒或其他载体的一部分，等等。

[0166] 给予抗体和融合蛋白的方法包括但不限于:肠胃外给予(例如，真皮内的、肌内的、腹膜内的、静脉内的以及皮下的)，硬膜外给予，以及粘膜给予(例如，鼻内途径和口腔途径)。在一个具体实施例中，肌内地、静脉内地、或皮下地给予抗体或融合蛋白。可以通过任何方便的途径给予这些组合物，例如通过输注或单次快速静脉注射(bolus injection)、通过经由上皮或皮肤粘膜内层(例如口腔粘膜、直肠和肠道粘膜，等等)吸收，并且可以将其与其他生物活性剂一起给予。给予可以是全身性的或局部的。此外，也可以例如通过使用吸入器或雾化器以及具有雾化剂的配制品而采用肺部给予。参见，例如美国专利号6,019,968;5,985,20;5,985,309;5,934,272;5,874,064;5,855,913;5,290,540;以及4,880,078;以及PCT公开号WO 92/19244;WO 97/32572;WO 97/44013;WO 98/31346;以及WO 99/66903。在一

个具体实施例中，会希望将药用组合物局部地给予至需要治疗的区域；这可以通过例如以下方式实现，但不仅限于这些方式：局部输注，通过注射、或借助植入物，所述植入物是多孔的、非多孔的或凝胶状材料，包括膜，如硅橡胶膜(sialastic membrane)、或纤维。优选地，当给予抗体或融合蛋白时，必须小心，要使用不吸收抗体或融合蛋白的材料。

[0167] 在一些实施例中，将抗体和或融合蛋白配制在脂质体中，用于抗体或融合蛋白的靶向递送。脂质体是由封装水相的同心有序的磷脂双分子层组成的囊泡。脂质体典型地包括不同类型的脂质、磷脂、和/或表面活性剂。脂质体的组分是以双分子层构型安排的，类似于生物膜的脂类安排。脂质体是特别优选的递送运载体，部分归因于其生物相容性、低免疫原性、以及低毒性。用于制备脂质体的方法是本领域已知的，并且涵盖在本发明内，参见例如爱泼斯坦(Epstein)等人，1985，美国国家科学院院刊(Proc.Natl.Acad.Sci.USA)82:3688；黄(Hwang)等人，1980美国国家科学院院刊(Proc.Natl.Acad.Sci.USA)，77:4030-4；美国专利号4,485,045和4,544,545。

[0168] 用于制备具有延长的血清半衰期(即增强的循环时间)的脂质体的方法，例如披露于美国专利号5,013,556中的那些，可以用于制造脂质体-抗体组合物。优选的脂质体并不从循环中迅速清除，即并不被摄取进单核巨噬细胞系统(MPS)中。本发明涵盖使用本领域普通技术人员已知的普通方法制备的立体稳定脂质体。虽然并不旨在受具体作用机制的束缚，但是立体稳定脂质体包含具有体积大并且高度柔性的亲水部分的脂质组分，这些组分减少了脂质体与血清蛋白的不想要的反应，减少了血清组分的调理作用，并且减少了被MPS识别。优选使用聚乙二醇来制备立体稳定脂质体。关于制备脂质体和立体稳定脂质体，参见例如本达斯(Bendas)等人，2001生物药物(BioDrugs)，15(4):215-224；艾伦(Allen)等人，1987欧洲生化学会联合会快报(FEBS Lett.)223:42-6；克利巴诺夫(Klibanov)等人，1990欧洲生化学会联合会快报(FEBS Lett.)268:235-7；布卢姆(Blum)等人，1990，生物化学与生物物理学报(Biochim.Biophys.Acta.)，1029:91-7；托基林(Torchilin)等人，1996，脂质体研究杂志(J.Liposome Res.)6:99-116；里辛格尔(Litzinger)等人，1994，生物化学与生物物理学报(Biochim.Biophys.Acta.)，1190:99-107；丸山(Maruyama)等人，1991，化学与药学通报(Chem.Pharm.Bull.)，39:1620-2；克利巴诺夫(Klibanov)等人，1991，生物化学与生物物理学报(Biochim.Biophys.Acta.)，1062;142-8；艾伦(Allen)等人，1994，先进药物递送评论(Adv.Drug Deliv.Rev)，13:285-309。本发明还涵盖适配为用于特定器官靶向(参见例如美国专利号4,544,545)、或特定细胞靶向(参见例如美国专利申请公开号2005/0074403)的脂质体。可以通过用包括磷脂酰胆碱、胆固醇、和PEG衍生化磷脂酰乙醇胺(PEG-PE)的脂质组合物的反相蒸发方法，来产生用于披露的组合物和方法的特别有用的脂质体。通过限定孔径大小的过滤器挤出脂质体，以生成具有希望的直径的脂质体。在一些实施例中，可以使用先前描述的方法，将抗体的片段例如F(ab')轭合至脂质体，参见例如马丁(Martin)等人，1982，生物化学杂志(J.Biol.Chem.)257:286-288。

[0169] 还可以将B7-H5抗体、或B7-H5或CD28H融合蛋白配制为免疫脂质体。免疫脂质体是指脂质体组合物，其中抗体或其片段被共价地或非共价地连接至脂质体表面。连接抗体至脂质体表面的化学是本领域已知的，并且涵盖在本发明内，参见例如美国专利号6,787,153；艾伦(Allen)等人，1995，隐形脂质体(Stealth Liposomes)博卡拉顿：CRC出版社，233-44；汉森(Hansen)等人，1995，生物化学与生物物理学报(Biochim.Biophys.Acta.)，1239:

133-144。在最优选的实施例中，用于披露的方法和组合物的免疫脂质体进一步被立体稳定化。优选地，抗体或融合蛋白共价地或非共价地连接至疏水锚，该锚稳定地扎根于脂质体的脂质双分子层中。疏水锚的实例包括但不限于磷脂，例如磷脂酰乙醇胺(PE)、磷脂酰肌醇(PI)。为了实现抗体和疏水锚之间的共价键，可以使用本领域中任何已知的生物化学策略，参见例如托马斯奥格斯格(J.Thomas August)编辑，1997，基因治疗：药理学进展(Gene Therapy:Advances in Pharmacology)，40卷，学术出版社(Academic Press)，圣迭戈，加利福尼亚州，399-435页。例如，抗体分子上的官能团可以与关联脂质体的疏水锚上的活性基团反应，例如抗体上的赖氨酸侧链的氨基可以偶联至关联脂质体的用水溶性碳二亚胺活化的N-戊二酰-磷脂酰乙醇胺；或经由硫醇反应性锚，例如吡啶基巯基丙酰基磷脂酰乙醇胺，还原的抗体的硫醇基可以偶联至脂质体。参见例如迪特里希(Dietrich)等人，1996，生物化学(Biochemistry)，35:1100-1105；罗贵尔(Loughrey)等人，1987，生物化学与生物物理学报(Biochim.Biophys.Acta.)，901:157-160；马丁(Martin)等人，1982，生物化学杂志(J.Biol.Chem.)257:286-288；马丁(Martin)等人，1981，生物化学(Biochemistry)，20:4429-38。虽然并不旨在受具体作用机制的束缚，但是包括抗体或融合蛋白的免疫脂质体配制品作为治疗剂是特别有效的，因为它们递送抗体或融合蛋白至靶细胞(即包括抗体或融合蛋白结合的受体的细胞)的细胞质。免疫脂质体优选具有血液(确切地靶细胞)中增加的半衰期，并且可以内化进靶细胞的细胞质中，由此避免了治疗剂的损失或被内吞溶酶体通路降解。

[0170] 免疫脂质体组合物包括一种或多种囊泡形成脂质、抗体或其片段或衍生物或融合蛋白，和任选地，亲水聚合物。囊泡形成脂质优选是具有两个烃链，例如酰基链和极性头基的脂质。囊泡形成脂质的实例包括磷脂，例如磷脂酰胆碱、磷脂酰乙醇胺、磷脂酸、磷脂酰肌醇、鞘磷脂、和糖脂，例如脑苷脂、神经节苷脂。在配制品中有用的另外的脂质是本领域普通技术人员已知的并且涵盖在本发明内。在一些实施例中，免疫脂质体组合物进一步包括亲水聚合物，例如聚乙二醇、和神经节苷脂GM1，这些亲水聚合物增加了脂质体的血清半衰期。将亲水聚合物轭合至脂质体的方法是本领域熟知的并且涵盖在本发明内。关于免疫脂质体和用于制备它们的方法的综述，参见例如美国专利申请号2003/0044407；PCT国际公开号WO 97/38731，温格霍茨(Vingerhoeads)等人，1994，免疫方法(Immunomethods)，4:259-72；丸山(Maruyama)，2000，生物与药学通报(Chem.Pharm.Bull.)23(7):791-799；阿布拉(Abra)等人，2002，脂质体研究杂志(Journal of Liposome Research)，12(1和2):1-3；帕克(Park)，2002，生物科学报道(Bioscience Reports)，22(2):267-281；本达斯(Bendas)等人，2001生物药物(BioDrugs)，14(4):215-224；托马斯奥格斯格(J.Thomas August)编辑，1997，基因治疗：药理学进展(Gene Therapy:Advances in Pharmacology)，40卷，学术出版社(Academic Press)，圣迭戈，加利福尼亚州，399-435页。

[0171] 可以将抗体和融合蛋白包装在指明抗体的量的密封容器中，例如安瓿或小药囊。在一个实施例中，这些抗体是作为一种处于密封容器中的干燥的无菌冻干粉或无水浓缩物来提供，并且可以用水或盐水重构至用于给予受试者的适当浓度。优选地，这些抗体或融合蛋白是作为一种干燥的无菌冻干粉在密封容器中按以下单位剂量提供的：至少5mg、更优选地至少10mg、至少15mg、至少25mg、至少35mg、至少45mg、至少50mg、或至少75mg。冻干的抗体或融合蛋白应在2℃与8℃之间在其原始容器中储存，并且这些抗体在重构之后应在12小时

内，优选地6小时内、5小时内、3小时内、或1小时内给予。在一个可替代实施例中，抗体或融合蛋白是以液体形式在指明该抗体、融合蛋白、或轭合分子的量和浓度的密封容器中提供的。优选地，液体形式的抗体或融合蛋白是在密封容器中以至少1mg/ml、更优选至少2.5mg/ml、至少5mg/ml、至少8mg/ml、至少10mg/ml、至少15mg/kg、至少25mg/ml、至少50mg/ml、至少100mg/ml、至少150mg/ml、至少200mg/ml的抗体或融合蛋白提供。

[0172] 配制品中采用的精确剂量还将取决于给药途径和病症的严重性，并且应当根据执业医师的判断和每一患者的情况来决定。可以根据源自体外或动物模型测试系统的剂量反应曲线推断有效剂量。对于抗体和融合蛋白，给予至患者的剂量典型地是0.0001mg/kg至100mg/kg患者体重。优选地，给予患者的剂量是在0.0001mg/kg和20mg/kg、0.0001mg/kg和10mg/kg、0.0001mg/kg和5mg/kg、0.0001和2mg/kg、0.0001和1mg/kg、0.0001mg/kg和0.75mg/kg、0.0001mg/kg和0.5mg/kg之间、0.0001mg/kg至0.25mg/kg、0.0001至0.15mg/kg、0.0001至0.10mg/kg、0.001至0.5mg/kg、0.01至0.25mg/kg或0.01至0.10mg/kg患者体重。通常，人类抗体在人体内相比其他物种具有更长的半衰期，归因于对外来多肽的免疫应答。因此，更低剂量的人类抗体以及更小频率给予常常是合适的。进一步，给予抗体或其片段或融合蛋白的剂量和频率可通过增强这些抗体或融合蛋白的摄取以及组织渗透性(通过修饰诸如，例如脂化)来减少。

[0173] 在又另一个实施例中，可以在控制释放或持续释放系统中递送这些组合物。可以使用本领域普通技术人员已知的任何技术来产生包括一种或多种抗体或融合蛋白的持续释放配制品。参见例如美国专利号4,526,938;PCT公开案WO 91/05548;PCT公开案WO 96/20698;宁(Ning)等人，1996，“使用持续释放凝胶的人类结肠癌异种移植物的瘤内放射免疫治疗(Intratumoral Radioimmunotheraphy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel)”，放射治疗&肿瘤学(Radiotherapy&Oncology)39:179-189;宋(Song)等人，1995，“长循环乳剂的抗体介导的肺靶向(Antibody Mediated Lung Targeting of Long-Circulating Emulsions)”，制药科学和技术的PDA杂志(PDA Journal of Pharmaceutical Science and Technology)50:372-397;克里克(Cleek)等人，1997，“用于心血管应用的bFGF抗体的生物可降解聚合物载体(Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application)”，控制脂肪生物活性材料的国际讨论会的会议记录(Pro.Int'l.Symp.Control.Rel.Bioact.Mater.)24:853-854;和拉姆(Lam)等人，1997，“用于局部递送的重组人源化单克隆抗体的微胶囊化(Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery)”，控制脂肪生物活性材料的国际讨论会的会议记录(Pro.Int'l.Symp.Control.Rel.Bioact.Mater.)24:759-760。在一个实施例中，可以将泵用于控制释放系统(参见兰格(Langer)，同上;塞夫顿(Sefton)，1987，生物医学工程CRC关键参考(CRC Crit.Ref.Biomed.Eng.)14:20;布赫瓦尔德(Buchwald)等人，1980，外科学(Surgery)88:507;和索德克(Saudek)等人，1989，新英格兰医学杂志(N.Engl.J.Med.)321:574)。在另一实施例中，聚合物材料可以用于实现抗体的控制释放(参见例如控制释放的医学应用(Medical Applications of Controlled Release)，兰格(Langer)和怀斯(Wise)(编辑)，CRC出版社(CRC Pres.)，波卡拉顿，佛罗里达州(1974);控制的药物生物利用率、药物产物涉及和性能(Controlled Drug Bioavailability,Drug Product Design and

Performance)，斯莫伦(Smolen)和鲍尔(Ball)(编辑)，威利出版社(Wiley)，纽约(1984)；兰杰(Ranger)和帕帕斯(Peppas)，1983，大分子科学综述和大分子化学杂志(J.，Macromol.Sci.Rev.Macromol.Chem.)23:61；还参见利维(Levy)等人，1985，科学(Science)228:190；迪兰(During)等人，1989，神经学年报(Ann.Neurol.)25:351；霍华德(Howard)等人，1989，神经外科杂志(J.Neurosurg.)71:105)；美国专利号5,679,377；美国专利号5,916,597；美国专利号5,912,015；美国专利号5,989,463；美国专利号5,128,326；PCT公开号WO 99/15154；和PCT公开号WO 99/20253)。在持续释放制剂中使用的聚合物的例子包括但不限于，聚(2-羟乙基甲基丙烯酸酯)、聚(甲基丙烯酸甲酯)、聚(丙烯酸)、聚(乙烯共乙酸乙烯酯)、聚(甲基丙烯酸)、聚乙交酯(PLG)、聚酐、聚(N-乙烯吡咯烷酮)、聚(乙烯醇)、聚丙烯酰胺、聚(乙二醇)、聚丙交酯(PLA)、聚(丙交酯共乙交酯)(PLGA)和聚原酸酯。在又另一个实施例中，可以将控制释放系统放置在治疗靶(例如肺)附近，因此仅要求一部分的全身性剂量(参见例如古德森(Goodson)，控制释放的医学应用(Medical Applications of Controlled Release)，同上，2卷，115-138页(1984))。在另一个实施例中，根据邓恩(Dunn)等人，使用作为控制释放植入物有用的聚合物组合物(参见U.S.5,945,155)。这一具体方法是基于来自聚合物系统的生物活性材料的原位控制释放的治疗效果。通常，植入发生在需要治疗性治疗的患者的身体内的任何地方。在另一实施例中，使用非聚合物持续递送系统，由此受试者体内的非聚合物植入物被用作药物递送系统。当在体内植入时，植入物的有机溶剂将从组合物消散、分散、或浸入至周围组织液，并且非聚合物材料将逐步凝结或沉淀以形成固体、微孔基质(参见U.S.5,888,533)。在兰格(Langer)的综述(1990，科学(Science)249:1527-1533)中讨论了控制释放系统。可以使用本领域普通技术人员已知的任何技术来产生包括一种或多种治疗剂，即B7-H5抗体或CD28H融合蛋白的持续释放配制品。参见例如美国专利号4,526,938；国际公开号WO 91/05548和WO 96/20698；宁(Ning)等人，1996，放射治疗&肿瘤学(Radiotherapy&Oncology)39:179-189；宋(Song)等人，1995，制药科学和技术的PDA杂志(PDA Journal of Pharmaceutical Science and Technology)50:372-397；克里克(Cleek)等人，1997，控制脂肪生物活性材料的国际讨论会的会议记录(Pro.Int'l.Symp.Control.Rel.Bioact.Mater.)24:853-854；和拉姆(Lam)等人，1997，控制脂肪生物活性材料的国际讨论会的会议记录(Pro.Int'l.Symp.Control.Rel.Bioact.Mater.)24:759-760。

[0174] 在一个具体实施例中，其中治疗的或预防的组合物是编码B7-H5抗体或其抗原结合片段的核酸，或编码CD28H融合蛋白(例如CD28H-Ig融合蛋白)的核酸，可以在体内给予该核酸，以促进其编码的抗体或融合体的表达，这是通过构建它为适当核酸表达载体的一部分并且给予它使得它变为细胞内的来实现的，例如通过使用逆转录病毒载体(参见美国专利号4,980,286)实现，或通过直接注射实现，或通过使用微粒轰击(例如基因枪，Biolistic，杜邦公司(Dupont))实现，或通过涂覆脂质或细胞表面受体或转染剂实现，或通过将其连接到已知进入细胞核的同源框样肽来给予它实现(参见例如约里奥(Joliot)等人，1991，美国国家科学院院刊(Proc.Natl.Acad.Sci.USA)88:1864-1868)等。可替代地，核酸可以引入细胞内且通过同源重组整合入宿主细胞DNA内用于表达。

[0175] 使用治疗有效量的或预防有效量的抗体或融合蛋白对受试者进行的治疗可以包括单一治疗、或优选地可以包括一系列治疗。

[0176] G.药用组合物

[0177] 这些组合物包括在药用组合物的制造中有用的散装药物组合物(例如不纯的或非无菌的组合物)以及可用于单位剂型的制备的药用组合物(即适合于给予受试者或病人的组合物)。这样的组合物包括在此披露的预防或治疗有效量的预防和/或治疗剂或那些药剂与药学上可接受的载体的组合。优选地,披露的组合物包括预防有效量的或治疗有效量的抗体或融合蛋白和药学上可接受的载体。

