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(54) Title: B7-H4 FUSION PROTEINS AND METHODS OF USE THEREOF

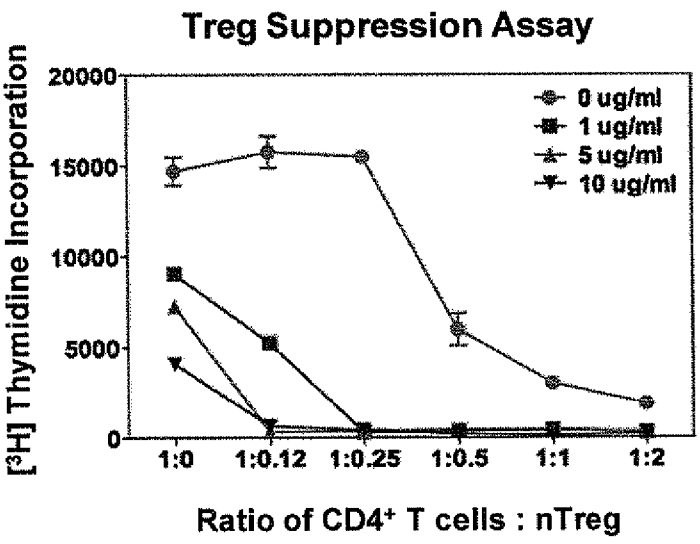

Figure 35

(57) Abstract: Fusion proteins containing B7-H4 polypeptides are disclosed. The B7-H4 fusion proteins can include full-length B7-H4 polypeptides, or can contain a fragment of a full-length B7-H4 polypeptide, including some or all of the extracellular domain of the B7-H4 polypeptide. Methods for using the fusion proteins to downregulate T cell activation and for the treatment of inflammatory and autoimmune diseases and disorders are also disclosed. The B7-H4 fusion proteins are useful for treating inflammation by inhibiting or reducing differentiation, proliferation, activity, and/or cytokine production and/or secretion by ThI, ThI 7, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules, including, but not limited to, IL-lβ, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs; or enhancing IL-IO secretion by Tregs, increasing the differentiation of Tregs, increasing the number of Tregs, or combinations thereof.

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B7-H4 FUSION PROTEINS AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

This invention relates to B7-H4 fusion proteins and methods for modulating immune responses in a subject using B7-H4 fusion proteins.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisonal Patent Application No. 61/238,605 filed on August 31, 2009, U.S. Provisional Patent Application No. 61/266,854, filed on December 4, 2009, U.S. Provisional Patent Application No. 61/254,930 filed on October 26, 2009, U.S. Provisional Patent Application No. 61/286,537 filed on December 15, 2009, and U.S. Provisional Patent Application No. 61/378,361 filed August 30, 2010.

BACKGROUND OF THE INVENTION

An antigen specific T cell response is mediated by two signals: first, engagement of the TCR with antigenic peptide presented in the context of MHC (signal 1), and second, a second antigen-independent signal delivered by contact between different receptor/ligand pairs (signal 2). This "second signal" is critical in determining the type of T cell response (activation versus tolerance) as well as the strength and duration of that response, and is regulated by both positive and negative signals from costimulatory molecules, such as the B7 family of proteins. The most extensively characterized T cell costimulatory pathway is B7-CD28, in which B7-1 (CD80) and B7-2 (CD86) each can engage the stimulatory CD28 receptor and the inhibitory CTLA-4 (CD152) receptor. In conjunction with signaling through the T cell receptor, CD28 ligation increases antigen-specific proliferation of T cells, enhances production of cytokines, stimulates differentiation and effector function, and promotes survival of T cells (Lenshow, et al., *Annu. Rev. Immunol.*, 14:233-258 (1996); Chambers and Allison, *Curr. Opin. Immunol.*, 9:396-404 (1997); and Rathmell and Thompson, *Annu. Rev. Immunol.*, 17:781-828 (1999)). In contrast, signaling through CTLA-4 is thought to deliver a negative signal that inhibits T cell proliferation, IL-2 production, and cell cycle progression (Krummel and Allison, *J. Exp. Med.*, 183:2533-2540 (1996); and Walunas, et al., *J. Exp. Med.*, 183:2541-2550 (1996)). Other members of the B7 family include B7-H1 (Dong, et al., *Nature Med.*, 5:1365-

1369 (1999); and Freeman, et al., *J. Exp. Med.*, 192:1-9 (2000)), B7-DC (Tseng, et al., *J. Exp. Med.*, 193:839-846 (2001); and Latchman, et al., *Nature Immunol.*, 2:261-268 (2001)), B7-H2 (Wang, et al., *Blood*, 96:2808-2813 (2000); Swallow, et al., *Immunity*, 11:423-432 (1999); and Yoshinaga, et al., *Nature*, 402:827-832 (l999)), B7-H3 (Chapoval, et al., *Nature Immunol.*, 2:269-274 (2001)) and B7-H4 (Choi, et al., *J. Immunol.*, 171:4650-4654 (2003); Sica, et al., *Immunity*, 18:849-861 (2003); Prasad, et al., *Immunity*, 18:863-873 (2003); and Zang, et al., *Proc. Natl. Acad. Sci. U.S.A.*, 100:10388-10392 (2003)). B7-H1 and B7-DC are ligands for PD-1, B7-H2 is a ligand for ICOS, and B7-H3 remains at this time an orphan ligand (Dong, et al., *Immunol. Res.*, 28:39-48 (2003)).

B7-H4 is member of the B7 family that is a negative regulator of T cell responses. Human and mouse B7-H4 share 87% amino acid identity, suggesting an important evolutionarily conserved function. Human and mouse B7-H4 mRNAs are expressed broadly in both lymphoid (spleen and thymus) and nonlymphoid organs (including lung, liver, testis, ovary, placenta, skeletal muscle, pancreas, and small intestine). Limited studies of B7-H4 protein expression indicate that B7-H4 is not expressed on freshly isolated human T cells, B cells, DC, and monocytes, but it can be induced on these cell types after *in vitro* stimulation. Immunohistochemical staining shows that B7-H4 is highly expressed in breast, renal, lung and ovarian tumors, and reverse transcriptase polymerase chain reaction (RT-PCR) analyses indicate that mouse B7-H4 also is highly expressed in a number of tumor cell lines, including prostate, lung, and colon carcinomas. B7-H4 is highly expressed by tumor associated macrophages (TAMs) and is present in tumor vasculature. Regulatory T cells (Tregs) induce upregulation of B7-H4 on TAMs via IL-6 and IL-10; this is thought to be one of the mechanisms by which Tregs contribute to immune suppression. (Kryczek, J.I., *J. Immunol.*, 177(1):40-44 (2006)). B7-H4 expression has also been observed in tubule epithelial cells of diseased kidneys (Chen, Y., *Kidney Int.*, 70(12):2092-9 (2006) Epub 2006 Oct 18.).

The receptor for B7-H4 has not been cloned. B7-H4 has been shown not to bind to known CD28 family members such as CD28, CTLA-4, ICOS, and PD-1 (Sica, et al., *Immunity*, 18:849-861 (2003)), and these are therefore

not potential receptors for B7-H4. Functional studies using B7-H4 transfectants and B7-H4-Ig fusion proteins demonstrate that B7-H4 delivers a signal that inhibits TCR-mediated $CD4^+$ and $CD8^+$ T cell proliferation, cell-cycle progression and IL-2 production. B7-1 costimulation cannot overcome B7-H4-Ig-induced inhibition. In agreement with the *in vitro* activity, B7-H4 knock-out mice develop autoimmunity. The broad and inducible expression of B7-H4, together with functional studies, suggests that B7-H4 serves to downregulate immune responses in peripheral tissues.