[0178] 在一个特定的实施例中,术语"药学上可接受的"是指由联邦政府或州政府的监管机构批准的或在美国药典或其他普遍认可的药典中列出的,用于在动物体内并且更特别地在人体内使用。术语"载体"是指与治疗剂一起给予的稀释剂、佐剂(如弗氏佐剂(完全和不完全的))、赋形剂或运载体。此类药物载体可以为无菌液体,如水和油,包括石油、动物、植物或合成来源的油,如花生油、豆油、矿物油、芝麻油等。当静脉内给予药用组合物时,水是优选的载体。还可以将盐水溶液、水性右旋糖和甘油溶液用作液体载体,特别是用于注射溶液。适合的药用赋形剂包括淀粉、葡萄糖、乳糖、蔗糖、明胶、麦芽、米、面粉、白垩、硅胶、硬脂酸钠、单硬脂酸甘油酯、滑石、氯化钠、脱脂奶粉、甘油、丙烯、二醇、水、乙醇以及类似物。如果希望的话,该组合物还可包含少量湿润剂或乳化剂或pH缓冲剂。这些组合物可以采取以下形式:溶液、悬浮液、乳液、片剂、丸剂、胶囊、粉剂、持续释放配制品以及类似物。

[0179] 一般地,组合物的成分分开或混合在一起以单位剂型提供,例如,作为在密封容器中的干燥的冻干粉或无水浓缩剂,所述密封容器例如是指明活性剂的量的安瓿或小药囊。在通过输注给予组合物时,可以用含有无菌药用级水或盐水的输液瓶配制。在通过注射给予组合物时,可以提供无菌注射用水或盐水的安瓿,使得可以在给药前将这些成分混合。

[0180] 可以将这些组合物配制为中性形式或盐形式。药学上可接受的盐包括但不限于由阴离子形成的那些,例如来源于盐酸、磷酸、乙酸、草酸、酒石酸等的那些,以及由阳离子形成的那些,例如来源于钠、钾、铵、钙、铁的氢氧化物,异丙胺,三乙胺,2-乙氨基乙醇,组氨酸,普鲁卡因等的那些。

H.试剂盒

[0181] 一个实施例提供了包括一个或多个填充有抗体或融合蛋白的容器的药物包或试剂盒。另外,对于治疗疾病有用的一种或多种其他预防剂或治疗剂也可以包括在药物包或试剂盒中。一个实施例提供了包括填充有一种或多种药用组合物的成分的一个或多个容器的药物包或试剂盒。可任选地,与这样一个或多个容器相关联的可以是由管理药物或生物制品的制造、使用或销售的政府机构规定的形式的公告,所述公告反映该机构针对人类给予,对制造、使用或销售的许可。

[0182] 本发明提供了可以在以上方法中使用的试剂盒。在一个实施例中,试剂盒包括一种或多种抗体或融合蛋白。在另一实施例中,试剂盒进一步包括在一个或多个容器中,对治疗癌症有用的一种或多种其他预防剂或治疗剂。在另一实施例中,试剂盒进一步包括结合与癌症有关的一种或多种癌症抗原的一种或多种细胞毒性抗体。在某些实施例中,其他预防剂或者治疗剂是化学治疗剂。在其他实施例中,预防剂或治疗剂是生物治疗剂或激素治疗剂。

I.诊断方法

[0183] B7-H5抗体及其抗原结合片段可以用于诊断目的,例如检测、诊断、或监测与B7-H5

表达有关的疾病、失调或感染。本发明提供了对于疾病、失调或感染，特别是自身免疫性疾病的检测或诊断，包括：(a)使用免疫特异性地结合至此类抗原的一种或多种抗体(或其片段)，测定在受试者的细胞或组织样品中B7-H5的表达；并且(b)将该抗原的水平与对照水平(例如，在正常组织样品中的水平)进行比较，借此相比于该抗原的对照水平而言该抗原的测定水平的增加或降低指示该疾病、失调或感染。在免疫测定，诸如酶联免疫吸附测定(ELISA)、放射性免疫测定(RIA)以及荧光激活的细胞分选(FACS)中优选采用此类抗体以及片段。

[0184] 一个实施例涉及将此类抗体以及片段、并且特别是结合到人B7-H5的此类抗体以及片段用于体外或原位组织样品或体内细胞中的IHC分析的试剂。因此，本发明的这些抗体以及片段在检测以及诊断人的疾病、失调、或感染中具有效用。在一个实施例中，此类诊断包括：a)将有效量的免疫特异性地结合到B7-H5的标记的抗体或抗原结合片段给予至受试者(例如，经肠胃外、皮下、或腹膜内)；b)在给药后等待一个时间间隔以允许该标记的分子优先集中在受试者中B7-H5表达的部位(并且未结合的标记的分子被清除至背景水平)；c)确定背景水平；并且d)检测受试者中的标记抗体，使得高于背景水平的标记抗体的检测指示该受试者患有疾病、失调、或感染。根据此实施例，用成像部分标记该抗体，该成像部分可使用本领域的普通技术人员已知的成像系统检测到。背景水平可以通过不同方法确定，这些方法包括将所检测到的标记的分子的量与之前针对具体系统测定的标准值进行对比。

[0185] 本领域将理解的是，受试者和使用的成像系统的大小将决定产生诊断图像所需的成像部分的量。在以下项中描述了体内肿瘤成像：S.W.布切尔(Burchiel)等人，“放射标记的抗体及其片段的免疫药代动力学(Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments)”，(肿瘤成像，第13章：癌症的生物化学检测(TUMOR IMAGING:THE RADIOCHEMICAL DETECTION OF CANCER)，S.W.布切尔(Burchiel)和B.A.罗德(Rhodes)编辑，马森出版公司(Masson Publishing Inc.)(1982)。

[0186] 依赖于若干变量，包括所使用的标记的类型以及给予模式，在给药后用以允许该标记的分子优先集中在受试者的部位并且未结合的标记的分子被清除至背景水平的一个时间间隔是6至48小时或6至24小时或6至12小时。在另一个实施例中，给药后的时间间隔是5至20天或5至10天。

[0187] 在一个实施例中，通过重复用于诊断疾病、失调或感染的方法对该疾病、失调或感染进行监测，例如，初次诊断一个月之后，初次诊断六个月之后，初次诊断一年之后等。

[0188] 可以使用本领域中已知的用于体内扫描的方法在受试者中检测该标记的分子的存在。这些方法依赖于所使用的标记的类型。技术人员将能够确定用于检测具体的标记的适当的方法。可用于这些披露的诊断方法的方法以及装置包括但不限于计算机断层摄影(CT)、全身扫描诸如正电子发射体层摄影(PET)、磁共振成像(MRI)、以及超声检查。

[0189] 在一个特定实施例中，该分子是用放射性同位素标记的，并且在患者中使用辐射响应外科仪器检测(瑟斯顿(Thurston)等人，美国专利号5,441,050)。在另一个实施例中，该分子是用荧光化合物标记的，并且在患者中使用荧光响应的扫描仪器检测。在另一个实施例中，该分子是用正电子发射金属标记的，并且在患者中使用正电子发射断层摄影检测。在又另一个实施例中，该分子是用顺磁标记来标记的，并且在患者中使用磁共振成像(MRI)检测。

[0190] 目前总体上已经描述了本发明，其内容可以通过参考下面的实例更容易理解，这些实例是以说明的方式提供，而并非旨在限制本发明，除非特别指明。

实例1

抗人类B7-H5抗体的分离和表征

[0191] 通过用B7-H5免疫仓鼠，并且分离表达B7-H5免疫反应性抗体的杂交瘤，获得抗人类B7-H5抗体。分离产生抗体的克隆2D3和18C3。图4A-4B示出由克隆2D3和18C3产生的抗体结合由CHO转染子表达的人类B7-H5的能力。

[0192] 通过在此类抗体的存在下孵育表达人类B7-H5的CHO转染子，并且增加CD28H Ig(SEQ ID NO:20)和抗人类Ig PE的浓度，来确定分离的抗体阻滞B7-H5:CD28H相互作用的能力。

[0193] 在这样一个实验中，在非阻滞抗体的存在下孵育的细胞能够结合CD28H Ig(参见如第二迁移峰)(图5A-5B)。发现抗体2D3和18C3阻滞B7-H5:CD28H相互作用。

[0194] 针对其阻滞B7-H5:CD28H相互作用的能力，测试本发明的抗B7-H5抗体。制备B7-H5Ig融合体并且发现它能够结合CD28H并且由此能够刺激T细胞应答(图6)。融合蛋白是融合至鼠IgG2a序列的B7-H5的胞外结构域(SEQ ID NO:26)。

IFPLAFFIYVPMNEQIVIGRLDEDIILPSSFERGSEVVIHWKYQDSYKVHSYYKGSDHLESQDPRYANRTSLFYNEIQNGNASLFFRRVSLLDEGIYTCYVGTAIQVITNKVVLKVGVFLTPVMKYEKRNTNSFLICSVLSVYPRPIITWKMDNTPISENNMEETGSLDSFSINSPLNITGSNSSYECTIENSLLKQTWTGRWTMKDGLHKMQSEHVSLSCQPVNDYFSPNQDFKVTWSRMKSGTFSVLAYYLSSSQNTIINESRFSWNKELINQSDFSMNLMDLNLSDSGEYLCNISSDEYTLLTIHTVHVEPSQET

IgG2a序列可以是(SEQ ID NO:27)

EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

[0195] mIgG2a并不要求S->P修饰(在以下更详细讨论并且如SEQ ID NO:20中示出)来稳定Fab臂或不要求缺失C末端Lys。还可以用其他Ig序列，例如人类IgG4来制造融合蛋白。对于人类IgG4，它可以优选包括S->P修饰和/或缺失最后的Lys残基，如在以上讨论的示例性hIgG4蛋白中说明。

[0196] 融合蛋白表达自编码CD33信号肽(不是天然信号肽)的载体。

[0197] 图7示出B7-H5 Ig诱导细胞因子(IL-2、IFN-γ和IL-10)表达的能力，由此确认该分子刺激免疫系统的能力。抗B7-H5抗体2D3的提供抑制了这种刺激(图8)。还发现该抗体能够抑制异体T细胞应答(图9)。这些结果指示能够结合B7-H5从而阻滞B7-H5的与其CD28H反受体相互作用的能力的本发明的那些抗体能够介导免疫系统活化中的生理学下降。

实例2

抗人类B7-H5抗体降低体内活化T细胞的百分比

[0198] 为了研究抗人类B7-H5抗体的体内作用，将2000万个外周血单核细胞(PBMC)注入NOD scid IL2受体γ链敲除(NSG)小鼠的腹膜腔中(参见例如艾奥恩斯(Iorns)，E.等人(2012年10月31日电子出版)“用于研究人类乳癌转移的新小鼠模型(A New Mouse Model

For The Study Of Human Breast Cancer Metastasis)",公共科学图书馆-(PLoS One)7(10):e47995;米沙林(Misharin),A.V.等人(2012)"开发新人源化小鼠模型来研究急性炎症性关节炎(Development Of A New Humanized Mouse Model To Study Acute Inflammatory Arthritis)"转化医学杂志(J.Transl.Med.)10:190:沃尔德伦(Waldron)-林奇(Lynch)等人(2012年8月17日电子出版)"用人源化小鼠中的小鼠皮肤移植模型分析人类生物学(Analysis Of Human Biologies With A Mouse Skin Transplant Model In Humanized Mice)",美国移植杂志(Amer.J.Transplant.)12(10):2652-2662;沃尔克(Volk),A.等人(2012)"用于T细胞过继转移的三个人源化小鼠模型的比较(Comparison Of Three Humanized Mouse Models For Adoptive T Cell Transfer)",基因医学杂志(J.Gene Med.)14(8):540-548;莱基(Racki),W.J.等人(2010)"人类皮肤移植和同种异体移植排斥的小鼠模型(NOD-scid IL2rgamma(null)Mouse Model Of Human Skin Transplantation And Allograft Rejection)",移植(Transplantation)89(5):527-536)。将磷酸盐缓冲盐水(PBS)或抗人类B7-H5抗体(2D3)注入动物的尾静脉并且确定$CD45RO^+$细胞中$CD28H^+$细胞的百分比。

[0199] 如图10A和图10B中示出,当用抗人类B7-H5抗体进行治疗时,在脾脏(图10A)和外周血(图10B)中,活化的($CD45RO^+$)人类T细胞中的$CD28H^+$细胞的百分比降低。因此用B7-H5抗体进行治疗降低了体内活化T细胞的百分比。相比之下,这种治疗对原初($CD45RO^-$)T细胞群体(图11A和图11B)仅具有有限的作用。

实例3

重组抗人类B7-H5抗体保持了抗原特异性

[0200] IgG4抗体具有独特的结构的和功能的特性并且在体内经历"半抗体交换",生成由两个不同结合特异性组成的重组抗体(尼如拉(Nirula),A.等人(2011)"什么是Igg4?独特的免疫球蛋白亚型的生物学的综述(What Is Igg4?A Review Of The Biology Of A Unique Immunoglobulin Subtype)",风湿病学新见(Curr.Opin.Rheumatol.)23(1):119-124;阿尔伯斯(Aalberse),R.C.等人(2009)"免疫球蛋白G4:古怪的抗体(Immunoglobulin G4:An Odd Antibody)"临床和实验过敏症(Clin.Exp.Allergy)39(4):469-477)。将Ser228突变为Pro以稳定该臂。嵌合hIgG4P并不做"半抗体交换"。

[0201] 将本发明的人类B7-H5结合抗体(抗体2D3和18C3)从其天然仓鼠同种型重组地转化为人类IgG4P同种型。然后在重组抗体的存在下,孵育表达人类B7-H5的CHO转染子。如图12A-C中示出,发现人类IgG4P衍生抗体都保持了其朝向人类B7-H5的免疫特异性结合能力并且阻滞了破坏B7-H5-CD28H相互作用的能力(图13,小图A-C)。图14A-14B示出抗体2D3和18C3识别B7-H5的第一IgV结构域,该结构域介导了B7-H5与CD28H的相互作用(图14C)。图15比较了抗体2D3和18C3结合至如表达在CHO细胞的表面上的人类B7-H5的亲和力。

实例4

B7-H5:CD28H相互作用的阻滞抑制破伤风类毒素(TT)特异性T细胞应答

[0202] 为了确定B7-H5:CD28H相互作用对记忆特异性T细胞的召回的影响,用破伤风类毒素("TT")疫苗来免疫用人类PBMC加上自体树突细胞重构的NSG小鼠。在同一天,用对照Ig、或抗人类B7-H5抗体2D3治疗小鼠。七天后,收获腹膜腔中的细胞,并且用TT抗原重新刺激。通过BrdU掺入来分析T细胞分裂。用人类Th1/Th2CBA试剂盒来检查培养上清液中的细胞因

子。

[0203] 在图16A-16B中示出此类研究的结果。如这些图中示出，对于TT特异性记忆T细胞的召回，内源B7-H5:CD28H相互作用显现是重要的，因为用阻滞mAb 2D3的B7-H5注射小鼠显著抑制了TT疫苗引发的T细胞增殖，如通过BrdU掺入所指示的(图16A，小图A-B)。其结果是，包括IFN-γ、IL-5和IL-10的培养上清液中的细胞因子都基本上被2D3mAb降低(图16B，小图A-B)。综上，结果表明B7-H5:CD28H相互作用在记忆T细胞的召回中起到重要作用。

实例5

B7-H5:CD28H相互作用的阻滞抑制异体T细胞应答

[0204] 为了确定B7-H5:CD28H相互作用对体内异体应答的作用，用异体人类巨噬细胞来激发人源化NSG小鼠。在同一天，用对照或2D3mAb接种每一小鼠。在第7天，收获脾细胞并且通过细胞内染色，用Ki-67表达来评估异体CD4和CD8T细胞增殖。

[0205] 在图17中示出此类研究的结果。如在图17中所示，对于异体T细胞应答，内源B7-H5:CD28H相互作用显现是重要的，因为用阻滞mAb 2D3的B7-H5注射小鼠显著抑制了CD8+(图17A)和CD4+(图17B)T细胞增殖，如通过Ki-67表达所指示的。

实例6

B7-H5:CD28H相互作用的阻滞抑制人类T细胞中的AKT活化

[0206] 在图18中示出此类研究的结果。如在图18中所示，对于存在TCR信号的AKT通路活化，内源B7-H5:CD28H相互作用显现是重要的。在刺激后30min，TCR和B7-H5融合蛋白的同时交联诱导AKT磷酸化，同时TCR交联单独诱导最小的AKT磷酸化。阻滞抗体2D3的B7-H5的包含阻止AKT活化，指示B7-H5阻滞可以阻滞T细胞AKT通路活化。

实例7

B7-H5:CD28H相互作用的阻滞抑制针对肿瘤细胞的细胞毒性T淋巴细胞杀伤

[0207] 在图19中示出此类研究的结果。如在图19中所示，肿瘤相关B7-H5与细胞毒性T细胞表达的CD28H的相互作用共刺激细胞毒性杀伤活性，因为在若干效应子与靶(E:T)的比率条件下，通过诱饵受体融合蛋白阻滞B7-H5-CD28H通路抑制了CTL杀伤。

实例8

B7-H5:CD28H相互作用的阻滞抑制自然杀伤细胞活化

[0208] 在图20A-B中示出此类研究的结果。如在图20中示出，在可溶性重组人类IL-2的存在下，自然杀伤细胞(NK)上的CD16和B7-H5融合蛋白的同时交联诱导NK活化和脱粒(CD107a表面上调)，而单独CD16接合并不诱导显著的NK脱粒。通过B7-H5抗体2D3阻滞B7-H5-CD28H通路抑制了NK脱粒(图20B)，指示B7-H5共刺激NK活化。

[0209] 本说明书提到的所有公开和专利均通过引用结合在此，引用程度就如同每个单独公开或专利申请特定地并且单独地指示通过引用以其全文结合在此一般。尽管已经联系其具体实施例描述了本发明，但是将理解，它能够进一步修改，并且本申请旨在覆盖按照本发明的一般原理，本发明的任何变化、用途、或改编，并且包括本披露的此类偏离，只要其落入本发明从属领域内的已知的或常规的实践内，并且可以适用于在此以上列出的基本特征。

序列表

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<120> 抗-B7-H5抗体及其用途

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<151> 2013-05-24

<160> 29

<170> PatentIn 3.5版

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Pro Met Asn Glu Gln Ile Val Ile Gly Arg Leu Asp Glu Asp Ile Ile
35 40 45

Leu Pro Ser Ser Phe Glu Arg Gly Ser Glu Val Val Ile His Trp Lys
50 55 60

Tyr Gln Asp Ser Tyr Lys Val His Ser Tyr Tyr Lys Gly Ser Asp His
65 70 75 80

Leu Glu Ser Gln Asp Pro Arg Tyr Ala Asn Arg Thr Ser Leu Phe Tyr
85 90 95

Asn Glu Ile Gln Asn Gly Asn Ala Ser Leu Phe Phe Arg Arg Val Ser
100 105 110

Leu Leu Asp Glu Gly Ile Tyr Thr Cys Tyr Val Gly Thr Ala Ile Gln
115 120 125

Val Ile Thr Asn Lys Val Val Leu Lys Val Gly Val Phe Leu Thr Pro
130 135 140

Val Met Lys Tyr Glu Lys Arg Asn Thr Asn Ser Phe Leu Ile Cys Ser
145 150 155 160

Val Leu Ser Val Tyr Pro Arg Pro Ile Ile Thr Trp Lys Met Asp Asn
165 170 175

Thr Pro Ile Ser Glu Asn Asn Met Glu Glu Thr Gly Ser Leu Asp Ser
180 185 190

Phe Ser Ile Asn Ser Pro Leu Asn Ile Thr Gly Ser Asn Ser Ser Tyr
195 200 205

Glu Cys Thr Ile Glu Asn Ser Leu Leu Lys Gln Thr Trp Thr Gly Arg
210 215 220

Trp Thr Met Lys Asp Gly Leu His Lys Met Gln Ser Glu His Val Ser
225 230 235 240

Leu Ser Cys Gln Pro Val Asn Asp Tyr Phe Ser Pro Asn Gln Asp Phe
245 250 255

Lys Val Thr Trp Ser Arg Met Lys Ser Gly Thr Phe Ser Val Leu Ala
260 265 270

Tyr Tyr Leu Ser Ser Ser Gln Asn Thr Ile Ile Asn Glu Ser Arg Phe
275 280 285

Ser Trp Asn Lys Glu Leu Ile Asn Gln Ser Asp Phe Ser Met Asn Leu
290 295 300

Met Asp Leu Asn Leu Ser Asp Ser Gly Glu Tyr Leu Cys Asn Ile Ser
305 310 315 320

Ser Asp Glu Tyr Thr Leu Leu Thr Ile His Thr Val His Val Glu Pro
325 330 335

Ser Gln Glu Thr Ala Ser His Asn Lys Gly Leu Trp Ile Leu Val Pro
340 345 350

[0002] Ser Ala Ile Leu Ala Ala Phe Leu Leu Ile Trp Ser Val Lys Cys Cys
355 360 365

Arg Ala Gln Leu Glu Ala Arg Arg Ser Arg His Pro Ala Asp Gly Ala
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Gln Gln Glu Arg Cys Cys Val Pro Pro Gly Glu Arg Cys Pro Ser Ala
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caaggcatat tccctttggc tttcttcatt tatgttccta tgaatgaaca aatcgtcatt 120

ggaagacttg atgaagatat aatttctccc tcttcatttg agaggggatc cgaagtcgta 180

atacactgga agtatcaaga tagctataag gttcatagtt actacaaagg cagtgaccat 240

ttggaaagcc aagatcccag atatgcaaac aggacatccc ttttctataa tgagattcaa 300

aatgggaatg cgtcactatt tttcagaaga gtaagccttc tggacgaagg aatttacacc 360

tgctatgtag gaacagcaat tcaagtgatt acaaacaaag tggtgctaaa ggtgggagtt 420

tttctcacac ccgtgatgaa gtatgaaaag aggaacacaa acagcttctt aatatgcagc 480

gtgttaagtg tttatcctcg tccaattatc acgtggaaaa tggacaacac acctatctct 540

gaaaacaaca tggaagaaac agggtctttg gattcttttt ctattaacag cccactgaat 600

attacaggat caaattcatc ttatgaatgt acaattgaaa attcactgct gaagcaaaca 660

tggacagggc gctggacgat gaaagatggc cttcataaaa tgcaaagtga acacgtttca 720

ctctcatgtc aacctgtaaa tgattatttt tcaccaaacc aagacttcaa agttacttgg 780

tccagaatga aaagtgggac tttctctgtc ctggcttact atctgagctc ctcacaaaat 840

acaattatca atgaatcccg attctcatgg aacaaagagc tgataaacca gagtgacttc 900

tctatgaatt tgatggatct taatctttca gacagtgggg aatatttatg caatatttct 960

tcggatgaat atactttact taccatccac acagtgcatg tagaaccgag ccaagaaaca 1020

gcttcccata acaaaggctt atggattttg gtgccctctg cgattttggc agcttttctg 1080

ctgatttgga gcgtaaaatg ttgcagagcc cagctagaag ccaggaggag cagacaccct 1140

gctgatggag cccaacaaga aagatgttgt gtccctcctg gtgagcgctg tcccagtgca 1200

cccgataatg gcgaagaaaa tgtgcctctt tcaggaaaag ta 1242

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Arg Gly Ser Glu Val Val Ile His Trp Lys Tyr Gln Asp Ser Tyr Lys
35 40 45

Val His Ser Tyr Tyr Lys Gly Ser Asp His Leu Glu Ser Gln Asp Pro
50 55 60

Arg Tyr Ala Asn Arg Thr Ser Leu Phe Tyr Asn Glu Ile Gln Asn Gly
65 70 75 80

Asn Ala Ser Leu Phe Phe Arg Arg Val Ser Leu Leu Asp Glu Gly Ile
85 90 95

Tyr Thr Cys Tyr Val Gly Thr Ala Ile Gln Val Ile Thr Asn Lys Val
100 105 110

Val Leu Lys Val Gly Val Phe Leu Thr Pro Val Met Lys Tyr Glu Lys
115 120 125

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe
130 135 140

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
145 150 155 160

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
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Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
180 185 190

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
195 200 205

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
210 215 220

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
225 230 235 240

Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
245 250 255

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
260 265 270

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
275 280 285

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
290 295 300

[0004] Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
305 310 315 320

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
325 330 335

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
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Leu Ser Pro Gly
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caggga 66

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<211> 1068
<212> DNA
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<220>
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tggaagtacc aggactccta caaggtgcac tcctactaca agggctccga ccacctggaa 180

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aacgcctccc tgttcttccg gcgagtgtcc ctgctggatg agggcatcta caccctgttc 300

gtgggcaccg ccatccaagt gatcaccaac aaggtggtgc tgaaagtggg cgtgttcctg 360

acccccgtga tgaaglacga gaaagagtct aagtacggcc ctccctgccc ccctlgtcct 420

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Gln Val Arg Gln Gly Ser Gln Ala Thr Leu Val Cys Gln Val Asp Gln
35 40 45

Ala Thr Ala Trp Glu Arg Leu Arg Val Lys Trp Thr Lys Asp Gly Ala
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Ile Leu Cys Gln Pro Tyr Ile Thr Asn Gly Ser Leu Ser Leu Gly Val
65 70 75 80

Cys Gly Pro Gln Gly Arg Leu Ser Trp Gln Ala Pro Ser His Leu Thr
85 90 95

Leu Gln Leu Asp Pro Val Ser Leu Asn His Ser Gly Ala Tyr Val Cys
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115 120 125

Thr Arg Leu Phe Val Asp Pro Asp Asp Pro Thr Gln Asn Arg Asn Arg
130 135 140

Ile Ala Ser Phe Pro Gly Phe Leu Phe Val Leu Leu Gly Val Gly Ser
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Met Gly Val Ala Ala Ile Val Trp Gly Ala Trp Phe Trp Gly Arg Arg
165 170 175

Ser Cys Gln Gln Arg Asp Ser Gly Asn Ser Pro Gly Asn Ala Phe Tyr
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Ser Asn Val Leu Tyr Arg Pro Arg Gly Ala Pro Lys Lys Ser Glu Asp
195 200 205

Cys Ser Gly Glu Gly Lys Asp Gln Arg Gly Gln Ser Ile Tyr Ser Thr
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[0006]

Ser Phe Pro Gln Pro Ala Pro Arg Gln Pro His Leu Ala Ser Arg Pro
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Cys Pro Ser Pro Arg Pro Cys Pro Ser Pro Arg Pro Gly His Pro Val
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Pro Lys Gly Phe Pro Lys Val Gly Glu Glu
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<211> 846
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accctggtct gccaggtgga ccaggccaca gcctgggaac ggctccgtgt taagtggaca 180

aaggatgggg ccatcctgtg tcaaccgtac atcaccaacg gcagcctcag cctgggggtc 240

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cctgtgagcc tcaaccacag cggggcgtac gtgtgctggg cggccgtaga gattcctgag 360

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aacagaaacc ggatcgcaag cttcccagga ttcctcttcg tgctgctggg ggtgggaagc 480

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tgccccagcc cgagaccctg ccccagcccc aggcccggcc acccccgtctc tatggtcagg 780

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gaggag 846

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35 40 45

Gly Trp Ile Phe Pro Gly Asp Gly Ser Thr Lys Phe Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Ile Gln Leu Ser Arg Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
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Ala Arg Asn Ser Phe Tyr Ser Met Asp Tyr Trp Gly Gln Gly Thr Ser
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Val Thr Val Ser Ser
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<210> 10
<211> 351
<212> DNA
<213> 人工序列

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cctgaactgg gacttgagtg gattggatgg attttttcctg gggatggtag tactaagttc 180

aatgagaagt tcaagggcaa ggccacactg actacagaca aatcctccag cacagcctac 240

atacagctca gcaggctgac gtctgaggac tctgctgtct atttctgtgc aagaaactcc 300

ttctactcta tggactattg gggtcaagga acctcagtca ccgtctcctc a 351

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Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30

Arg Thr Arg Lys Asn Gln Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Phe Ile Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

[0008]

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Ser Tyr Asn Leu Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
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<210> 12
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tggtaccagc agaaaccagg acagtctcct aaattactga tctactgggc attcattagg 180

gaatctgggg tccctgatcg cttcacaggc agtggatctg ggacagattt cactctcacc 240

atcagcagtg tgcaggctga agacctggca gtttattact gcaagcaatc ttataatctt 300

cggacgttcg gtggaggcac caagctggaa atcaaac 337

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<213> 人工序列

<220>
<223> 合成的多肽 - 重链可变区抗-人类B7-H5克隆18C3

<400> 13

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
20 25 30

Asp Ile Asn Trp Val Arg Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Phe Pro Gly Asp Gly Ser Thr Lys Phe Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Ile Gln Leu Ser Arg Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Asn Ser Phe Tyr Ser Met Asp Tyr Trp Gly Gln Gly Thr Ser
100 105 110

Val Thr Val Ser Ser
115

<210> 14
<211> 351
<212> DNA
<213> 人工序列

[0009]

<220>
<223> 合成的多核苷酸- 编码重链可变区抗-人类B7-H5克隆18C3的多核苷酸

<400> 14
caggttcaac tgcagcagtc tggagctgaa ctggtaaagc ctggggcttc agtgaagttg 60

tcctgcaagg cttctggcta caccttcaca agccatgata taaactgggt gaggcagagg 120

cctgaacagg gacttgagtg gattggatgg attttttcctg gggatggtag tactaagttc 180

aatgagaagt tcaagggcaa ggccacactg actacagaca aatcctccag cacagcctac 240

atacagctca gcaggctgac atctgaggac tctgctgtct atttctgtgc aagaaactcc 300

ttctattcta tggactactg gggtcaagga acctcagtca ccgtctcctc a 351

<210> 15
<211> 112
<212> PRT
<213> 人工序列

<220>
<223> 合成的多肽 - 轻链可变区抗-人类B7-H5克隆18C3

<400> 15

Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30

Arg Thr Arg Lys Asn Gln Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Lys Gln
85 90 95

Ser Tyr Asn Leu Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 16
<211> 4
<212> PRT
<213> 人工序列

<220>
<223> 合成的多肽 - 接头

<400> 16

Gly Ser Gly Ser
1

<210> 17
<211> 4
<212> PRT
<213> 人工序列

<220>
<223> 合成的多肽 - 接头

[0010]

<400> 17

Gly Gly Gly Ser
1

<210> 18
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 合成的多肽 - 接头

<400> 18

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 19
<211> 20
<212> PRT
<213> 人工序列

<220>
<223> 合成的多肽 - 接头

<400> 19

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser
20

<210> 20
<211> 347
<212> PRT
<213> 人工序列

<220>
<223> 合成的多肽 - CD28H-hIgG4融合蛋白

<400> 20

Leu Ser Val Gln Gln Gly Pro Asn Leu Leu Gln Val Arg Gln Gly Ser
1 5 10 15

Gln Ala Thr Leu Val Cys Gln Val Asp Gln Ala Thr Ala Trp Glu Arg
20 25 30

Leu Arg Val Lys Trp Thr Lys Asp Gly Ala Ile Leu Cys Gln Pro Tyr
35 40 45

Ile Thr Asn Gly Ser Leu Ser Leu Gly Val Cys Gly Pro Gln Gly Arg
50 55 60

Leu Ser Trp Gln Ala Pro Ser His Leu Thr Leu Gln Leu Asp Pro Val
65 70 75 80

Ser Leu Asn His Ser Gly Ala Tyr Val Cys Trp Ala Ala Val Glu Ile
85 90 95

Pro Glu Leu Glu Glu Ala Glu Gly Asn Ile Thr Arg Leu Phe Val Asp
100 105 110

[0011]

Pro Asp Asp Pro Thr Gln Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro
115 120 125

Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
130 135 140

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
145 150 155 160

Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
165 170 175

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
180 185 190

Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
195 200 205

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
210 215 220

Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
225 230 235 240

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
245 250 255

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
260 265 270

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
275 280 285

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
290 295 300

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
305 310 315 320

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
325 330 335

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
340 345

<210> 21
<211> 20
<212> PRT
<213> 人工序列

<220>
<223> 合成的多肽 - 信号序列

<400> 21

Met Ser Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr
1 5 10 15

Asp Ala Arg Cys
20

[0012]

<210> 22
<211> 22
<212> PRT
<213> 人工序列

<220>
<223> 合成的多肽 - 信号序列

<400> 22

Met Gly Ser Pro Gly Met Val Leu Gly Leu Leu Val Gln Ile Trp Ala
1 5 10 15

Leu Gln Glu Ala Ser Ser
20

<210> 23
<211> 1104
<212> DNA
<213> 人工序列

<220>
<223> 合成的多核苷酸- 编码CD28H-Ig的多核苷酸

<400> 23
atgtccgtgc ccacccaggt gctgggattg ctgctgctgt ggctgaccga cgccagatgc 60

ctgtctgtgc agcagggccc taacctgctg caagtgcggc agggctctca ggctacactc 120

gtgtgtcagg tggaccaggc caccgcctgg gagagactga gagtgaagtg gaccaaggac 180

ggcgccatcc tgtgccagcc ctacatcacc aacggctccc tgtccctggg cgtgtgtgga 240

cctcagggca gactgtcttg gcaggccccc tctcacctga ccctgcagct ggaccctgtg 300

tccctgaatc actccggcgc ctacgtgtgt tgggccgctg tggaaatccc cgagctggaa 360

gaggccgagg gcaacatcac ccggctgttc gtggaccctg acgaccctac ccaggaatct 420

aagtacggcc ctcccctgcc cttcttgccca gcccctgaat ttctgggcgg accctccgtg 480

ttcctgttcc ccccaaagcc caaggacacc ctgatgatct cccggacccc cgaagtgacc 540

tgcgtggtgg tggatgtgtc ccaggaagat cccgaggtgc agttcaattg gtacgtggac 600

ggcgtggaag tgcacaacgc caagaccaag cccagagagg aacagttcaa ctccacctac 660

cgggtggtgt ccgtgctgac cgtgctgcac caggattggc tgaacggcaa agagtacaag 720

tgcaaggtgt ccaacaaggg cctgcccagc tccatcgaaa agaccatctc caaggccaag 780

ggccagcccc gggaacccca ggtgtacaca ctgcctccaa gccaggaaga gatgaccaag 840

aaccaggtgt cactgacctg tctcgtgaag ggcttctacc cctccgatat cgccgtggaa 900

tgggagtcca acggccagcc tgagaacaac tacaagacca ccccccctgt gctggactcc 960

gacggctcct tcttcctgta ctcccgcctg accgtggaca agtccagatg gcaggaaggc 1020

aacgtgttct cctgctccgt gatgcacgag gccctgcaca accactacac ccagaagtcc 1080

ctgagcctgt cccccggcaa gtga 1104

<210> 24
<211> 546
<212> PRT
<213> 人工序列

<220>
<223> 合成的多肽 - B7-H5ECD-hIgG4P融合蛋白

<400> 24

[0013]