More recent results demonstrate that B7-H4 also acts as a negative regulator of neutrophil response. Neutrophils are a key component of the innate immune system and are a first line of host defense against pathogens. However, neutrophils can also contribute to chronic inflammation and autoimmune disease. B7-H4 knockout mice display increased Th1 responses and are more resistant to infection by *Listeria monocytogenes* due to an augmented immune response that is neutrophil dependent (Suh. W.K., et al., *Mol Cell Biol.*, 26(17):6403-11 (2006) and Zhu, G., et al., *Blood*, 113(8):1759-67 (2009) Epub 2008 Dec 24.). Mice hydrodynamically transfected with monomeric B7-H4 IgV domain or extracellular domain (ECD) increased neutrophil response to lipopolysaccharide (LPS) and *Listeria* infection, while dimeric B7-H4-Ig reduces proliferation of bone marrow derived neutrophil precursors (Zhu, G., et al., *Blood*, 113(8):1759-67 (2009) Epub 2008 Dec 24).

Certain immune cells and immune cell signal transduction pathways are promising targets for new agents for treating immune disorders. For example Th1, Th17, Th22, and regulatory T cells (Tregs) play important roles in mediating autoimmunity and inflammation. Mounting evidence from numerous studies shows the importance of these immune cells in disorders such as rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, psoriasis, lupus erythematosus and uveitis. Most existing therapies target only one pathway at a time. Thus, there is a need for therapies that target multiple cells and pathways involved in autoimmunity and inflammation, such as Th1, Th17, Th22, Tregs, or other cells that secrete, or cause other cells to secrete, inflammatory molecules such as cytokines,

metalloproteases, chemokines and other molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, IL-10 and MMPs.

Therefore it is an object of the invention to provide compositions and methods for modulating Th1, Th17, Th22, or other cells that secrete, or cause other cells to secrete, inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs.

It is another object of the invention to provide compositions and methods for modulation of at least two immune pathways that result in the secretion of one or more inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs, preferably by Th1, Th17 or Th22 cells.

It is another object of the invention to provide compositions and methods for modulation of the Treg cells and pathways, such as IL-10 and TGF-beta secretion.

It is another object of the invention to provide compositions and methods for modulating the proinflammatory activity of Th1, Th17 or Th22 T cells while simultaneously increasing or promoting the activity of Tregs.

It is an object of the invention to provide compositions containing B7-H4 polypeptides that function to decrease or inhibit antigen-specific proliferation of T cells, decrease or inhibit production of pro-inflammatory molecules by T cells, decrease or inhibit differentiation and effector function of Th1, Th17 or Th22 cells, and decrease or inhibit survival of Th1, Th17 or Th22 cells.

It is another object of the invention to provide compositions containing B7-H4 polypeptides that function to increase or promote the activity of Tregs, increase the production of inflammatory molecules such as IL-10 from Tregs, increase the differentiation of naïve T cells intoTregs, increase the number of Tregs, or increase the survival of Tregs.

It is another object of the invention to provide compositions containing B7-H4 polypeptides that function to inhibit or decrease the

proinflammatory activity of Th1, Th17 or Th22 T cells while simultaneously increasing or promoting the activity of Tregs.

It is another object of the invention to provide isolated nucleic acid molecules encoding B7-H4 compositions.

It is another object of the invention to provide cells containing vectors that express nucleic acid molecules encoding B7-H4 compositions.

It is still a further object of the invention to provide methods for decreasing or inhibiting pro-inflammatory T cell activation by contacting them with B7-H4 compositions.

It is still a further object of the invention to provide methods for the treatment of inflammatory and autoimmune diseases and disorders.

It is still a further object of the invention to provide methods for administering B7-H4 compositions, nucleic acids encoding the same, or cells transfected or transduced with nucleic acids encoding B7-H4 compositions to a mammal in need thereof.

It is another object to provide compositions and methods for increasing Treg biological activity.

It is yet another object to provide compositions and methods for inhibiting or reducing eptitope spreading.

It is another object to provide compositions and methods for inhibiting differentiation of naïve T cells into Th1, Th17, Th22, or other cells that secrete, or cause other cells to secrete, pro-inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs.

It is another object to provide compositions and methods for inhibiting the differentiation and maturation of immature antigen-presenting cells.

It is another object of the invention to monitor patients who would benefit from treatment with the compositions and methods disclosed by measuring the levels of biomarkers such as inflammatory chemokines, cytokines or other molecules, or gene expression of biomarkers in the patient.

It is another object of the invention to identify patients who would benefit from treatment with the compositions and methods disclosed by measuring the levels of biomarkers such as inflammatory chemokines, cytokines or other molecules in the patient.

It is another object of the invention to identify patients who would benefit from treatment with the compositions and methods disclosed by identifying patients with polymorphisms in genes encoding biomarkers such as inflammatory chemokines, cytokines or other molecules.

It is another object of the invention to provide combination therapies for treating patients with inflammatory and autoimmune diseases and disorders.

It is another object of the invention to provide compositions for treating patients who do not respond to TNF blockers.

It is another object of the invention to provide compositions for treating chronic and persistent inflammation.

SUMMARY OF THE INVENTION

Fusion proteins containing B7-H4 polypeptides are disclosed. B7-H4 fusion polypeptides have a first fusion partner comprising all or a part of a B7-H4 protein fused to a second polypeptide directly or indirectly via a linker peptide sequence that is fused to the second polypeptide. The B7-H4 polypeptide may be of any species of origin. In preferred embodiments, the B7-H4 polypeptide is of murine, non-human primate or human origin. In one embodiment the B7-H4 fusion protein inhibits the inflammatory activity of Th1, Th17, Th22, or other cells that secrete, or cause other cells to secrete, inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs. The B7-H4 fusion protein can also increase the suppressive capacity of Tregs by increasing the production of molecules such as the cytokine IL-10.

The B7-H4 fusion proteins can include full-length B7-H4 polypeptides, or a fragment thereof. In one embodiment, the B7-H4 polypeptide is a soluble fragment of full-length B7-H4. Fragments include those that retain the ability to bind to their natural receptors and incorporate some, or all, of the extracellular domain of the B7-H4 polypeptide, and lack

some or all of the intracellular and/or transmembrane domains. In one embodiment, B7-H4 polypeptide fragments include the entire extracellular domain of the B7-H4 polypeptide. In other embodiments, the soluble fragments of B7-H4 polypeptides include fragments of the extracellular domain that retain B7-H4 biological activity. B7-H4 polypeptide extracellular domains can include 1, 2, 3, 4, 5 or more contiguous amino acids from the transmembrane domain, and/or 1, 2, 3, 4, 5 or more contiguous amino acids from the signal sequence. Alternatively, the extracellular domain can have 1, 2, 3, 4, 5, or more contiguous amino acids removed from the C-terminus, N-terminus, or both. Variants of B7-H4 polypeptides and fragments thereof may also be used.

In one embodiment, the B7-H4 polypeptide may be fused to one or more domains of an Ig 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.

The fusion proteins can be dimerized or multimerized to form homodimers, heterodimers, homomultimers or heteromultimers. Dimerization/multimerization partners can be arranged either in parallel or antiparallel orientations.

Isolated nucleic acids molecules encoding the fusion proteins, vectors and host cells incorporating the nucleic acids, and pharmaceutical and immunogenic compositions containing the fusion proteins are also provided. Immunogenic compositions contain antigens, a source of fusion protein and, optionally, adjuvant.

Methods for using the fusion proteins to decrease or inhibit pro-inflammatoryT cell activation are disclosed. Therapeutic uses for the disclosed compositions include the treatment or alleviation of one or more symptoms of inflammatory and autoimmune diseases and disorders. The B7-H4 fusion proteins are useful for treating inflammation by any or all of the following: inhibiting or reducing differentiation of Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules; inhibiting or reducing activity of Th1, Th17, Th22, and/or other

cells that secrete, or cause other cells to secrete, inflammatory molecules; inhibiting or reducing the Th1 and/or Th17 pathways; inhibiting or reducing inflammatory molecule production and/or secretion by Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules; inhibiting or reducing proliferation of Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules; interacting with Tregs; enhancing Treg activity; enhancing IL-10 secretion by Tregs; increasing the number of Tregs; increasing the suppressive capacity of Tregs; or combinations thereof.

In one embodiment, B7-H4 polypeptides or fusion proteins enhance the suppressive activity of Tregs on the immune system. Tregs can suppress differentiation, proliferation, activity, and/or cytokine production and/or secretion by Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules. In a preferred embodiment the B7-H4 polypeptides or fusion proteins enhance the suppressive activity of Tregs on naïve T cells to inhibit or reduce naïve T cells from differentiating into Th1, Th17 or Th22 cells and thereby reduce the number of Th1 or Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs in a subject.

One embodiment provides a method to inhibit or reduce epitope spreading in a subject by administering to the subject an effective amount of B7-H4 polypeptide or fusion proteins thereof. A preferred embodiment provides a method of administering an effective amount of B7-H4 polypeptide or fusion protein thereof to inhibit or reduce epitope spreading in patients with Multiple Sclerosis (MS) of systemic lupus erythematosus (SLE).

B7-H4 polypeptides, fragments or fusions thereof can be administered in combination with one or more additional therapeutic agents, including, but not limited to, antibodies against other lymphocyte surface markers (e.g., CD40, alpha-4 integrin) or against cytokines, other fusion proteins, e.g., CTLA4-Ig (Orencia®, belatacept), TNFR-Ig (Enbrel®), TNF-α blockers such as Remicade, Cimzia and Humira, CD73-Ig,

cyclophosphamide (CTX) (i.e. Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune™), methotrexate (MTX) (i.e. Rheumatrex®, Trexall®), belimumab (i.e. Benlysta®), Tysabri or other immunosuppressive drugs, anti-proliferatives, cytotoxic agents, or other compounds that may assist in immunosuppression.

In one embodiment, the additional therapeutic agent targets a different pathway involved in immune activation. In a preferred embodiment, the additional therapeutic agent is a CTLA-4 fusion protein, such as CTLA-4 Ig (abatacept). In a preferred embodiment, the additional therapeutic agent is a CTLA4-Ig fusion protein known as belatacept that contains two amino acid substuitutions (L104E and A29Y) that markedly increase its avidity to CD86 *in vivo*.

In another embodiment, the second therapeutic agent is cyclophosphamide (CTX). In a preferred embodiment, B7-H4-Ig and CTX are coadministered in an effective amount to treat a chronic autoimmune disease or disorder such as Systemic lupus erythematosus (SLE).

In another embodiment, the second therapeutic agent increases the amount of adenosine in the serum. In a preferred embodiment, the second therapeutic is CD73-Ig, recombinant CD73, or another agent (e.g. a cytokine or monoclonal antibody or small molecule) that increases the expression of CD73. In another embodiment the second therapeutic agent is Interferon-beta.

In another embodiment, the second therapeutic is Tysabri or another therapeutic for MS. In a preferred embodiment, B7-H4-Ig is cycled with Tysabri or used during a drug holiday in order to allow less frequent dosing with the second therapeutic and reduce the risk of side effects such as PML and to prevent resistance to the second therapeutic.

In another embodiment, the second therapeutic agent is a small molecule that inhibits or reduces differentiation, proliferation, activity, and/or cytokine production and/or secretion by Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules. In another embodiment, the second therapeutic agent is a small molecule that interacts with Tregs, enhances Treg activity, promotes or

enhances IL-10 secretion by Tregs, increases the number of Tregs, increases the suppressive capacity of Tregs, or combinations thereof. In one embodiment, the small molecule is retinoic acid or a derivative thereof.

In another embodiment, the B7-H4 polypeptides, fusion proteins, or fragments thereof can be used to treat patients who do not respond to TNF blockers.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B are diagrams illustrating modes of action for inhibiting inflammation using B7-H4-Ig. In Figure 1A, B7-H4-Ig targets T cells. In Figure 1B, B7-H4-Ig blocks maturation of dendritic cells (DC).

Figure 2 is a diagram illustrating arthritis induction and treatment for the collagen induced arthritis (CIA) prophylactic model.

Figure 3 is a line graph of arthritis scores versus post collagen II (CII) challenge in mice treated with B7-H4-Ig (filled circles) and mice treated with vehicle (open circles) in the prophylactic model.

Figure 4 is a diagram illustrating arthritis induction and treatment for the CIA therapeutic model.

Figure 5 is a line graph of arthritis scores versus post CII challenge in mice treated with B7-H4-Ig (filled circles) and mice treated with vehicle (open circles) in the therapeutic model.

Figure 6 is a diagram illustrating representative disease induction and dosing regimen for the therapeutic CIA model.

Figure 7 is a line graph showing the Arthritis Score in female mice (AA#79) as a function of time post CII immunization (days). Vehicle, murine IgG, murine B7-H4-Ig, Synagis®, B7-H4-Ig, and RPA110010 treatments are shown.

Figure 8 is a line graph showing the Arthritis Score in male mice (AA#80) as a function of time post CII immunization (days). Vehicle, murine IgG, murine B7-H4-Ig, Synagis®, B7-H4-Ig, and RPA110010 treatments are shown.

Figure 9 is a chart showing the first half of a complete data set following quantitative immunoassay multi-analyte profiling of serum samples from mice treated with B7-H4-Ig, or Synagis® to identify

biomarkers that could be used to monitor disease progression and response to B7-H4-Ig treatment.

Figure 10 is a chart showing the second half of a complete data set following quantitative immunoassay multi-analyte profiling analysis of serum samples from mice treated with B7-H4-Ig, or Synagis® to identify biomarkers that could be used to monitor disease progression and response to B7-H4-Ig treatment.

Figure 11 is a chart showing the quantitative immunoassay multi-analyte profiling Data Analysis Summary for the complete data set of Figures 7 and 8. Analytes are shaded if there is a difference (p less than 0.05) between Synagis® and B7-H4-Ig treated groups at Day 27, Day 34, Day 41, or overall. Analytes are also shaded if the correlation coefficient between the analyte and the clinical score is less than 0.3.

Figure 12 is a line graph showing serum levels of C-Reactive Protein (CRP) (μg/ml) versus clinical score.

Figure 13 is a plot showing the serum levels of Endothelin 1 (ET-1) (pg/ml) in mice treated with B7-H4-Ig (-■-) or Synagis® (-•-) at day 27, day 34, and day 41 post CII immunization.

Figure 14 is a line graph showing serum levels of IL-6 (pg/ml) versus clinical score.

Figure 15 is a plot showing the serum levels of Monocyte Chemotactic Protein-1 (MPC-1) (pg/ml) in mice treated with B7-H4-Ig (-■-) or Synagis® (-•-) at day 27, day 34, and day 41 post CII immunization.

Figure 16 is a line graph showing serum levels of Monocyte Chemotactic Protein-3 (MPC-3) (pg/ml) versus clinical score.

Figure 17 is a plot showing the serum levels of Monocyte Chemotactic Protein-3 (MPC-3) (pg/ml) in mice treated with B7-H4-Ig (-■-) or Synagis® (-•-) at day 27, day 34, and day 41 post CII immunization.

Figure 18 is a plot showing the serum levels of phage Inflammatory Protein-2 (MIP-2) (pg/ml) in mice treated with B7-H4-Ig (-■-) or Synagis® (-•-) at day 27, day 34, and day 41 post CII immunization.

Figures 20A, 20B, and 20C are bar graphs showing plasma proinflammatory cytokine and chemokine levels of mice treated with B7-H4-

Ig (solid bar) or vehicle control (open bar) for the indicated proinflammatory cytokine/chemokine. TNFα (Figure 6a), IL-6 (Figure 6b), and MCP-1 (Figure 6c)

Figure 21 is a bar graph of mouse T cell proliferation ([^{3}H] thymidine incorporation) of naïve T cells cultured under $CD4^+$ Th1 promoting conditions with control IgG (open bars) or B7-H4-Ig (closed bars) bound to the culture plate, or in solution, and activated with either anti-CD3/CD28 bound to beads or with antigen presenting cells pulsed with ovalbumin peptide.