Ile Phe Pro Leu Ala Phe Phe Ile Tyr Val Pro Met Asn Glu Gln Ile
1 5 10 15

Val Ile Gly Arg Leu Asp Glu Asp Ile Ile Leu Pro Ser Ser Phe Glu
20 25 30

Arg Gly Ser Glu Val Val Ile His Trp Lys Tyr Gln Asp Ser Tyr Lys
35 40 45

Val His Ser Tyr Tyr Lys Gly Ser Asp His Leu Glu Ser Gln Asp Pro
50 55 60

Arg Tyr Ala Asn Arg Thr Ser Leu Phe Tyr Asn Glu Ile Gln Asn Gly
65 70 75 80

Asn Ala Ser Leu Phe Phe Arg Arg Val Ser Leu Leu Asp Glu Gly Ile
85 90 95

Tyr Thr Cys Tyr Val Gly Thr Ala Ile Gln Val Ile Thr Asn Lys Val
100 105 110

Val Leu Lys Val Gly Val Phe Leu Thr Pro Val Met Lys Tyr Glu Lys
115 120 125

Arg Asn Thr Asn Ser Phe Leu Ile Cys Ser Val Leu Ser Val Tyr Pro
130 135 140

Arg Pro Ile Ile Thr Trp Lys Met Asp Asn Thr Pro Ile Ser Glu Asn
145 150 155 160

Asn Met Glu Glu Thr Gly Ser Leu Asp Ser Phe Ser Ile Asn Ser Pro
165 170 175

Leu Asn Ile Thr Gly Ser Asn Ser Ser Tyr Glu Cys Thr Ile Glu Asn
180 185 190

Ser Leu Leu Lys Gln Thr Trp Thr Gly Arg Trp Thr Met Lys Asp Gly
195 200 205

Leu His Lys Met Gln Ser Glu His Val Ser Leu Ser Cys Gln Pro Val
210 215 220

Asn Asp Tyr Phe Ser Pro Asn Gln Asp Phe Lys Val Thr Trp Ser Arg
225 230 235 240

Met Lys Ser Gly Thr Phe Ser Val Leu Ala Tyr Tyr Leu Ser Ser Ser
245 250 255

Gln Asn Thr Ile Ile Asn Glu Ser Arg Phe Ser Trp Asn Lys Glu Leu
260 265 270

Ile Asn Gln Ser Asp Phe Ser Met Asn Leu Met Asp Leu Asn Leu Ser
275 280 285

Asp Ser Gly Glu Tyr Leu Cys Asn Ile Ser Ser Asp Glu Tyr Thr Leu
290 295 300

[0014] Leu Thr Ile His Thr Val His Val Glu Pro Ser Gln Glu Thr Glu Ser
305 310 315 320

Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly
325 330 335

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
340 345 350

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln
355 360 365

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
370 375 380

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr
385 390 395 400

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
405 410 415

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile
420 425 430

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
435 440 445

Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser
450 455 460

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
465 470 475 480

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
485 490 495

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val
500 505 510

Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met
515 520 525

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
530 535 540

Pro Gly
545

<210> 25
<211> 1707
<212> PRT
<213> 人工序列

<220>
<223> 合成的多核苷酸- 编码B7-H5ECD-hIgG4P的多核苷酸

<400> 25

Ala Thr Gly Ala Ala Gly Gly Cys Cys Cys Ala Gly Ala Cys Cys Gly
1 5 10 15

[0015]

Cys Cys Cys Thr Gly Thr Cys Cys Thr Thr Cys Thr Thr Cys Cys Thr
20 25 30

Gly Ala Thr Cys Cys Thr Gly Ala Thr Cys Ala Cys Cys Thr Cys Cys
35 40 45

Cys Thr Gly Thr Cys Cys Gly Gly Cys Ala Gly Cys Cys Ala Gly Gly
50 55 60

Gly Ala Ala Thr Cys Thr Thr Cys Cys Cys Thr Cys Thr Gly Gly Cys
65 70 75 80

Cys Thr Thr Cys Thr Thr Cys Ala Thr Cys Thr Ala Cys Gly Thr Gly
85 90 95

Cys Cys Cys Ala Thr Gly Ala Ala Cys Gly Ala Gly Cys Ala Gly Ala
100 105 110

Thr Cys Gly Thr Gly Ala Thr Cys Gly Gly Cys Cys Gly Gly Cys Thr
115 120 125

Gly Gly Ala Cys Gly Ala Gly Gly Ala Thr Ala Thr Thr Ala Thr Cys
130 135 140

Cys Thr Gly Cys Cys Cys Thr Cys Cys Ala Gly Cys Thr Thr Cys Gly
145 150 155 160

Ala Gly Cys Gly Gly Gly Gly Cys Thr Cys Cys Gly Ala Gly Gly Thr
165 170 175

Cys Gly Thr Gly Ala Thr Cys Cys Ala Cys Thr Gly Gly Ala Ala Gly
180 185 190

Thr Ala Cys Cys Ala Gly Gly Ala Cys Thr Cys Cys Thr Ala Cys Ala
195 200 205

Ala Gly Gly Thr Gly Cys Ala Cys Thr Cys Cys Thr Ala Cys Thr Ala
210 215 220

Cys Ala Ala Gly Gly Gly Cys Thr Cys Cys Gly Ala Cys Cys Ala Cys
225 230 235 240

Cys Thr Gly Gly Ala Ala Thr Cys Cys Cys Ala Gly Gly Ala Cys Cys
245 250 255

Cys Cys Ala Gly Ala Thr Ala Cys Gly Cys Cys Ala Ala Cys Cys Gly
260 265 270

Gly Ala Cys Cys Ala Gly Cys Cys Thr Gly Thr Thr Cys Thr Ala Cys
275 280 285

Ala Ala Cys Gly Ala Gly Ala Thr Cys Cys Ala Gly Ala Ala Cys Gly
290 295 300

Gly Cys Ala Ala Cys Gly Cys Cys Thr Cys Cys Cys Thr Gly Thr Thr
305 310 315 320

[0016] Cys Thr Thr Cys Cys Gly Gly Cys Gly Ala Gly Thr Gly Thr Cys Cys
325 330 335

Cys Thr Gly Cys Thr Gly Gly Ala Thr Gly Ala Gly Gly Gly Cys Ala
340 345 350

Thr Cys Thr Ala Cys Ala Cys Cys Thr Gly Thr Thr Ala Cys Gly Thr
355 360 365

Gly Gly Gly Cys Ala Cys Cys Gly Cys Cys Ala Thr Cys Cys Ala Ala
370 375 380

Gly Thr Gly Ala Thr Cys Ala Cys Cys Ala Ala Cys Ala Ala Gly Gly
385 390 395 400

Thr Gly Gly Thr Gly Cys Thr Gly Ala Ala Ala Gly Thr Gly Gly Gly
405 410 415

Cys Gly Thr Gly Thr Thr Cys Cys Thr Gly Ala Cys Cys Cys Cys Cys
420 425 430

Gly Thr Gly Ala Thr Gly Ala Ala Gly Thr Ala Cys Gly Ala Gly Ala
435 440 445

Ala Gly Cys Gly Gly Ala Ala Thr Ala Cys Cys Ala Ala Cys Thr Cys
450 455 460

Thr Thr Thr Cys Cys Thr Gly Ala Thr Cys Thr Gly Cys Thr Cys Cys
465 470 475 480

Gly Thr Gly Cys Thr Gly Thr Cys Cys Gly Thr Gly Thr Ala Cys Cys
485 490 495

Cys Thr Cys Gly Gly Cys Cys Cys Ala Thr Cys Ala Thr Cys Ala Cys
500 505 510

Cys Thr Gly Gly Ala Ala Gly Ala Thr Gly Gly Ala Cys Ala Ala Cys
515 520 525

Ala Cys Cys Cys Cys Cys Ala Thr Cys Thr Cys Cys Gly Ala Gly Ala
530 535 540

Ala Cys Ala Ala Cys Ala Thr Gly Gly Ala Ala Gly Ala Gly Ala Cys
545 550 555 560

Ala Gly Gly Cys Thr Cys Cys Cys Thr Gly Gly Ala Cys Thr Cys Cys
565 570 575

Thr Thr Cys Thr Cys Cys Ala Thr Cys Ala Ala Cys Thr Cys Cys Cys
580 585 590

Cys Cys Cys Thr Gly Ala Ala Cys Ala Thr Thr Ala Cys Cys Gly Gly
595 600 605

Cys Thr Cys Cys Ala Ala Cys Thr Cys Cys Thr Cys Cys Thr Ala Cys
610 615 620

[0017]

Gly Ala Gly Thr Gly Cys Ala Cys Cys Ala Thr Cys Gly Ala Gly Ala
625 630 635 640

Ala Cys Thr Cys Cys Cys Thr Gly Cys Thr Gly Ala Ala Gly Cys Ala
645 650 655

Gly Ala Cys Cys Thr Gly Gly Ala Cys Cys Gly Gly Cys Ala Gly Ala
660 665 670

Thr Gly Gly Ala Cys Thr Ala Thr Gly Ala Ala Gly Gly Ala Cys Gly
675 680 685

Gly Cys Cys Thr Gly Cys Ala Cys Ala Ala Gly Ala Thr Gly Cys Ala
690 695 700

Gly Thr Cys Cys Gly Ala Gly Cys Ala Cys Gly Thr Gly Thr Cys Cys
705 710 715 720

Cys Thr Gly Thr Cys Cys Thr Gly Cys Cys Ala Gly Cys Cys Cys Gly
725 730 735

Thr Gly Ala Ala Cys Gly Ala Cys Thr Ala Cys Thr Thr Cys Ala Gly
740 745 750

Cys Cys Cys Cys Ala Ala Cys Cys Ala Gly Gly Ala Cys Thr Thr Cys
755 760 765

Ala Ala Ala Gly Thr Gly Ala Cys Cys Thr Gly Gly Thr Cys Cys Cys
770 775 780

Gly Gly Ala Thr Gly Ala Ala Gly Thr Cys Cys Gly Gly Cys Ala Cys
785 790 795 800

Cys Thr Thr Cys Ala Gly Cys Gly Thr Gly Cys Thr Gly Gly Cys Cys
805 810 815

Thr Ala Cys Thr Ala Cys Cys Thr Gly Thr Cys Cys Ala Gly Cys Thr
820 825 830

Cys Cys Cys Ala Gly Ala Ala Cys Ala Cys Cys Ala Thr Cys Ala Thr
835 840 845

Cys Ala Ala Cys Gly Ala Gly Thr Cys Cys Cys Gly Gly Thr Thr Cys
850 855 860

Thr Cys Cys Thr Gly Gly Ala Ala Cys Ala Ala Ala Gly Ala Gly Cys
865 870 875 880

Thr Gly Ala Thr Cys Ala Ala Cys Cys Ala Gly Thr Cys Cys Gly Ala
885 890 895

Cys Thr Thr Cys Thr Cys Cys Ala Thr Gly Ala Ala Cys Cys Thr Gly
900 905 910

Ala Thr Gly Gly Ala Cys Cys Thr Gly Ala Ala Cys Cys Thr Gly Thr
915 920 925

Cys Cys Gly Ala Cys Ala Gly Cys Gly Gly Cys Gly Ala Gly Thr Ala
930 935 940

[0018]

Cys Cys Thr Gly Thr Gly Cys Ala Ala Cys Ala Thr Cys Thr Cys Cys
945 950 955 960

Ala Gly Cys Gly Ala Cys Gly Ala Gly Thr Ala Cys Ala Cys Cys Cys
965 970 975

Thr Gly Cys Thr Gly Ala Cys Cys Ala Thr Cys Cys Ala Cys Ala Cys
980 985 990

Cys Gly Thr Gly Cys Ala Cys Gly Thr Gly Gly Ala Ala Cys Cys Cys
995 1000 1005

Thr Cys Cys Cys Ala Gly Gly Ala Ala Ala Cys Cys Gly Ala Gly
1010 1015 1020

Thr Cys Thr Ala Ala Gly Thr Ala Cys Gly Gly Cys Cys Cys Thr
1025 1030 1035

Cys Cys Cys Thr Gly Cys Cys Cys Ala Cys Cys Thr Thr Gly Thr
1040 1045 1050

Cys Cys Cys Gly Cys Cys Cys Cys Thr Gly Ala Ala Thr Thr Thr
1055 1060 1065

Cys Thr Gly Gly Gly Cys Gly Gly Ala Cys Cys Cys Thr Cys Thr
1070 1075 1080

Gly Thr Gly Thr Thr Cys Cys Thr Gly Thr Thr Cys Cys Cys Cys

1085 1090 1095

Cys Cys Ala Ala Ala Gly Cys Cys Cys Ala Ala Gly Gly Ala Cys
1100 1105 1110

Ala Cys Cys Cys Thr Gly Ala Thr Gly Ala Thr Cys Thr Cys Cys
1115 1120 1125

Cys Gly Gly Ala Cys Cys Cys Cys Cys Gly Ala Ala Gly Thr Gly
1130 1135 1140

Ala Cys Ala Thr Gly Cys Gly Thr Gly Gly Thr Gly Gly Thr Gly
1145 1150 1155

Gly Ala Thr Gly Thr Gly Thr Cys Cys Cys Ala Gly Gly Ala Ala
1160 1165 1170

Gly Ala Thr Cys Cys Cys Gly Ala Gly Gly Thr Gly Cys Ala Gly
1175 1180 1185

Thr Thr Cys Ala Ala Thr Thr Gly Gly Thr Ala Cys Gly Thr Gly
1190 1195 1200

Gly Ala Cys Gly Gly Cys Gly Thr Gly Gly Ala Ala Gly Thr Gly
1205 1210 1215

Cys Ala Cys Ala Ala Cys Gly Cys Cys Ala Ala Gly Ala Cys Cys
1220 1225 1230

[0019]

Ala Ala Gly Cys Cys Cys Ala Gly Ala Gly Ala Gly Gly Ala Ala
1235 1240 1245

Cys Ala Gly Thr Thr Cys Ala Ala Cys Thr Cys Cys Ala Cys Cys
1250 1255 1260

Thr Ala Cys Cys Gly Gly Gly Thr Gly Gly Thr Gly Thr Cys Thr
1265 1270 1275

Gly Thr Gly Cys Thr Gly Ala Cys Cys Gly Thr Gly Cys Thr Gly
1280 1285 1290

Cys Ala Cys Cys Ala Gly Gly Ala Cys Thr Gly Gly Cys Thr Gly
1295 1300 1305

Ala Ala Cys Gly Gly Cys Ala Ala Ala Gly Ala Gly Thr Ala Cys
1310 1315 1320

Ala Ala Gly Thr Gly Cys Ala Ala Gly Gly Thr Gly Thr Cys Cys
1325 1330 1335

Ala Ala Cys Ala Ala Gly Gly Gly Cys Cys Thr Gly Cys Cys Cys
1340 1345 1350

Ala Gly Cys Thr Cys Cys Ala Thr Cys Gly Ala Ala Ala Ala Gly
1355 1360 1365

Ala Cys Cys Ala Thr Cys Thr Cys Cys Ala Ala Gly Gly Cys Cys
1370 1375 1380

Ala Ala Gly Gly Gly Cys Cys Ala Gly Cys Cys Cys Cys Gly Gly
1385 1390 1395

Gly Ala Ala Cys Cys Cys Cys Ala Gly Gly Thr Gly Thr Ala Cys
1400 1405 1410

Ala Cys Ala Cys Thr Gly Cys Cys Thr Cys Cys Ala Ala Gly Cys
1415 1420 1425

Cys Ala Gly Gly Ala Ala Gly Ala Gly Ala Thr Gly Ala Cys Cys
1430 1435 1440

Ala Ala Gly Ala Ala Cys Cys Ala Gly Gly Thr Gly Thr Cys Cys
1445 1450 1455

Cys Thr Gly Ala Cys Thr Thr Gly Cys Cys Thr Cys Gly Thr Gly
1460 1465 1470

Ala Ala Gly Gly Gly Cys Thr Thr Cys Thr Ala Cys Cys Cys Cys
1475 1480 1485

Thr Cys Cys Gly Ala Thr Ala Thr Cys Gly Cys Cys Gly Thr Gly
1490 1495 1500

Gly Ala Ala Thr Gly Gly Gly Ala Gly Thr Cys Cys Ala Ala Cys
1505 1510 1515

[0020]

Gly Gly Cys Cys Ala Gly Cys Cys Thr Gly Ala Gly Ala Ala Cys
1520 1525 1530

Ala Ala Cys Thr Ala Cys Ala Ala Gly Ala Cys Cys Ala Cys Cys
1535 1540 1545

Cys Cys Cys Cys Cys Thr Gly Thr Gly Cys Thr Gly Gly Ala Cys
1550 1555 1560

Thr Cys Cys Gly Ala Cys Gly Gly Cys Thr Cys Thr Thr Thr Cys
1565 1570 1575

Thr Thr Cys Cys Thr Gly Thr Ala Cys Thr Cys Cys Cys Gly Cys
1580 1585 1590

Cys Thr Gly Ala Cys Cys Gly Thr Gly Gly Ala Cys Ala Ala Gly
1595 1600 1605

Thr Cys Cys Ala Gly Ala Thr Gly Gly Cys Ala Gly Gly Ala Ala
1610 1615 1620

Gly Gly Cys Ala Ala Cys Gly Thr Gly Thr Thr Cys Thr Cys Cys
1625 1630 1635

Thr Gly Cys Ala Gly Cys Gly Thr Gly Ala Thr Gly Cys Ala Cys
1640 1645 1650

Gly Ala Gly Gly Cys Cys Cys Thr Gly Cys Ala Cys Ala Ala Cys
1655 1660 1665

Cys Ala Cys Thr Ala Cys Ala Cys Cys Cys Ala Gly Ala Ala Gly
1670 1675 1680

Thr Cys Cys Cys Thr Gly Ala Gly Cys Cys Thr Gly Thr Cys Cys
1685 1690 1695

Cys Cys Cys Gly Gly Cys Thr Gly Ala
1700 1705

<210> 26
<211> 318
<212> PRT
<213> 人工序列

<220>
<223> 人工多肽 - 胞外结构域

<400> 26

Ile Phe Pro Leu Ala Phe Phe Ile Tyr Val Pro Met Asn Glu Gln Ile
1 5 10 15

Val Ile Gly Arg Leu Asp Glu Asp Ile Ile Leu Pro Ser Ser Phe Glu
20 25 30

Arg Gly Ser Glu Val Val Ile His Trp Lys Tyr Gln Asp Ser Tyr Lys
35 40 45

Val His Ser Tyr Tyr Lys Gly Ser Asp His Leu Glu Ser Gln Asp Pro
50 55 60

[0021]

Arg Tyr Ala Asn Arg Thr Ser Leu Phe Tyr Asn Glu Ile Gln Asn Gly
65 70 75 80

Asn Ala Ser Leu Phe Phe Arg Arg Val Ser Leu Leu Asp Glu Gly Ile
85 90 95

Tyr Thr Cys Tyr Val Gly Thr Ala Ile Gln Val Ile Thr Asn Lys Val
100 105 110

Val Leu Lys Val Gly Val Phe Leu Thr Pro Val Met Lys Tyr Glu Lys
115 120 125

Arg Asn Thr Asn Ser Phe Leu Ile Cys Ser Val Leu Ser Val Tyr Pro
130 135 140

Arg Pro Ile Ile Thr Trp Lys Met Asp Asn Thr Pro Ile Ser Glu Asn
145 150 155 160

Asn Met Glu Glu Thr Gly Ser Leu Asp Ser Phe Ser Ile Asn Ser Pro
165 170 175

Leu Asn Ile Thr Gly Ser Asn Ser Ser Tyr Glu Cys Thr Ile Glu Asn
180 185 190

Ser Leu Leu Lys Gln Thr Trp Thr Gly Arg Trp Thr Met Lys Asp Gly
195 200 205

Leu His Lys Met Gln Ser Glu His Val Ser Leu Ser Cys Gln Pro Val
210 215 220

Asn Asp Tyr Phe Ser Pro Asn Gln Asp Phe Lys Val Thr Trp Ser Arg
225 230 235 240

Met Lys Ser Gly Thr Phe Ser Val Leu Ala Tyr Tyr Leu Ser Ser Ser
245 250 255

Gln Asn Thr Ile Ile Asn Glu Ser Arg Phe Ser Trp Asn Lys Glu Leu
260 265 270

Ile Asn Gln Ser Asp Phe Ser Met Asn Leu Met Asp Leu Asn Leu Ser
275 280 285

Asp Ser Gly Glu Tyr Leu Cys Asn Ile Ser Ser Asp Glu Tyr Thr Leu
290 295 300

Leu Thr Ile His Thr Val His Val Glu Pro Ser Gln Glu Thr
305 310 315

<210> 27
<211> 233
<212> PRT
<213> 人工序列

<220>
<223> 合成的多肽 - 鼠IgG2a结构域

<400> 27

Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro
1 5 10 15

[0022]

Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys
20 25 30

Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val
35 40 45

Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe
50 55 60

Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu
65 70 75 80

Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln His
85 90 95

Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys
100 105 110

Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser
115 120 125

Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met
130 135 140

Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro
145 150 155 160

Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn

165 170 175

Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met
180 185 190

Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser
195 200 205

Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr Thr
210 215 220

Lys Ser Phe Ser Arg Thr Pro Gly Lys
225 230

<210> 28
<211> 337
<212> DNA
<213> 人工序列

<220>
<223> 合成的多核苷酸- 编码轻链可变区抗-人类B7-H5克隆18C3的多核苷酸

<400> 28
[0023] gacattgtga tgtcacagtc tccatcctcc ctggctgtgt cagcaggaga gaaggtcact 60

atgagctgca aatccagtca gagtctgctc aacagtagaa cccgaaagaa ccagttggct 120

tggtaccagc agaaaccagg gcagtctcct aaactgctga tctactgggc atccactagg 180

gaatctgggg tccctgatcg cttcacaggc agtggatctg ggacagattt cactctcacc 240

atcagcagtg tgcaggctga agacctggca gtttattact gcaagcaatc ttataatctt 300

cggacgttcg gtggaggcac caagctggaa atcaaac 337

<210> 29
<211> 22
<212> PRT
<213> 人工序列

<220>
<223> 合成的多肽 - 信号序列

<400> 29

Met Lys Ala Gln Thr Ala Leu Ser Phe Phe Leu Ile Leu Ile Thr Ser
1 5 10 15

Leu Ser Gly Ser Gln Gly
20

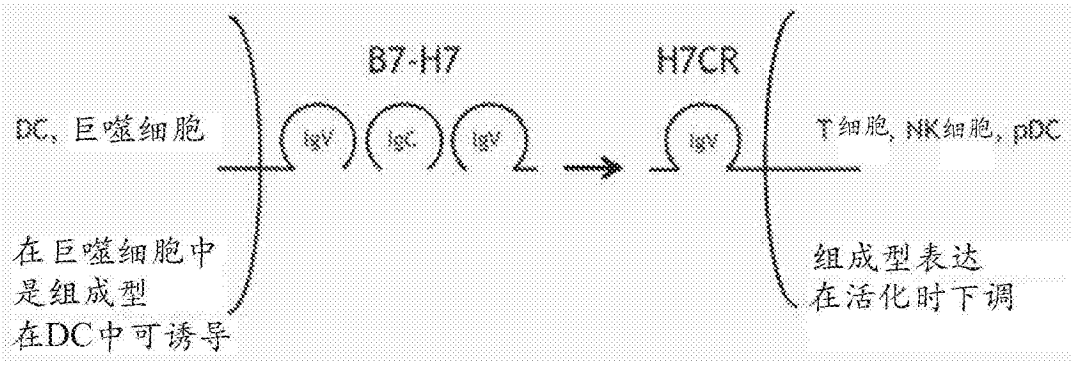

B7-H7
H7CR
DC、巨噬细胞
IgV
IgC
IgV
IgV
T细胞、NK细胞、pDC
在巨噬细胞中
是组成型
在DC中可诱导
组成型表达
在活化时下调

图1

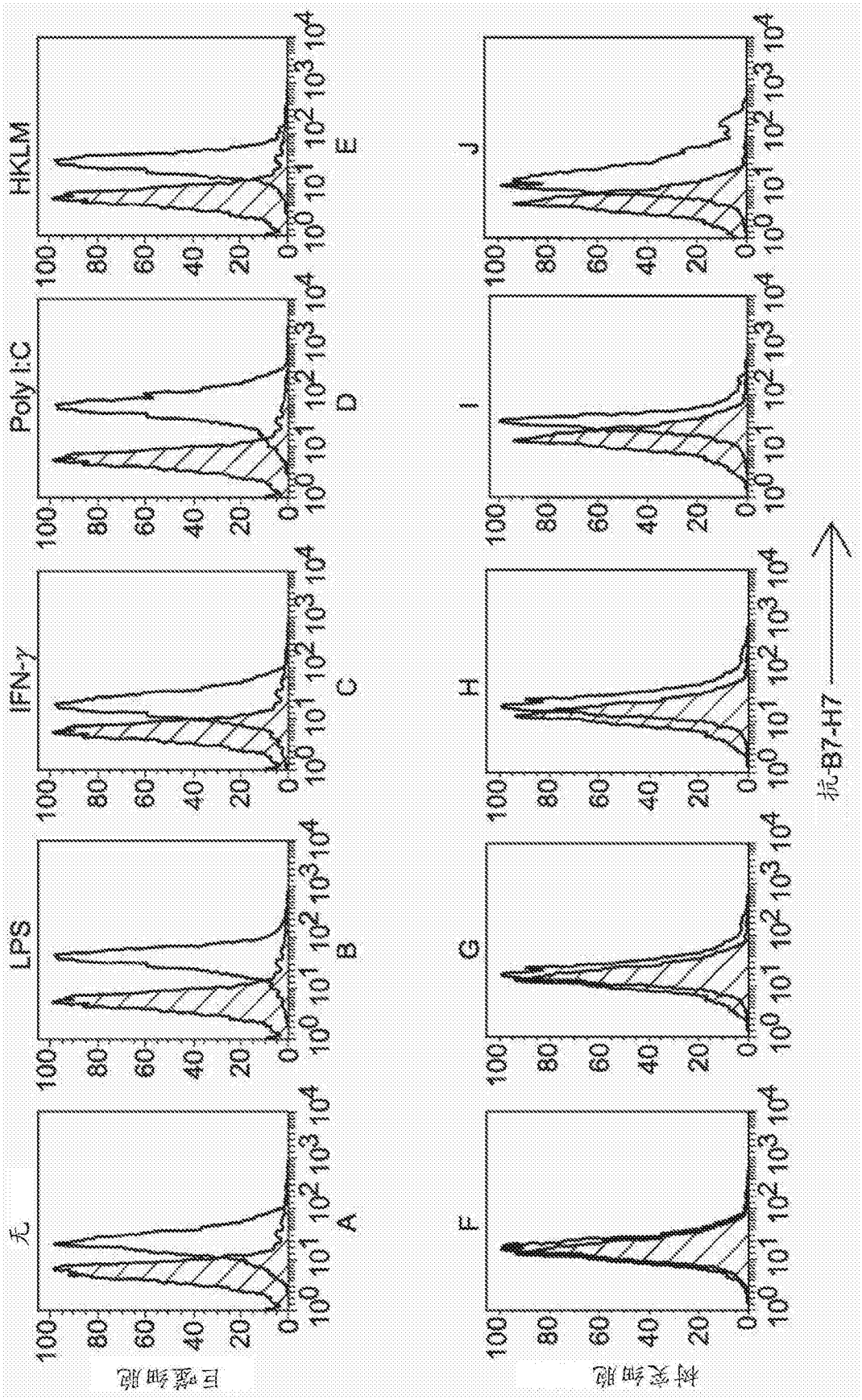

无
LPS
IFN-γ
Poly I:C
HKLM
A
B
C
D
E
F
G
H
I
J
巨噬细胞
树突细胞
抗-B7-H7
100
80
60
40
20
0
10^0 10^1 10^2 10^3 10^4