Figure 22 is a bar graph of IFN-γ (ng/ml) produced by mouse naïve $CD4^+$ T cells cultured under Th1 promoting conditions with control IgG (open bars) or B7-H4-Ig (closed bars) bound to the culture plate, or in solution, and activated with either anti-CD3/CD28 bound to beads or with antigen presenting cells pulsed with ovalbumin peptide.

Figure 23 is a bar graph mouse T cell proliferation ([^{3}H] thymidine incorporation) of naïve $CD4^+$ T cells cultured under Th17 promoting conditions with control IgG (open bars) or B7-H4-Ig (closed bars) bound to the culture plate, or in solution, and activated with either anti-CD3/CD28 bound to beads or with antigen presenting cells pulsed with ovalbumin peptide.

Figure 24 is a bar graph of IL17 (ng/ml) produced by mouse naïve T cells cultured under Th17 promoting conditions with control IgG (open bars) or B7-H4-Ig (closed bars) bound to the culture plate, or in solution, and activated with either anti-CD3/CD28 bound to beads or with antigen presenting cells pulsed with ovalbumin peptide.

Figure 25 is a bar graph of TNF-α (ng/ml) produced by mouse naïve T cells cultured under Th17 promoting conditions with control IgG (open bars) or B7-H4-Ig (closed bars) bound to the culture plate or in solution, and activated with either anti-CD3/CD28 bound to beads or with antigen presenting cells pulsed with ovalbumin peptide.

Figure 26 is a bar graph of mouse T cell proliferation ([^{3}H] thymidine incorporation) of naïve $CD4^+$ T cells cultured under Th2 promoting conditions with control IgG (open bars) or B7-H4-Ig (closed bars) bound to

the culture plate, or in solution, and activated with either anti-CD3/CD28 bound to beads or with antigen presenting cells pulsed with ovalbumin peptide.

Figure 27 is a line graph showing INF-γ (ng/ml) produced by mouse naïve T cells cultured under Th1 promoting conditions and stimulated with ovalbumin peptide, as a function of concentration (μg/ml) of control IgG (-○-) or B7-H4-Ig Lot #22 (-•-) or B7-H4-Ig Lot #23 (-▲-).

Figure 28 is a line graph showing IL-17 (ng/ml) produced by mouse naïve T cells cultured under Th17 promoting conditions and stimulated with ovalbumin peptide, as a function of concentration (μg/ml) of control IgG (-○-) or B7-H4-Ig Lot #22 (-•-) or B7-H4-Ig Lot #23 (-▲-).

Figure 29 is a line graph of mouse T cell proliferation ([^{3}H] thymidine incorporation) of naïve CD4$^+$ T cells cultured under Th1 (-•-) or Th17 (-▲-) promoting conditions, stimulated with anti-CD3/CD28 bound to beads, and treated with increasing concentrations of human B7-H4-Ig (μg/ml) added directly to the culture (solution).

Figure 30 is a line graph of IFN-γ (ng/ml) produced by mouse naïve CD4$^+$ T cells cultured under Th1 (-•-) or Th17 (-▲-) promoting conditions, stimulated with anti-CD3/CD28 bound to beads, and treated with increasing concentrations of human B7-H4-Ig (μg/ml) added directly to the culture (solution).

Figure 31 is a line graph of IL17 (ng/ml) produced by mouse naïve CD4$^+$ T cells cultured under Th1 (-•-) or Th17 (-▲-) promoting conditions, stimulated with anti-CD3/CD28 bound to beads, and treated with increasing concentrations of human B7-H4-Ig (μg/ml) added directly to the culture (solution).

Figures 32A and 32B are line graphs of IFN-γ (ng/ml) in (A) and IL-10 (ng/ml) in (B) produced by CD4$^+$CD62$^+$ naïve mouse T cells in the presence or absence of CD25$^+$ T cells cultured under Th1 promoting conditions, stimulated with antigen presenting cells pulsed with ovalbumin peptide, and treated with different concentrations (μg/ml) of control IgG or B7-H4-Ig. T cells containing CD25$^+$ T cells treated with control IgG are shown as (•) or B7-H4-Ig are shown as (▲). T cells with CD25$^+$ T cells

depeleted and treated with IgG are shown as (○) or B7-H4-Ig are shown as (△).

Figures 33A and 33B are a line graphs of IL-17A (ng/ml) in (A) and IL-10 (ng/ml) in (B) produced by $CD4^+CD62^+$ naïve mouse T cells in the presence or absence of $CD25^+$ T cells cultured under Th17 promoting conditions, stimulated with antigen presenting cells pulsed with ovalbumin peptide, and treated with different concentrations (μg/ml) of control IgG or B7-H4-Ig. T cells containing $CD25^+$ T cells treated with control IgG are shown as (●) or B7-H4-Ig are shown as (▲). T cells with $CD25^+$ T cells depeleted and treated with IgG are shown as (○) or B7-H4-Ig are shown as (△).

Figure 34 is a flow chart illustrating the nTreg suppression assay.

Figure 35 is a line graph of T cell proliferation ($[^3H]$ thymidine incorporation) for naïve $CD4^+/GFP^-$ responder T cells and $CD4^+/GFP^+$ nTreg cells at ratios of 1:0, 1:0.12, 1:0.25, 1:0.5, 1:1 and 1:2, plus irradiated non purified splenocytes as antigen presenting cells (APC), anti-CD3 antibody, and varying amounts of murine B7-h4-Ig (0 (-•-), 1 (-■-), 5 (-▲-) ,or 10 (-▼-) μg/mL)).

Figure 36 is a schematic illustration of experimental autoimmune encephalomyelitis (EAE) induction and treatment regimen in an *in vivo* study utilizing an auto-immune R-EAE murine model for Multiple Sclerosis (MS).

Figure 37 is a line graph of mean clinical score versus days post disease induction in EAE induced SJL mice at treatment day = 0 with 60 μg (3mg/kg) control IgG (○); or 60 μg (3mg/kg) B7-H4-Ig (•).

Figure 38 is a line graph of mean clinical score versus days post disease induction in EAE induced SJL mice at treatment day = 21 with 60 μg (3mg/kg) control IgG (○); or 60 μg (3mg/kg) B7-H4-Ig (•).

Figure 39 is a bar graph of Delayed-Type Hypersensitivity (DTH) Responses at day 55 as measured by Mean Ear Swelling ($x10^{-4}$ inches) in EAE mice treated with control IgG or murine B7-H4-Ig at day 0 or day 21, following ear challenge with $PLP_{139-151}$ or $PLP_{178-191}$ on day 50. Open rectangle is control IgG administered at t=0, solid rectangle is B7-H4-Ig administered at t=0, right hatched rectangle is Control IgG at t=21, and left

hatched rectangle is B7-H4-Ig administered at t=21. *DTH response significantly less than Control IgG injected mice, *p less than 0.01*.

Figure 40 is a bar graph of T cell proliferation (ΔCPU (counts per minute)) of T cells isolated from lymph nodes of EAE mice treated with B7-H4-Ig and Control IgG at day 0 or day 21, following treatment with with $PLP_{139-151}$, the disease inducing dominant epitope, and $PLP_{178-191}$, the spread epitope-specific peptide, *in vitro*. Open rectangle is control IgG administered at t=0, solid rectangle is B7-H4-Ig administered at t=0, right hatched rectangle is Control IgG at t=21, and left hatched rectangle is B7-H4-Ig administered at t=21. *[^{3}H]-thymidine incorporation significantly less than Control IgG injected mice, *p less than 0.01*.

Figure 41 is a bar graph of IFN-γ (pg/ml) produced by T cells isolated from lymph nodes of EAE mice treated with B7-H4-Ig and Control IgG at day 0 or day 21, following treatment with with $PLP_{139-151}$, the disease inducing dominant epitope, and $PLP_{178-191}$, the spread epitope-specific peptide, *in vitro*. Open rectangle is control IgG administered at t=0, solid rectangle is B7-H4-Ig administered at t=0, right hatched rectangle is Control IgG at t=21, and left hatched rectangle is B7-H4-Ig administered at t=21. *IFN-γ production significantly less than Control IgG injected mice, *p less than 0.01*.

Figure 42 is a bar graph of Δ Mean Ear Swelling ($x10^{-4}$ inches) minus OVA response in EAE mice treated with 5 injections of 60μg B7-H4-Ig, 300μg B7-H4-Ig, or Control IgG between day 0 and day 10 post disease induction, following ear challenge with $PLP_{139-151}$ or $OVA_{323-339}$ peptide (negative control) on day 10. . *DTH response significantly less than Control IgG injected mice.

Figure 43 is a line graph of proliferation (ΔCPU (counts per minute)) of T cells isolated from lymph nodes of EAE mice described in Figure 42, reactivated *in vitro* in the presence of anti-CD3 (0.1-10 μg/mL), $PLP_{139-151}$ (1-20 μg/mL), or $OVA_{323-339}$ (1-20 μg/mL).

Figure 44 is a schematic illustration of experimental autoimmune encephalomyelitis (EAE) induction and treatment regimen in an *in vivo* study utilizing an auto-immune R-EAE murine model for Multiple Sclerosis (MS).

Figures 45A, 45B, and 45C are bar graphs showing the total lymphocyte cell number ($x10^6$) in the spleen (A), draining lymph nodes (B), and CNS isolated on day 50 from SJL mice immunized with 50 μg of PLP peptide emulsified in CFA. Mice were treated with Control IgG or B7-H4-Ig during remission: 60 or 300 μg per dose, 3 doses/wk, for 2 weeks (6 doses).

Figure 46A, 46B, and 46C are bar graphs showing T cell subset number ($x10^6$) isolated from the spleen (A), draining lymph node (B), or CNS as described in Figure 45, which were $CD4^+$ (T cells), CD4+/FoxP3+ (Treg), and CD4+/CD44+ (effector/memory T cells). The data is presented as the mean number of cells for each phenotype from individual mouse.

Figure 47 is a bar graph showing the percentage of Treg in CD4+ T cells from Figure 46, for Control IgG, 60, or 300 μg B7-H4-Ig treatments.

Figure 48A and 48B are bar graphs showing the proliferation (CPU (counts per minute)) of total splenocytes (A) and lymph node cells (B) isolated on day 35 from SJL mice immunized with 50 μg of PLP peptide emulsified in CFA, and activated *in vitro* in the presence of anti-CD3 (1ug/ml), $PLP_{139-151}$ or $PLP_{178-191}$ (20 μg/mL). Mice were treated with Control IgG or B7-H4-Ig during remission: 60 or 300 μg per dose, 3 doses/wk, for 2 weeks (6 doses).

Figure 49 is a line graph showing the mean clinical score of SJL mice over the 50 day time course (days) following disease induction. Mice were treated with 60μg B7-H4-Ig (-•-), 300μg B7-H4-Ig (-▲-), or Control IgG (-Δ-) three time a week beginning on day 23 post disease induction.

Figure 50 is a schematic illustration of experimental autoimmune encephalomyelitis (EAE) induction and treatment regimen of human B7-H4-Ig in an *in vivo* study utilizing an auto-immune R-EAE murine model for Multiple Sclerosis (MS).

Figures 51A and 51B are line graphs showing the mean clinical score (A) and long term relapse rate (B) of SJL mice over the 55 day time course (days) following disease induction. Mice were treated with 100μg human B7-H4-Ig (5mg/kg), 500μg human B7-H4-Ig (25mg/kg), or 100μg Control IgG, Synagis®, (5mg/kg) three times a week for 4 weeks beginning on day 23 post disease induction.

Figure 52 is a line graph of % $CTLA_4KD$/NOD mice with diabetes versus weeks post treatment with B7-H4-Ig 3 times per week for 4 weeks (15 mg/kg, per i.p. injection). Mice were two weeks old.

Figure 53 is a line graph of % $CTLA_4KD/BDC_{2.5}$/NOD mice with diabetes versus weeks post treatment with B7-H4-Ig 3 times per week for 4 weeks (15 mg/kg, per i.p. injection). Mice were eleven weeks old.

Figure 54 is a bar graph of percent proliferation in Treg cells from control mice and mice treated with different doses of B7-H4-Ig. Treg cells were titrated into a 5 day enteroantigen specific CD4+CD25- proliferation assay. Each column represents mean cpm values of four replicate cultures expressed in percent of cultures not exposed to Treg and bars represent SD. Each group of three columns in the graph from left to right represents Tregs from mice treated with a control, B7-H4-Ig (60 μg) or B7-H4-Ig (300 μg).

Figure 55 is a bar graph of proliferation (cpm) in CD4+CD25- cells recovered from control mice or mice treated with B7-H4-Ig and exposed to enteroantigen pulsed splenocyte APC for 5 days. Each column represents mean cpm values of four replicates cultures and bars represent SD. Each group of three columns in the graph from left to right represents Tregs from mice treated with a control, B7-H4-Ig (60 μg) or B7-H4-Ig (300 μg).

Figure 56 is a bar graph showing the amount of IL-17A (ng/ml) secreted by Balb/C mouse naïve T cells activated by anti-CD3/CD28 in the presence of Th17 differentiation cocktail and either murine B7-H4-Ig, murine isotype IgG2a control, or retinoic acid (RA) added to the culture at day 9, day 1, or day 2 of the four day culture.

Figure 57 is a bar graph showing the fold up- or down-regulation of mRNAs in mouse naïve T cells activated by anti-CD3/CD28 and murine B7-H4-Ig versus murine isotype control. All cells were cultured in the presence of Th17 differentiation cocktail. Expression of mRNAs involved in Th17 cells, Tregs, autoimmune disorders, and/or inflammation was tested by quantitative RT-PCR.

Figure 58 is a bar graph showing the fold up- or down-regulation of mRNAs in human T cells activated by anti-CD3/CD28 and human B7-H4-Ig (1 μg/mL) with a Q or an L at position 46 of the fusion protein, or a

humanized monoclonal IgG antibody directed against an epitope in the A antigenic site of the F protein of the Respiratory Syncytial Virus (Synagis), as an isotype control. All cells were cultured in the presence of Th17 differentiation cocktail. Expression of mRNAs involved in Th17 cells, Tregs, autoimmune disorders, and/or inflammation was tested by quantitative RT-PCR.

Figure 59 is a bar graph of IL-17A (ng/ml) concentrations produced by TH17 cells treated with indicated concentrations of murine B7-H4-Ig, murine IgG control, or retinoic acid.

Figure 60 is a line graph of IL-17A (ng/ml) versus protein concentration (μg/ml) showing activity of B7-H4-IgQ (■), B7-H4-IgL (▲) versus Synagis (♦) (an irrelevant human IgG_1 antibody) on mouse Th17 cells.

Figure 61 a line graph of IL-17A (ng/ml) versus protein concentration (μg/ml) showing activity of murine B7-H4-Ig (lots 22 (■) and 23 (▲)) versus mouse IgG (♦) and retinoic acid controls (X)on Th17 cells.

Figure 62 is a schematic illustration of a treatment schedule for an *in vivo* study utilizing an MRL/*lpr* lupus mouse model.

Figure 63 is a bar graph showing anti-ds DNA auto antibodies (units) detected in plasma collected from MRL/*lpr* mice pre-treatment (4 week) and periodically up to 21 weeks of age in control, B7-H4-Ig and cyclophosphamide (CTX) combination treatment, B7-H4-Ig only treatment, and CTX only treatment groups. For each treatment group, bars from left to right show anti-ds DNA auto antibodies at 4, 7, 8, 9, 10, 11, 15, 19, and 21 weeks.

Figure 64 is a bar graph showing the protein index (units) in control, B7-H4-Ig and cyclophosphamide (CTX) combination treatment, B7-H4-Ig only treatment, and CTX only treatment groups at 21 weeks.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

As used herein the term "isolated" refers to a compound of interest (e.g., either a polynucleotide or a polypeptide) that is in an environment different from that in which the compound naturally occurs e.g. separated from its natural milieu such as by concentrating a peptide to a concentration

at which it is not found in nature. "Isolated" includes compounds that are within samples that are substantially enriched for the compound of interest and/or in which the compound of interest is partially or substantially purified.