图2

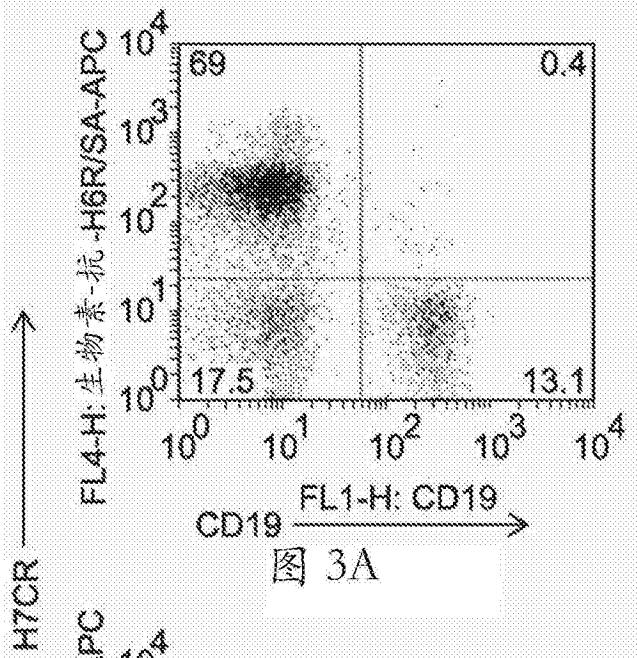


图 3A

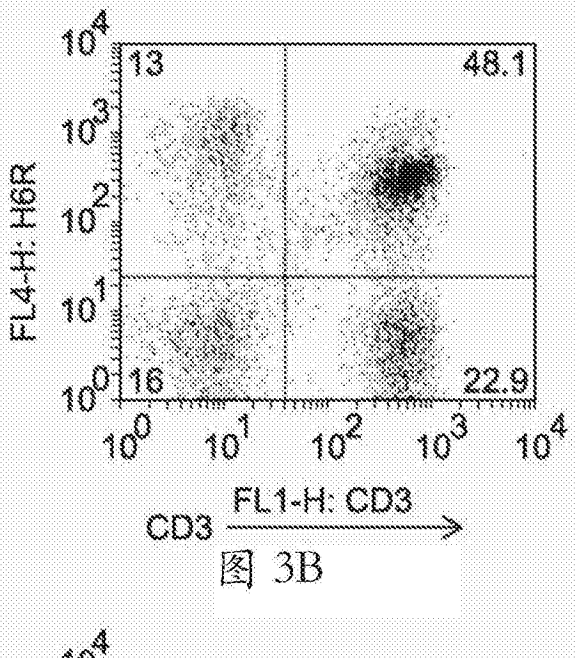


图 3B

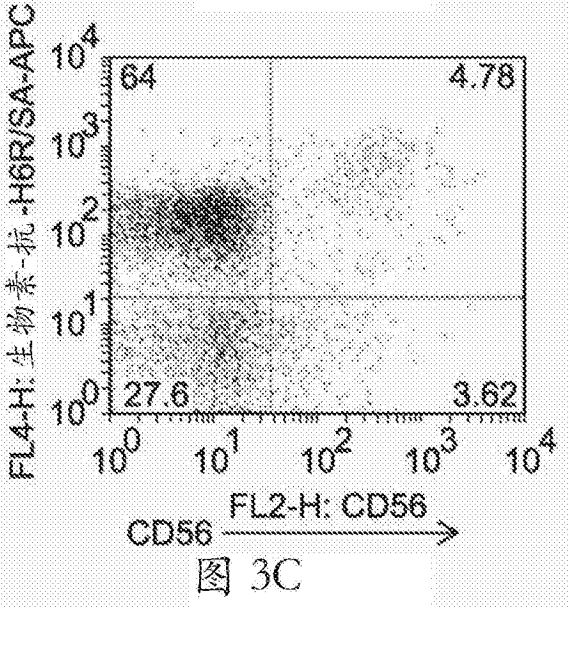


图 3C

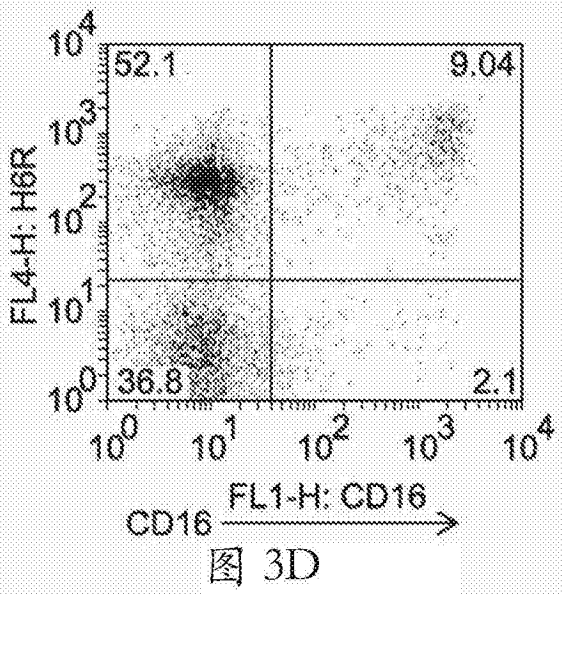


图 3D

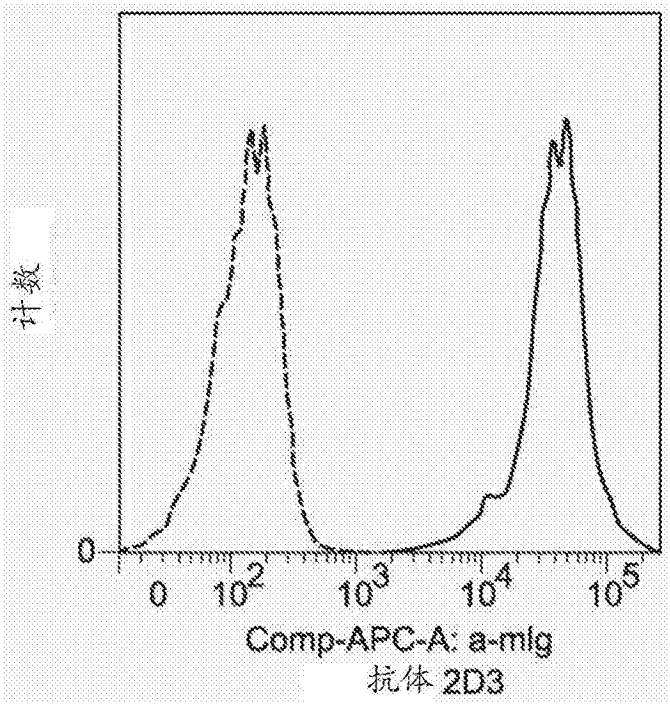

计数
0
0
10^2
10^3
10^4
10^5
Comp-APC-A: a-mIg
抗体 2D3

图4A

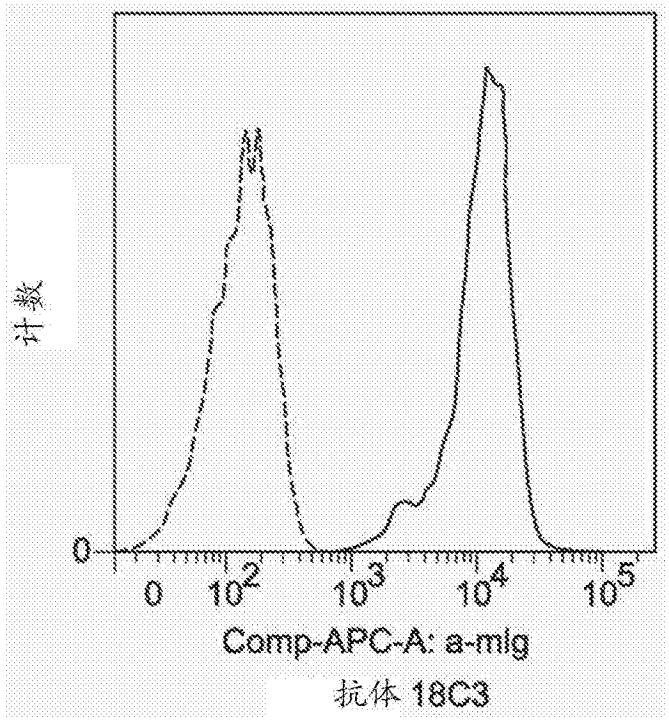

计数
0
0
10^2
10^3
10^4
10^5
Comp-APC-A: a-mIg
抗体 18C3

图4B

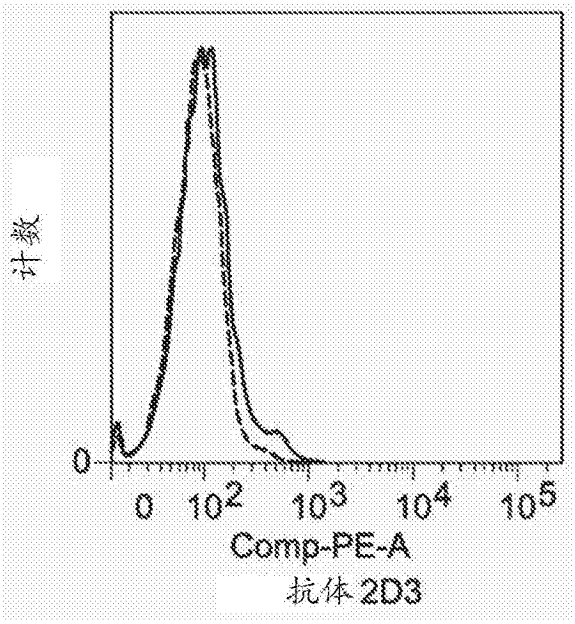

计数
0
0 10^2 10^3 10^4 10^5
Comp-PE-A
抗体 2D3

图5A

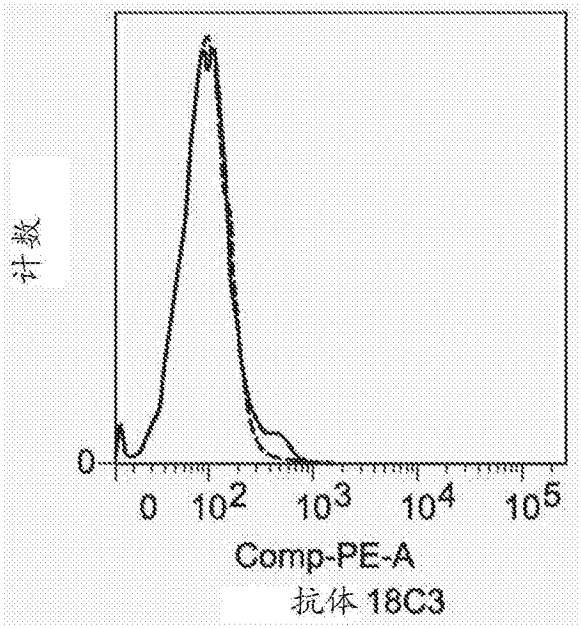

计数
0
0 10^2 10^3 10^4 10^5
Comp-PE-A
抗体 18C3

图5B

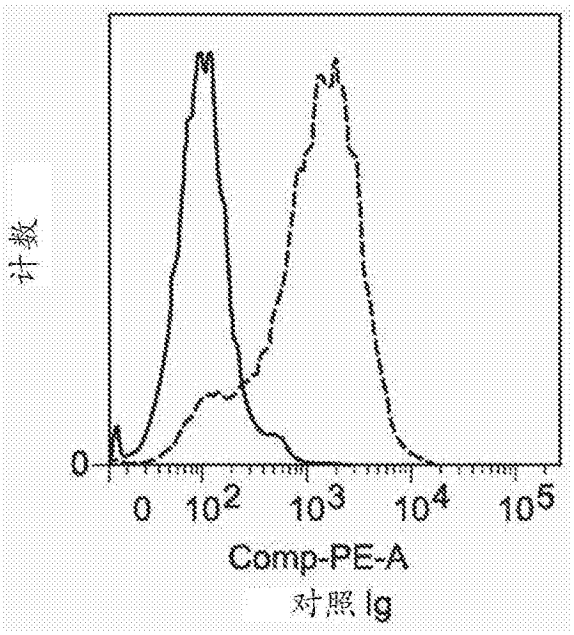

计数
0
0 10^2 10^3 10^4 10^5
Comp-PE-A
对照 Ig

图5C

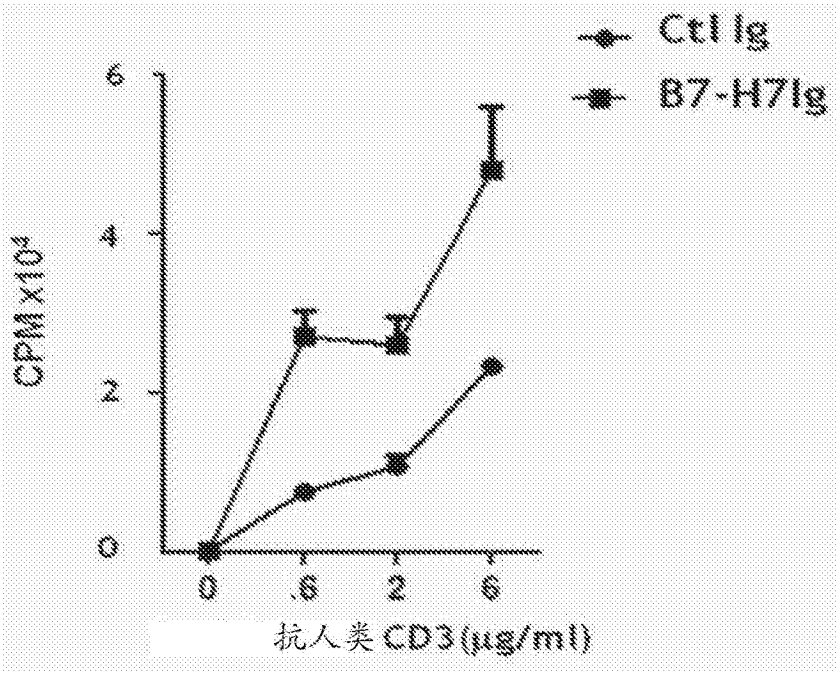

Ctl Ig
B7-H7Ig
CPM x10³
6
4
2
0
0
.6
2
6
抗人类CD3(μg/ml)

图6

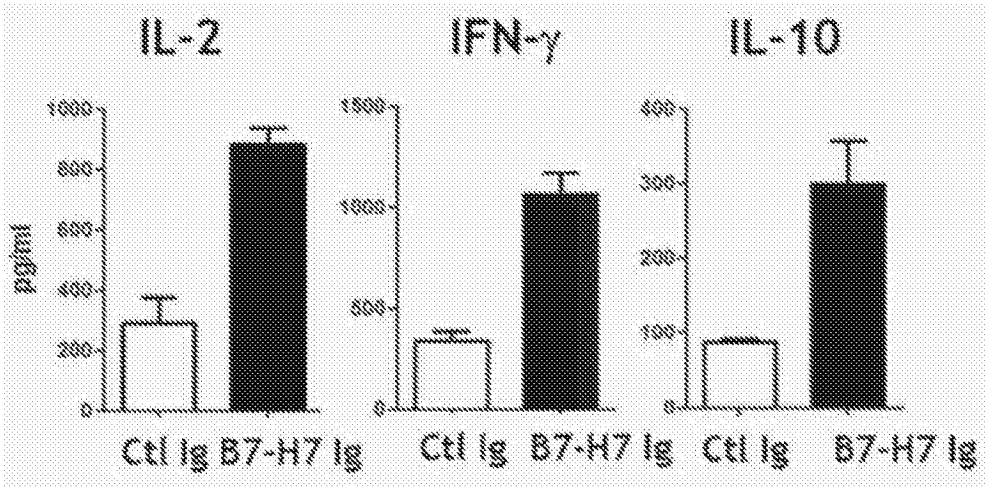

IL-2
IFN-γ
IL-10
pg/ml
Ctl Ig
B7-H7 Ig
Ctl Ig
B7-H7 Ig
Ctl Ig
B7-H7 Ig

图7

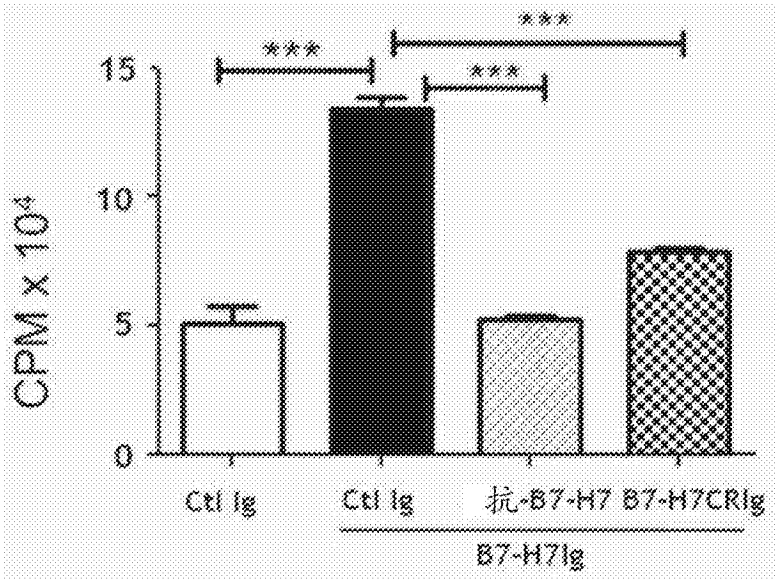

***
***
***
15
10
5
0
CPM x 10⁴
Ctl Ig
Ctl Ig
抗-B7-H7
B7-H7CRIg
B7-H7Ig

图8

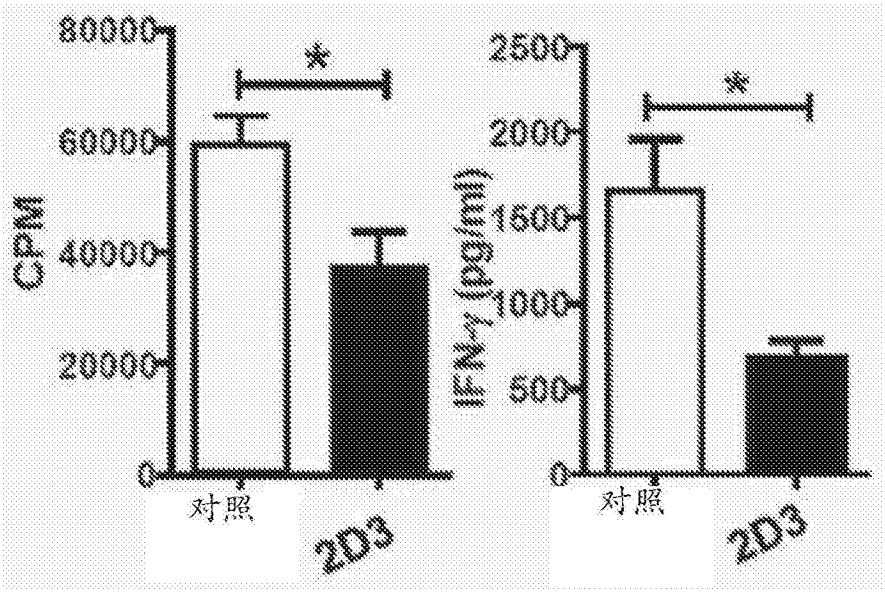

80000
60000
40000
20000
0
CPM
*
对照
2D3
2500
2000
1500
1000
500
0
IFN-γ (pg/ml)
*
对照
2D3

图9

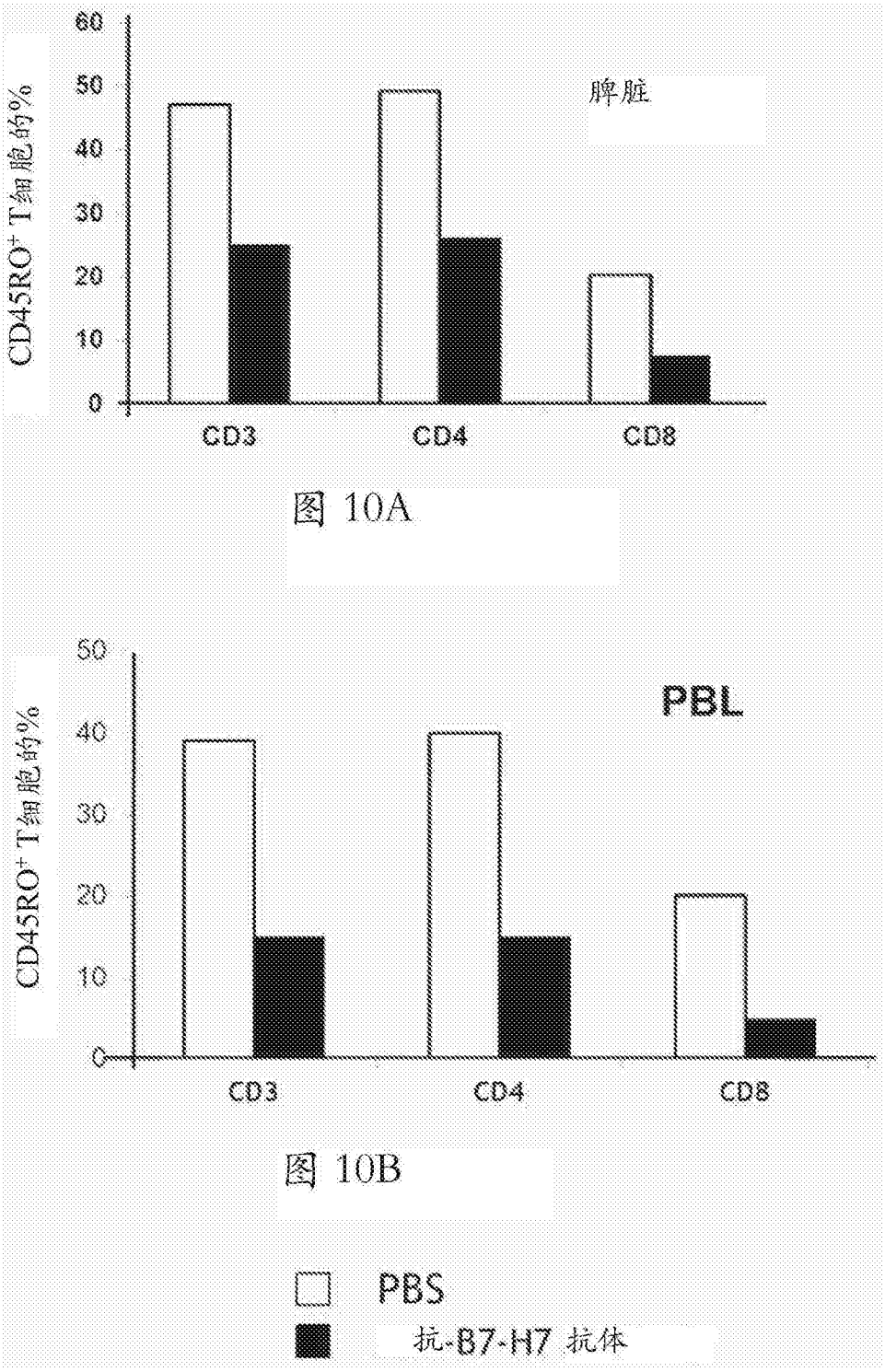

CD45RO+ T细胞的%
60
50
40
30
20
10
0
脾脏
CD3
CD4
CD8
CD45RO+ T细胞的%
50
40
30
20
10
0
PBL
CD3
CD4
CD8
PBS
抗-B7-H7 抗体

图 10A

图 10B

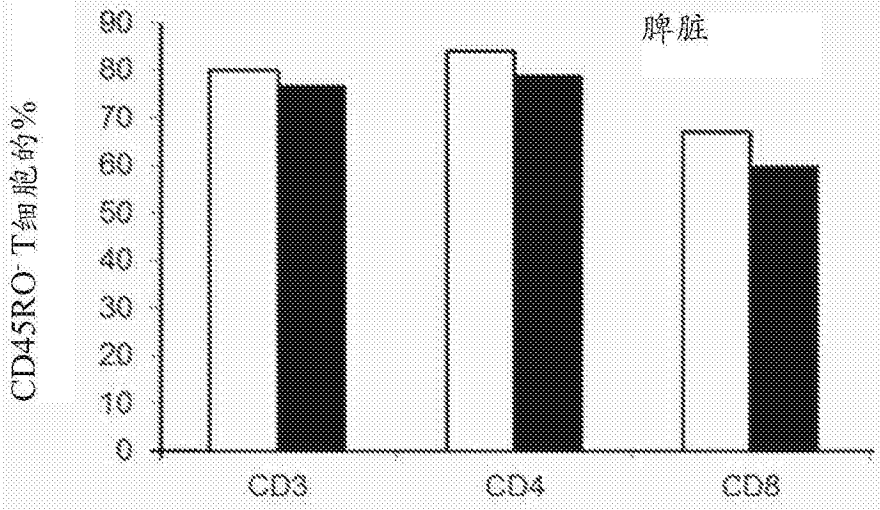

脾脏
CD45RO⁻T细胞的%
90
80
70
60
50
40
30
20
10
0
CD3
CD4
CD8

图 11A

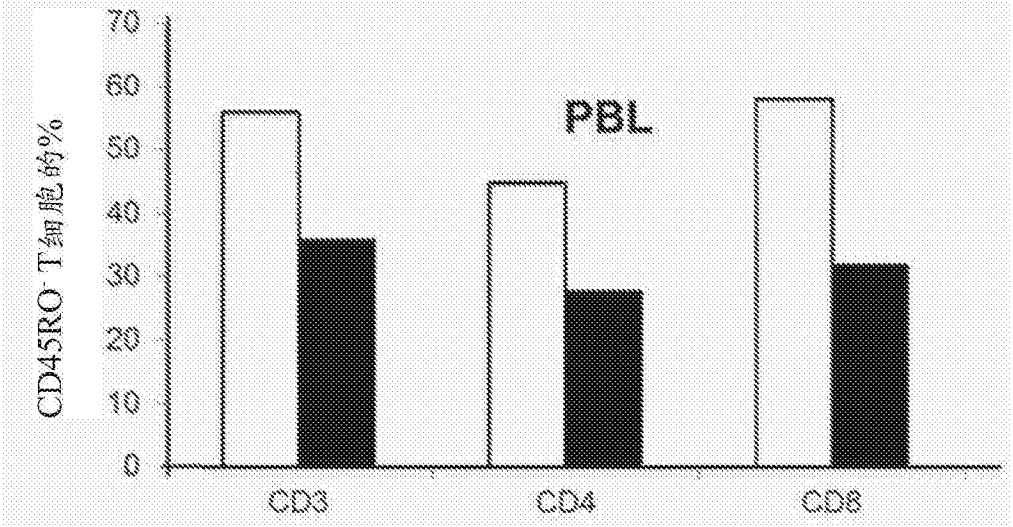
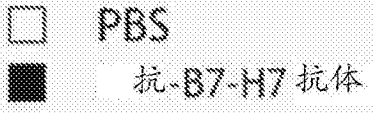