An "immune cell" refers to any cell from the hemopoietic origin including but not limited to T cells, B cells, monocytes, dendritic cells, and macrophages.

As used herein, the term "polypeptide" refers to a chain of amino acids of any length, regardless of modification (e.g., phosphorylation or glycosylation).

As used herein, a "costimulatory polypeptide" or "costimulatory molecule" is a polypeptide that, upon interaction with a cell-surface molecule on T cells, modulates T cell responses.

As used herein, a "costimulatory signaling" is the signaling activity resulting from the interaction between costimulatory polypeptides on antigen presenting cells and their receptors on T cells during antigen-specific T cell responses. Antigen-specific T cell response mediated by two signals: 1) engagement of the T cell Receptor (TCR) with antigenic peptide presented in the context of MHC (signal 1), and 2) a second antigen-independent signal delivered by contact between different costimulatory receptor/ligand pairs (signal 2). This "second signal" is critical in determining the type of T cell response (activation vs inhibition) as well as the strength and duration of that response, and is regulated by both positive and negative signals from costimulatory molecules, such as the B7 family of proteins.

As used herein, the term "B7 polypeptide" means a member of the B7 family of proteins that costimulate T cells including, but not limited to B7-1, B7-2, B7-DC, B7-H5, B7-H1, B7-H2, B7-H3, B7-H4 and biologically active fragments and/or variants thereof. Representative biologically active fragments include the extracellular domain or fragments of the extracellular domain that costimulate T cells.

As used herein "soluble B7-H4" or "sH4" refers to fragments of B7-H4 that may be shed, secreted or otherwise extracted from cells that express B7-H4. Soluble fragments of B7-H4 include some or all of the extracellular

domain of the B7-H4 polypeptide, and lack some or all of the intracellular and/or transmembrane domains. In one embodiment, soluble B7-H4 polypeptide fragments include the entire extracellular domain of the B7-H4 polypeptide. In other embodiments, the soluble fragments of B7-H4 polypeptides include fragments of the extracellular domain. Extracellular domains of B7-H4 polypeptides can be readily determined by those of skill in the art using standard methodologies such as hydropathy plotting.

As used herein, "inflammatory molecules" refers to molecules that results inflammatory responses including, but not limited to, cytokines and metalloproteases such as including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs.

As used herein, a "vector" is a replicon, such as a plasmid, phage, or cosmid, into which another DNA segment may be inserted so as to bring about the replication of the inserted segment. The vectors described herein can be expression vectors.

As used herein, an "expression vector" is a vector that includes one or more expression control sequences

As used herein, an "expression control sequence" is a DNA sequence that controls and regulates the transcription and/or translation of another DNA sequence.

"Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual or intended function. Thus, two different polypeptides operably linked together retain their respective biological functions while physically linked together.

As used herein, "valency" refers to the number of binding sites available per molecule.

As used herein, a "variant" polypeptide contains at least one amino acid sequence alteration as compared to the amino acid sequence of the corresponding wild-type polypeptide.

As used herein, "conservative" amino acid substitutions are substitutions wherein the substituted amino acid has similar structural or chemical properties.

As used herein, the term "host cell" refers to prokaryotic and eukaryotic cells into which a recombinant vector can be introduced.

As used herein, "transformed" and "transfected" encompass the introduction of a nucleic acid (e.g. a vector) into a cell by a number of techniques known in the art.

As used herein, the phrase that a molecule "specifically binds" or "displays specific binding" to a target refers to a binding reaction which is determinative of the presence of the molecule in the presence of a heterogeneous population of other biologics. Under designated immunoassay conditions, a specified molecule binds preferentially to a particular target and does not bind in a significant amount to other biologics present in the sample. Specific binding of an antibody to a target under such conditions requires the antibody be selected for its specificity to the target. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

As used herein, the terms "immunologic", "immunological" or "immune" response is the development of a beneficial humoral (antibody mediated) and/or a cellular (mediated by antigen-specific T cells or their secretion products) response directed against a peptide in a recipient patient. Such a response can be an active response induced by administration of immunogen or a passive response induced by administration of antibody or primed T-cells. A cellular immune response is elicited by the presentation of polypeptide epitopes in association with Class I or Class II MHC molecules to activate antigen-specific $CD4^+$ T helper cells and/or $CD8^+$ cytotoxic T cells. The response may also involve activation of monocytes, macrophages, NK cells, basophils, dendritic cells, astrocytes, microglia cells, eosinophils, activation or recruitment of neutrophils or other components of innate immunity. The presence of a cell-mediated immunological response can be

determined by proliferation assays ($CD4^+$ T cells) or CTL (cytotoxic T lymphocyte) assays. The relative contributions of humoral and cellular responses to the protective or therapeutic effect of an immunogen can be distinguished by separately isolating antibodies and T-cells from an immunized syngeneic animal and measuring protective or therapeutic effect in a second subject.

An "immunogenic agent" or "immunogen" is capable of inducing an immunological response against itself on administration to a mammal, optionally in conjunction with an adjuvant.

The terms "individual", "host", "subject", and "patient" are used interchangeably herein, and refer to a mammal, including, but not limited to, humans, rodents, such as mice and rats, and other laboratory animals.

II. Fusion proteins

B7-H4 fusion polypeptides have a first fusion partner comprising all or a part of a B7-H4 protein fused to a second polypeptide directly or via a linker peptide sequence that is fused to the second polypeptide. The fusion proteins optionally contain a domain that functions to dimerize or multimerize two or more fusion proteins. The peptide/polypeptide linker domain can either be a separate domain, or alternatively can be contained within one of the other domains (B7-H4 polypeptide or second polypeptide) of the fusion protein. Similarly, the domain that functions to dimerize or multimerize the fusion proteins can either be a separate domain, or alternatively can be contained within one of the other domains (B7-H4 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.

Fusion proteins disclosed herein are of formula I:

$$N\text{-}R_1\text{-}R_2\text{-}R_3\text{-}C$$

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 B7-H4 polypeptide, "R_2" is an optional peptide/polypeptide linker domain,

and "R_3" is a second polypeptide. Alternatively, R_3 may be a B7-H4 polypeptide and R_1 may be a second polypeptide.

Dimerization or multimerization can occur between or among two or more fusion proteins through dimerization or multimerization domains. Alternatively, dimerization or multimerization of fusion proteins can occur by chemical crosslinking. The dimers or multimers that are formed can be homodimeric/homomultimeric or heterodimeric/heteromultimeric.

A. B7-H4 polypeptides

In a preferred embodiment the B7- H4 polypeptide is from a mammalian species. In the most preferred embodiment, the B7- H4 polypeptide is of murine, non-human primate (*Pan troglodytes*, *Macaca mulatta* or *Macaca fascicularis*), or human origin. Useful murine B7-H4 polypeptides have at least about 80, 85, 90, 95 or 100% sequence identity to the B7-H4 polypeptide encoded by the nucleic acid having GenBank Accession Number NM_178594 or AY280973. Useful murine B7-H4 polypeptides have at least about 80, 85, 90, 95 or 100% sequence identity to the B7-H4 polypeptide according to GenBank Accession Number AAH32925.1 or NP_848709.2. Useful human B7-H4 polypeptides have at least about 80, 85, 90, 95 or 100% sequence identity to the B7-H4 polypeptide encoded by the nucleic acid having GenBank Accession Number AK026071. Useful human B7-H4 polypeptides have at least about 80, 85, 90, 95 or 100% sequence identity to the B7-H4 polypeptide according to GenBank Accession Number NP_078902.2 or BAB15349.1.

Murine B7-H4 polypeptides can be encoded by a nucleotide sequence having at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

```
atggcttcct tggggcagat catcttttgg agtattatta acatcatcat catcctggct    60
ggggccatcg cactcatcat tggctttggc atttcaggca agcacttcat cacggtcacg   120
accttcacct cagctggaaa cattggagag gacgggaccc tgagctgcac ttttgaacct   180
gacatcaaac tcaacggcat cgtcatccag tggctgaaag aaggcatcaa aggtttggtc   240
cacgagttca aagaaggcaa agacgacctc tcacagcagc atgagatgtt cagaggccgc   300
acagcagtgt ttgctgatca ggtggtagtt ggcaatgctt ccctgagact gaaaaacgtg   360
cagctcacgg atgctggcac ctacacatgt tacatccgca cctcaaaagg caaagggaat   420
gcaaaccttg agtataagac cggagccttc agtatgccag agataaatgt ggactataat   480
gccagttcag agagtttacg ctgcgaggct cctcggtggt tcccccagcc cacagtggcc   540
tgggcatctc aagtcgacca aggagccaat ttctcagaag tctccaacac cagctttgag   600
ttgaactctg agaatgtgac catgaaggtc gtatctgtgc tctacaatgt cacaatcaac   660
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aacacatact cctgtatgat tgaaaacgac attgccaaag ccaccgggga catcaaagtg    720
acagattcag aggtcaaaag gcgaagtcag ctgcagttgc tgaactctgg gccttccccg    780
tgtgtttttt cttctgcctt tgtggctggc tgggcactcc tatctctctc ctgttgcctg    840
atgctaagat ga                                                       852
```

(SEQ ID NO:1).

Murine B7-H4 polypeptides can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

```
MASLGQIIFW SIINIIIILA GAIALIIGFG ISGKHFITVT TFTSAGNIGE DGTLSCTFEP     60
DIKLNGIVIQ WLKEGIKGLV HEFKEGKDDL SQQHEMFRGR TAVFADQVVV GNASLRLKNV    120
QLTDAGTYTC YIRSSKGKGN ANLEYKTGAF SMPEINVDYN ASSESLRCEA PRWFPQPTVA    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TDSEVKRRSQ LQLLNSGPSP CVSSSAFVAG WALLSLSCCL MLR                      283
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(SEQ ID NO:2),

```
MEWSWVFLFF LSVTTGVHSG FGISGKHFIT VTTFTSAGNI GEDGTLSCTF EPDIKLNGIV     60
IQWLKEGIKG LVHEFKEGKD DLSQQHEMFR GRTAVFADQV VVGNASLRLK NVQLTDAGTY    120
TCYIRSSKGK GNANLEYKTG AFSMPEINVD YNASSESLRC EAPRWFPQPT VAWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTDSEVKRR    240
SQLQLLNSGP SPCVSSSAFV AGWALLSLSC CLMLR                               275
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(SEQ ID NO:3),

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK     60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRSSKG KGNANLEYKT    120
GAFSMPEINV DYNASSESLR CEAPRWFPQP TVAWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTDSEVKR RSQLQLLNSG PSPCVSSSAF    240
VAGWALLSLS CCLMLR                                                    256
```

(SEQ ID NO:4),

```
MASLGQIIFW SIINIIIILA GAIALIIGFG ISGKHFITVT TFTSAGNIGE DGTLSCTFEP     60
DIKLNGIVIQ WLKEGIKGLV HEFKEGKDDL SQQHEMFRGR TAVFADQVVV GNASLRLKNV    120
QLTDAGTYTC YIRTSKGKGN ANLEYKTGAF SMPEINVDYN ASSESLRCEA PRWFPQPTVA    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TDSEVKRRSQ LQLLNSGPSP CVFSSAFVAG WALLSLSCCL MLR                      283
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(SEQ ID NO:5),

```
MEWSWVFLFF LSVTTGVHSG FGISGKHFIT VTTFTSAGNI GEDGTLSCTF EPDIKLNGIV     60
IQWLKEGIKG LVHEFKEGKD DLSQQHEMFR GRTAVFADQV VVGNASLRLK NVQLTDAGTY    120
TCYIRTSKGK GNANLEYKTG AFSMPEINVD YNASSESLRC EAPRWFPQPT VAWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTDSEVKRR    240
SQLQLLNSGP SPCVFSSAFV AGWALLSLSC CLMLR                               275
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(SEQ ID NO:6), or

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK     60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRTSKG KGNANLEYKT    120
GAFSMPEINV DYNASSESLR CEAPRWFPQP TVAWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTDSEVKR RSQLQLLNSG PSPCVFSSAF    240
VAGWALLSLS CCLMLR                                                    256
```

(SEQ ID NO:7)

Human B7-H4 polypeptides can be encoded by a nucleotide sequence having at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

```
atggcttccc tggggcagat cctcttctgg agcataatta gcatcatcat tattctggct     60
ggagcaattg cactcatcat tggctttggt atttcaggga gacactccat cacagtcact    120
actgtcgcct cagctgggaa cattggggag gatggaatcc tgagctgcac ttttgaacct    180
gacatcaaac tttctgatat cgtgatacaa tggctgaagg aaggtgtttt aggcttggtc    240
catgagttca aagaaggcaa agatgagctg tcggagcagg atgaaatgtt cagaggccgg    300
acagcagtgt ttgctgatca agtgatagtt ggcaatgcct ctttgcggct gaaaaacgtg    360
caactcacag atgctggcac ctacaaatgt tatatcatca cttctaaagg caaggggaat    420
gctaaccttg agtataaaac tggagccttc agcatgccgg aagtgaatgt ggactataat    480
gccagctcag agaccttgcg gtgtgaggct ccccgatggt tcccccagcc cacagtggtc    540
tgggcatccc aagttgacca gggagccaac ttctcggaag tctccaatac cagctttgag    600
ctgaactctg agaatgtgac catgaaggtt gtgtctgtgc tctacaatgt tacgatcaac    660
aacacatact cctgtatgat tgaaaatgac attgccaaag caacagggga tatcaaagtg    720
acagaatcgg agatcaaaag gcggagtcac ctacagctgc taaactcaaa ggcttctctg    780
tgtgtctctt ctttctttgc catcagctgg gcacttctgc ctctcagccc ttacctgatg    840
ctaaaataa                                                            849
```

(SEQ ID NO:8).

Human B7-H4 polypeptides can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

```
MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRSH LQLLNSKASL CVSSFFAISW ALLPLSPYLM LK                       282
```

(SEQ ID NO:9),

```
MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV     60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY    120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR    240
SHLQLLNSKA SLCVSSFFAI SWALLPLSPY LMLK                                274
```

(SEQ ID NO:10),

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK ASLCVSSFFA    240
ISWALLPLSP YLMLK                                                     255
```

(SEQ ID NO:11),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSH LQLLNSKASL CVSSFFAISW ALLPLSPYLM LK 282

(SEQ ID NO:12),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SHLQLLNSKA SLCVSSFFAI SWALLPLSPY LMLK 274

(SEQ ID NO:13), or

GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK 60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT 120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT 180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK ASLCVSSFFA 240
ISWALLPLSP YLMLK 255

(SEQ ID NO:14).

Non-human primate B7-H4 polypeptides can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

MKPLTSRIIS IIIILAGAIA LIIGFGISGR HSITVTTVAS AGNIGEDGIL SCTFEPDIKL 60
SDIVIQWLKE GVLGLVHEFK EGKDELSEQD EMFRGRTAVF ADQVIVGNAS LRLKNVQLTD 120
AGTYKCYIIT SKGKGNANLE YKTGAFSMPE VNVDYNASSE TLRCEAPRWF PQPTVVWASQ 180
IDQGANFSEV SNTSFELNSE NVTMKVVSVL YNATINNTYS CMIENDIAKA TGDIKVTESE 240
IKRRSHLQLL NSKASLCVSS FFAISWALLP LSPYLMLK 278

(SEQ ID NO:15), or

GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK 60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT 120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQIDQ GANFSEVSNT SFELNSENVT 180
MKVVSVLYNA TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK ASLCVSSFFA 240
ISWALLPLSP YLMLK 255

(SEQ ID NO:16), or

MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP 60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSH LQLLNSKASL CVSSFLAISW ALLPLAPYLM LK 282

(SEQ ID NO:17), or

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK ASLCVSSFLA    240
ISWALLPLAP YLMLK                                                     255
```