PBL
CD45RO⁻T细胞的%
70
60
50
40
30
20
10
0
CD3
CD4
CD8

图 11B


PBS
抗-B7-H7 抗体

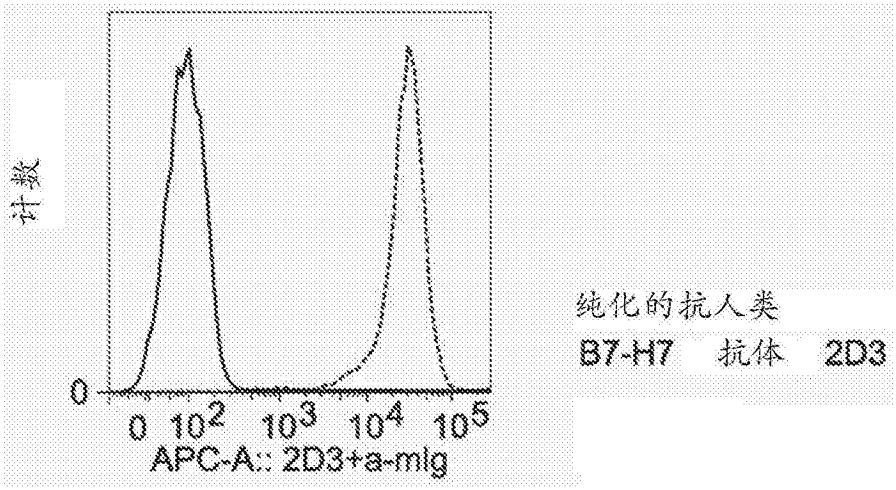


图12A

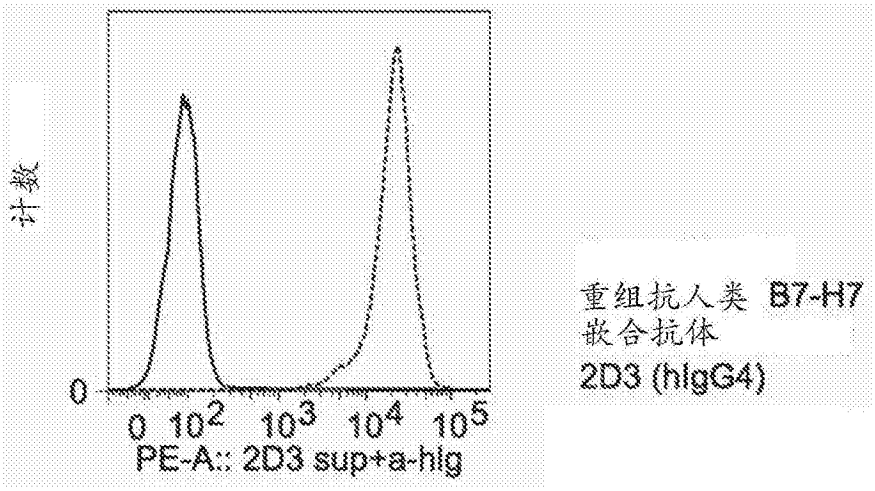


图12B

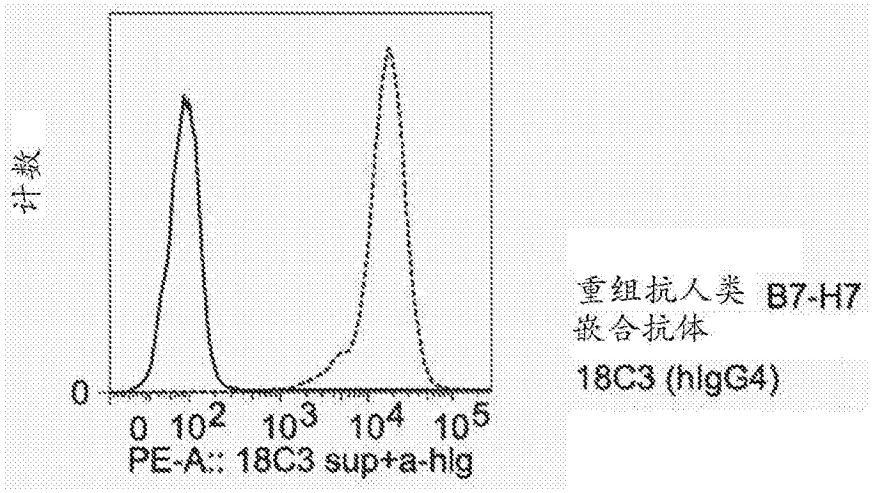


图12C

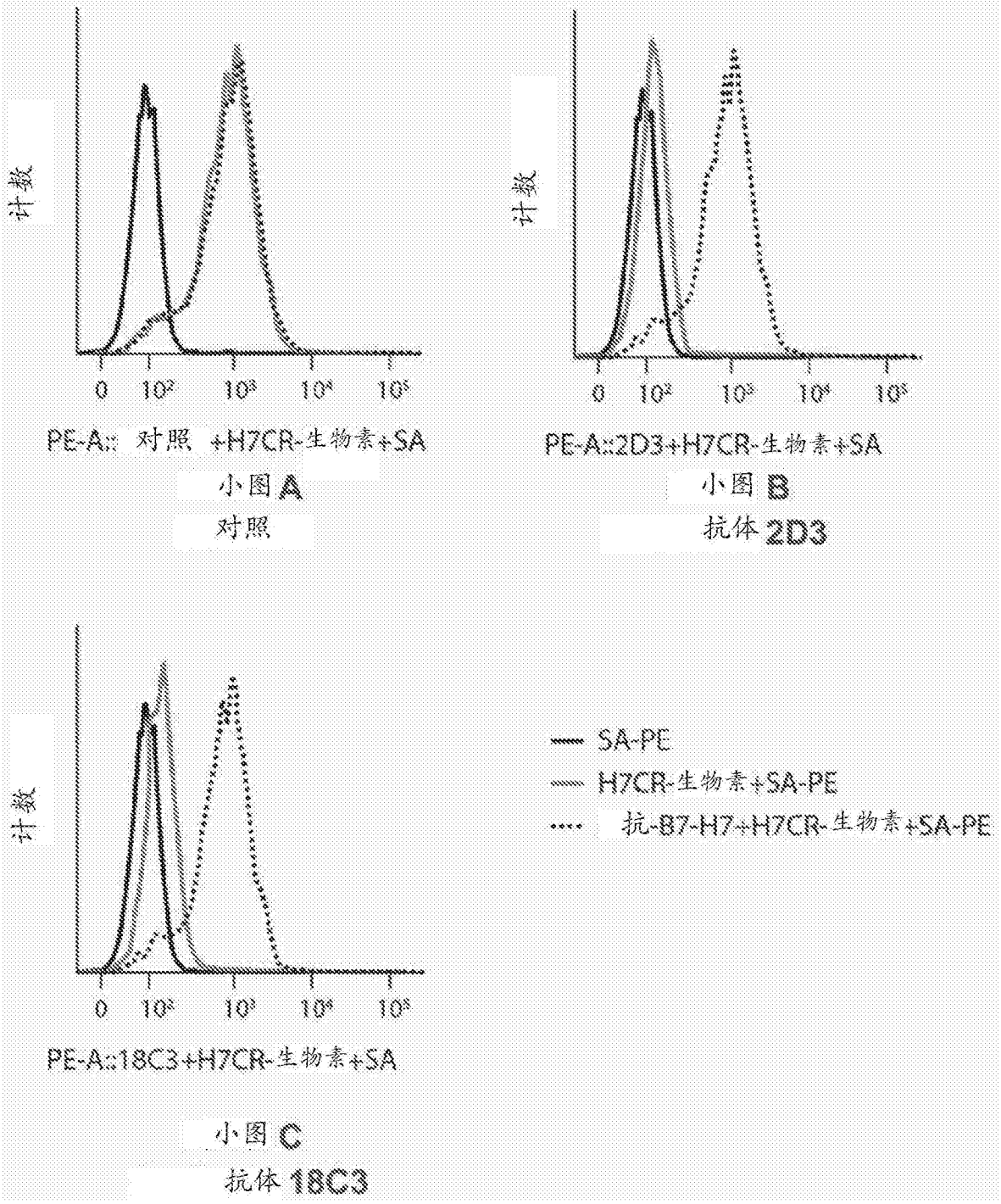

计数
0 10² 10³ 10⁴ 10⁵
PE-A:: 对照 +H7CR-生物素+SA
小图 A
对照
PE-A::2D3+H7CR-生物素+SA
小图 B
抗体 2D3
PE-A::18C3+H7CR-生物素+SA
小图 C
抗体 18C3
SA-PE
H7CR-生物素+SA-PE
抗-B7-H7+H7CR-生物素+SA-PE

图13

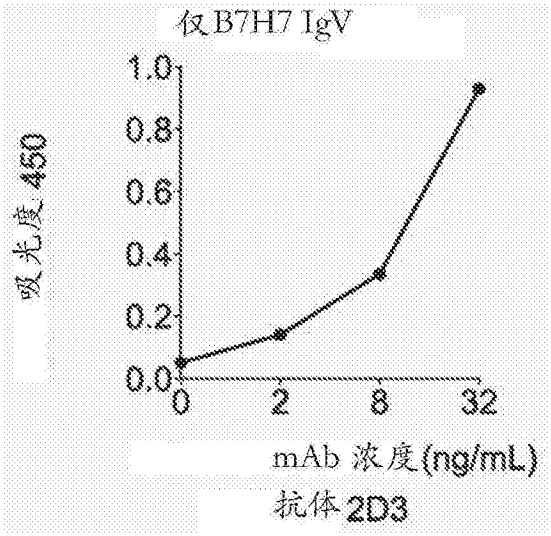


图14A

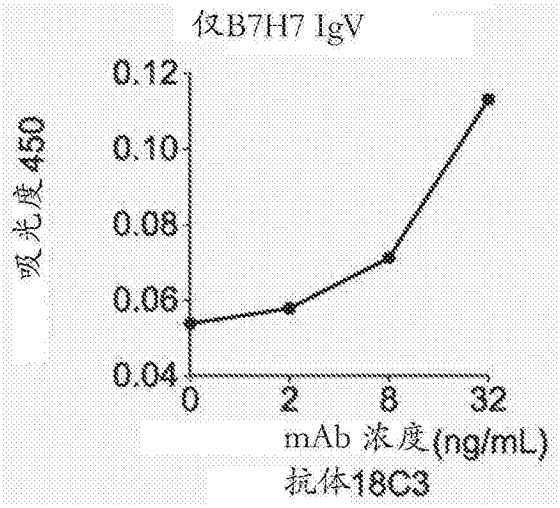


图14B

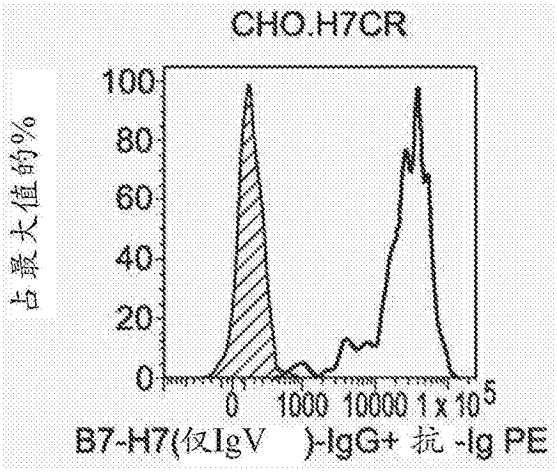


图14C

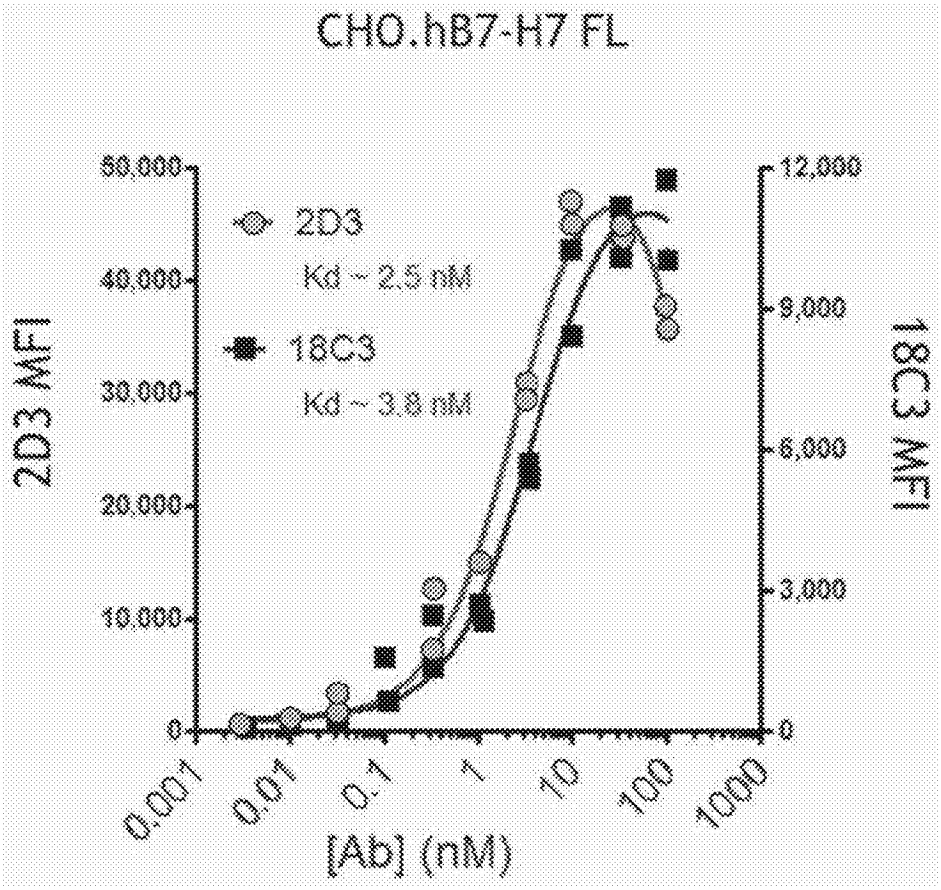

CHO.hB7-H7 FL
2D3 MFI
18C3 MFI
2D3
Kd ~ 2.5 nM
18C3
Kd ~ 3.8 nM
50,000
40,000
30,000
20,000
10,000
0
12,000
9,000
6,000
3,000
0
0.001
0.01
0.1
1
10
100
1000
[Ab] (nM)

图15

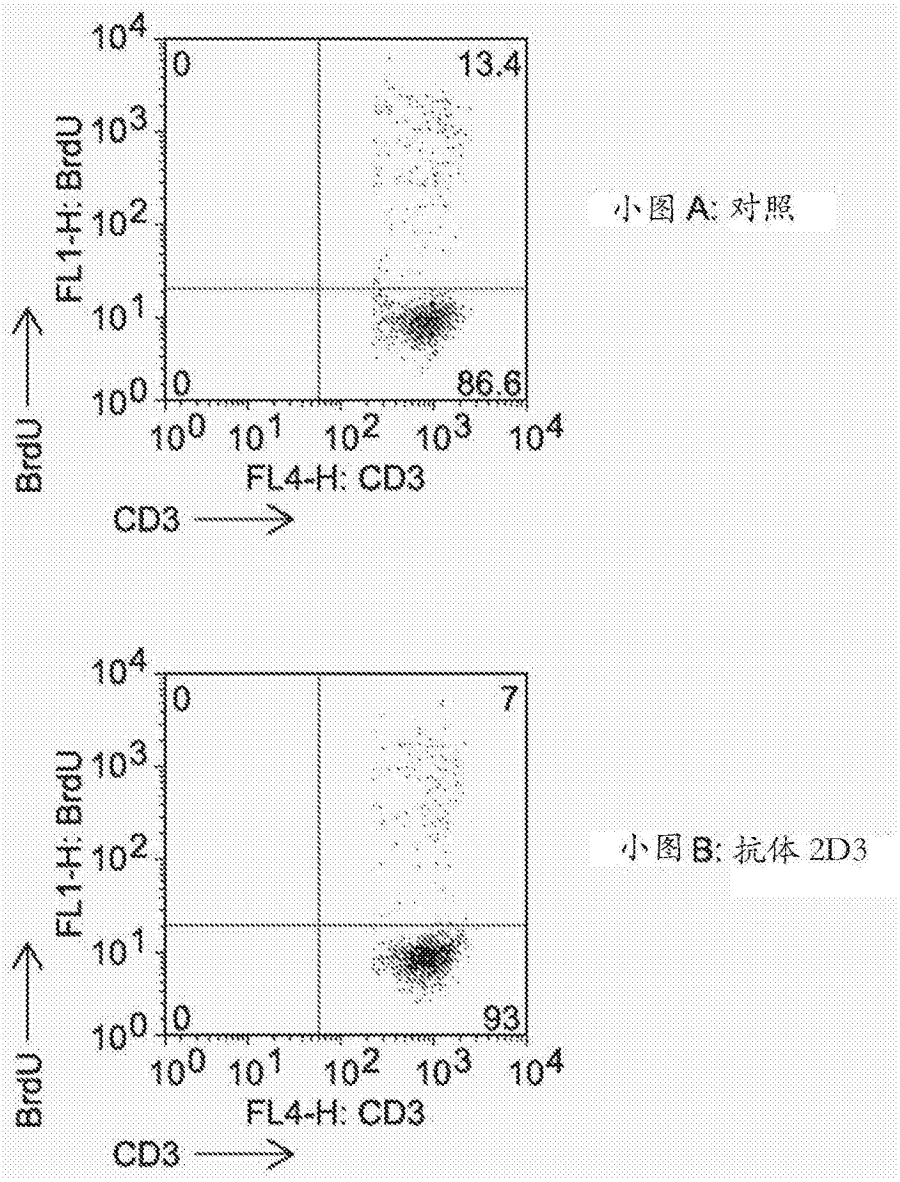


图16A

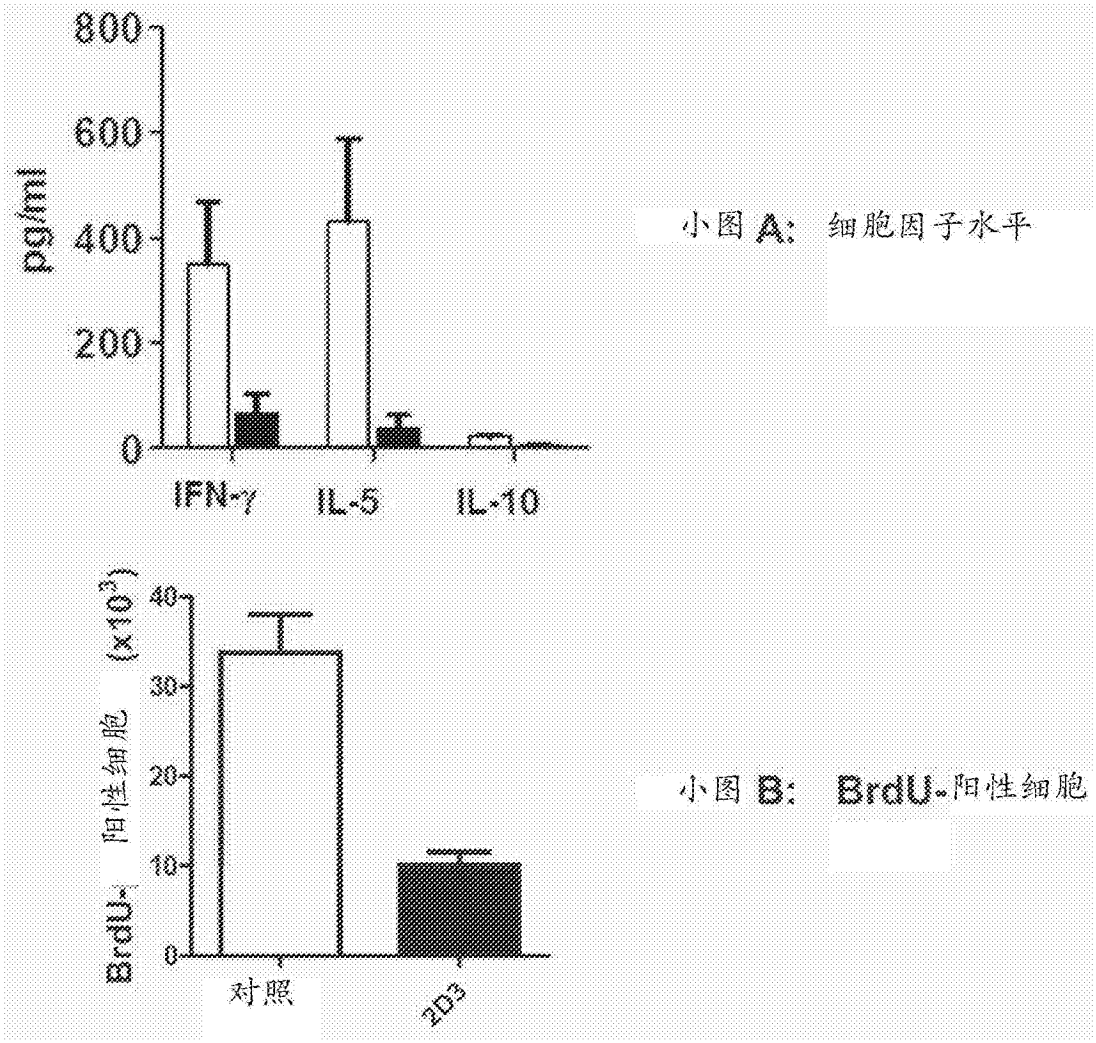

800
600
400
200
0
pg/ml
IFN-γ
IL-5
IL-10
小图 A: 细胞因子水平
40
30
20
10
0
BrdU-阳性细胞 (x10³)
对照
2D3
小图 B: BrdU-阳性细胞

图16B

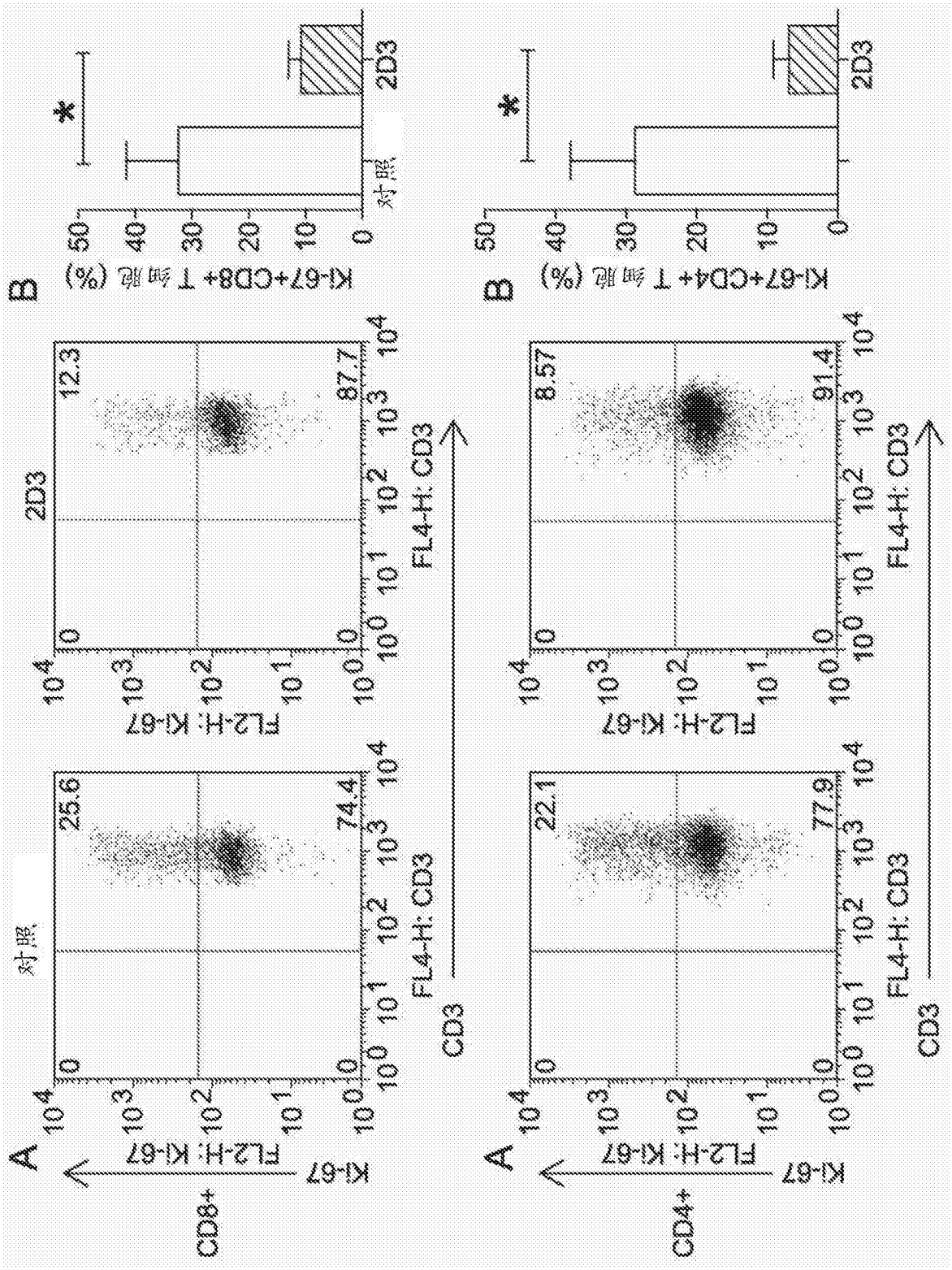

A
对照
2D3
CD8+
CD4+
Ki-67
FL2-H: Ki-67
FL4-H: CD3
CD3
25.6
74.4
12.3
87.7
22.1
77.9
8.57
91.4
B
Ki-67+CD8+ T 细胞 (%)
Ki-67+CD4+ T 细胞 (%)
*
0
10
20
30
40
50

图17A-B

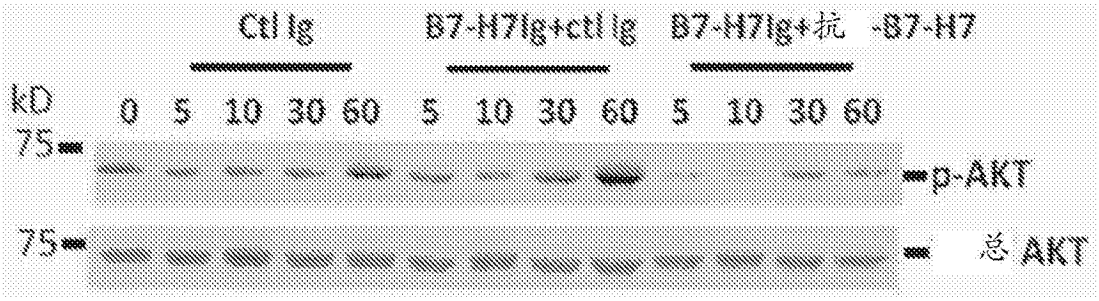

Ctl Ig
B7-H7Ig+ctl Ig
B7-H7Ig+抗 -B7-H7
kD
0 5 10 30 60 5 10 30 60 5 10 30 60
75
p-AKT
75
总 AKT

图18

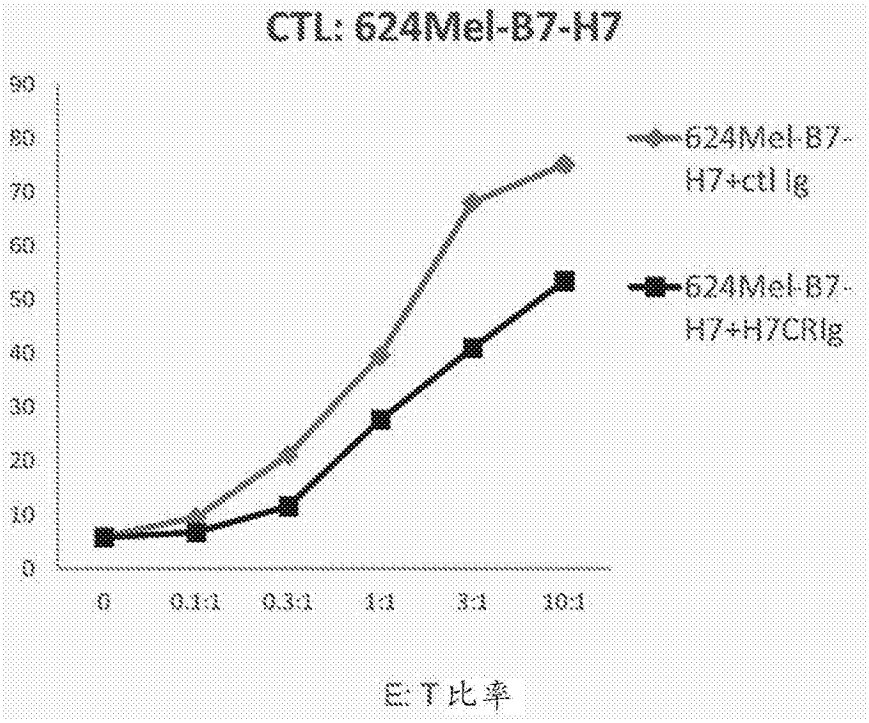

CTL: 624Mel-B7-H7
90
80
70
60
50
40
30
20
10
0
624Mel-B7-H7+ctl Ig
624Mel-B7-H7+H7CRIg
0
0.1:1
0.3:1
1:1
3:1
10:1
E:T比率

图19

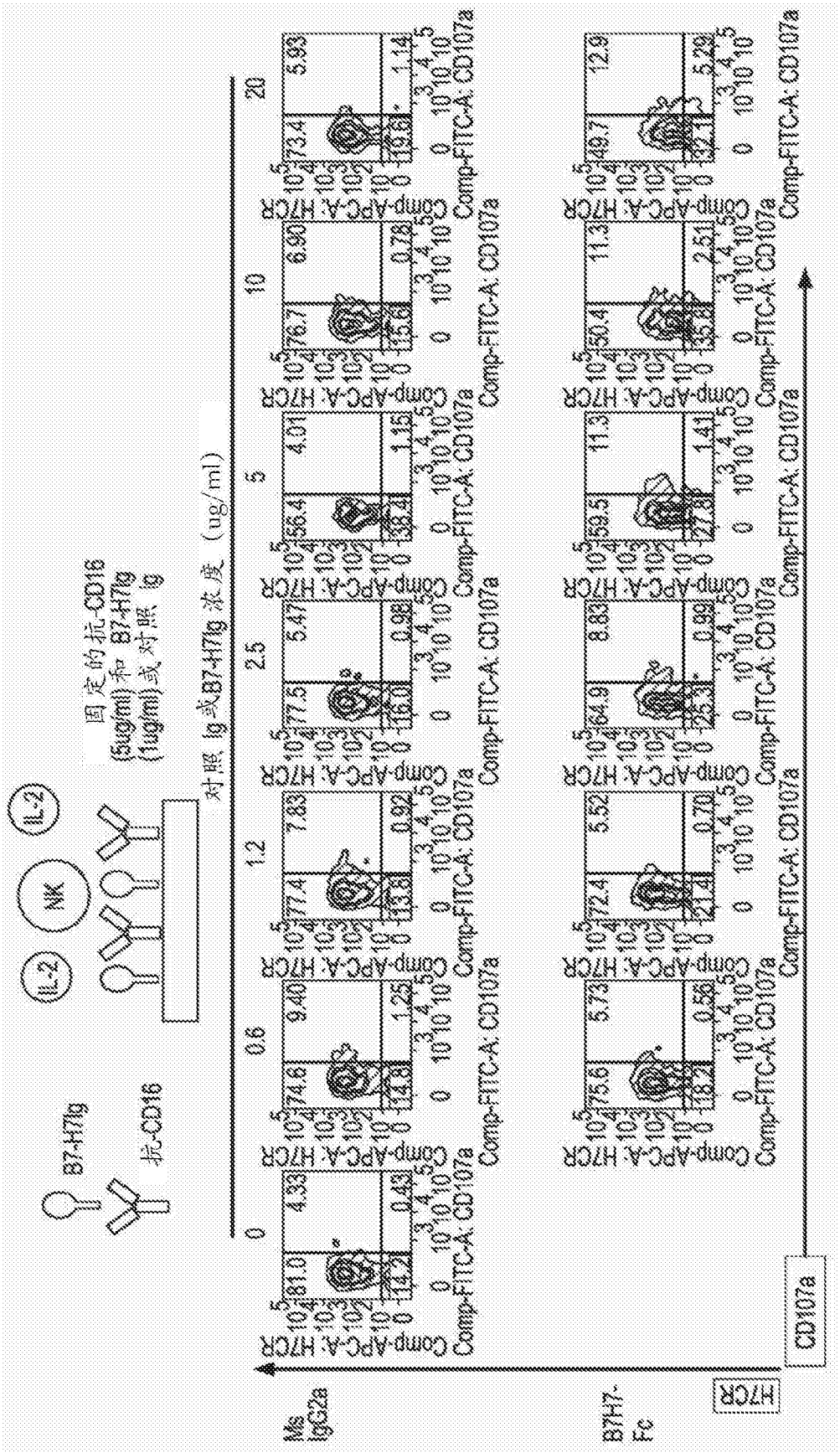

B7-H7Ig
抗-CD16
IL-2
NK
IL-2
固定的抗-CD16
(5ug/ml)和 B7-H7Ig
(1ug/ml)或对照 Ig
对照 Ig或B7-H7Ig 浓度 (ug/ml)
0
0.6
1.2
2.5
5
10
20
Ms IgG2a
B7H7-Fc
H7CR
CD107a
Comp-APC-A: H7CR
Comp-FITC-A: CD107a

图20A

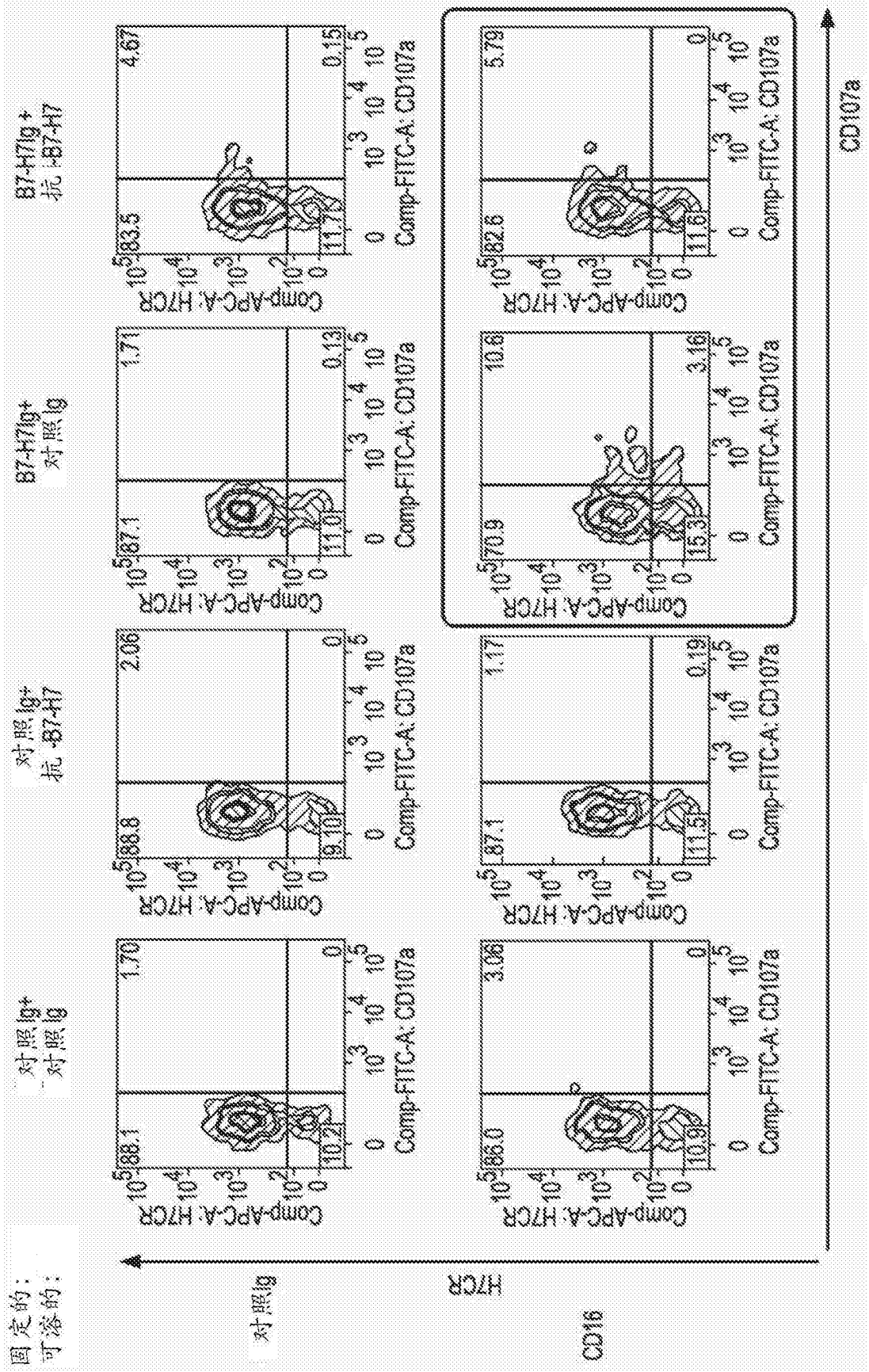

固定的：
可溶的：
对照Ig
对照Ig+
对照Ig
对照Ig+
抗-B7-H7
B7-H7Ig+
对照Ig
B7-H7Ig+
抗-B7-H7
CD16
HTCR
CD107a
Comp-APC-A: HTCR
Comp-FITC-A: CD107a

图20B