(SEQ ID NO:18), or

```
MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRSH LQLLNSKASL CVSSFLAISW ALPPLAPYLM LK                       282
```

(SEQ ID NO:19), or

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK ASLCVSSFLA    240
ISWALPPLAP YLMLK                                                     255
```

(SEQ ID NO:20),

where SEQ ID NOs:15 and 16 are chimapanzee (*Pan troglodytes*) polypeptide sequences, SEQ ID NOs:17 and 18 are rhesus monkey (*Macaca mulatta*) polypeptide sequences, and SEQ ID NOs:19 and 20 are cynomolgus monkey (*Macaca fascicularis*) polypeptide sequences.

Nucleic acids encoding B7-H4 polypeptides may be optimized for expression in the expression host of choice. Codons may be substituted with alternative codons encoding the same amino acid to account for differences in codon usage between the mammal from which the B7-H4 nucleic acid sequence is derived and the expression host. In this manner, the nucleic acids may be synthesized using expression host-preferred codons.

1. Fragments of B7-H4 polypeptides

The B7-H4 proteins contain two immunoglobulin domains within the extracellular, the IgV domain (or V domain) and the IgC domain (or C domain), which are related to the variable and constant domains of antibodies. The domains can be identified by anyone skilled in the art by searching against family and domain databases. The IgV domain is believed to be responsible for receptor binding, based on functional data from the isolated IgV domain as well as by analogy to the other B7 family members. Each Ig domain of extracellular domain includes one disulfide bond formed

between intradomain cystein residues, as is typical for this fold and may be important for structure-function. In SEQ ID NOS: 2, 5, 9 and 12 these cysteines are located at residues 56 and 130 for the IgV domain, and 168 and 225 for the IgC domain. In addition, there is one predicted N-linked glycosylation site in the IgV domain and six glycosylation sites in the IgC domain, which are conserved between mouse and human B7-H4 sequences.

In one embodiment, the first fusion partner is a fragment of B7-H4. As used herein, a fragment of B7-H4 refers to any subset of the polypeptide that is at least one amino acid shorter than full length protein. Useful fragments are those that retain the ability to bind to their natural receptor or receptors. A B7-H4 polypeptide that is a fragment of full-length B7-H4 typically has at least 20 percent, 30 percent, 40 percent, 50 percent, 60 percent, 70 percent, 80 percent, 90 percent, 95 percent, 98 percent, 99 percent, 100 percent, or even more than 100 percent of the ability to bind its natural receptor(s) as compared to full-length B7-H4.

Fragments of B7-H4 polypeptides include soluble fragments. Soluble B7-H4 polypeptide fragments are fragments of B7-H4 polypeptides that may be shed, secreted or otherwise extracted from the producing cells. Soluble fragments of B7-H4 polypeptides include some or all of the extracellular domain of the receptor polypeptide, and lack some or all of the intracellular and/or transmembrane domains. In one embodiment, B7-H4 polypeptide fragments include the entire extracellular domain of the B7-H4 polypeptide. In other embodiments, the soluble fragments of B7-H4 polypeptides include fragments of the extracellular domain that retain B7-H4 biological activity. The extracellular domain can include 1, 2, 3, 4, or 5 contiguous amino acids from the transmembrane domain, and/or 1, 2, 3, 4, or 5 contiguous amino acids from the signal sequence. Alternatively, the extracellular domain can have 1, 2, 3, 4, 5 or more amino acids removed from the C-terminus, N-terminus, or both. In some embodiments the extracellular domain is only the IgV domain , or the region between the conserved cysteines of the IgV domain located at residues 56 and 130 of the full-length protein.

Generally, the B7-H4 polypeptides or fragments thereof are expressed from nucleic acids that include sequences that encode a signal sequence. The signal sequence is generally cleaved from the immature polypeptide to produce the mature polypeptide lacking the signal sequence. SEQ ID NOs: 4, 7, 11, 14, 16, 18 and 20 each lack a signal peptide. The signal sequence of B7-H4 can be replaced by the signal sequence of another polypeptide using standard molecule biology techniques to affect the expression levels, secretion, solubility, or other property of the polypeptide. The signal sequence that is used to replace the B7-H4 signal sequence can be any known in the art. SEQ ID NOs: 2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19 each contain a signal peptide.

In a preferred embodiment, the fusion protein includes the extracellular domain of B7-H4, or a fragment thereof fused to an Ig Fc region. Recombinant B7-H4-Ig fusion proteins can be prepared by fusing the coding region of the extracellular domain of B7-H4 or a fragment thereof to the Fc region of human IgG1 or mouse IgG2a, as described previously (Chapoval, et al., *Methods Mol. Med.*, 45:247-255 (2000)).

a. Murine B7-DC extracellular domain fusion partners

In one embodiment, the first fusion partner of the fusion protein includes the extracellular domain of murine B7-H4 or a fragment thereof. The first fusion partner can be encoded by a nucleotide sequence having at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

```
atggcttcct tggggcagat catcttttgg agtattatta acatcatcat catcctggct     60
ggggccatcg cactcatcat tggctttggc atttcaggca agcacttcat cacggtcacg    120
accttcacct cagctggaaa cattggagag gacgggaccc tgagctgcac ttttgaacct    180
gacatcaaac tcaacggcat cgtcatccag tggctgaaag aaggcatcaa aggtttggtc    240
cacgagttca aagaaggcaa agacgacctc tcacagcagc atgagatgtt cagaggccgc    300
acagcagtgt ttgctgatca ggtggtagtt ggcaatgctt ccctgagact gaaaaacgtg    360
cagctcacgg atgctggcac ctacacatgt tacatccgca cctcaaaagg caaagggaat    420
gcaaaccttg agtataagac cggagccttc agtatgccag agataaatgt ggactataat    480
gccagttcag agagtttacg ctgcgaggct cctcggtggt tcccccagcc cacagtggcc    540
tgggcatctc aagtcgacca aggagccaat ttctcagaag tctccaacac cagctttgag    600
ttgaactctg agaatgtgac catgaaggtc gtatctgtgc tctacaatgt cacaatcaac    660
aacacatact cctgtatgat tgaaaacgac attgccaaag ccaccgggga catcaaagtg    720
acagattcag aggtcaaaag gcgaagtcag ctgcagttgc tgaactctgg g              771
```

(SEQ ID NO:21),

```
atggagtggt catgggtttt tctgttcttt cttagcgtga ctacaggcgt ccattcagga     60
ttcggcataa gcggcaagca cttcatcaca gttacaacgt ttacaagtgc ggggaacatt    120
ggggaagatg gaacattgtc atgtacattt gagccagata tcaaactcaa tggaatagta    180
attcagtggc ttaaggaggg catcaagggc ctggtccacg aatttaagga ggggaaagac    240
gatctgtctc agcagcacga gatgttcagg ggcagaaccg ccgtcttcgc agaccaggtt    300
gtggtaggca acgccagttt gcggctgaaa aacgtgcagc tgactgacgc cggcacctac    360
acatgctata tccggtcctc taagggcaag gggaacgcta atctcgagta caaaacaggc    420
gccttttcta tgccagagat caacgtggac tataacgcaa gctctgaaag tctgagatgc    480
gaggcgccaa ggtggttccc tcagcccacc gtcgcgtggg cttcccaggt ggatcaaggc    540
gccaactttt ctgaggtttc taacaccagc ttcgaactga acagcgaaaa tgtgacaatg    600
aaggtagtca gcgttctgta taacgtgacc atcaacaata cttactcctg tatgatagaa    660
aatgatatag ccaaggctac aggagatatt aaagtgacgg attcagaagt gaaaaggagg    720
agtcaactgc aactcttgaa tagcggc                                        747
```

(SEQ ID NO:22) or

```
atggagtggt catgggtttt tctgttcttt cttagcgtga ctacaggcgt ccattcagga     60
ttcggcataa gcggcaagca cttcatcaca gttacaacgt ttacaagtgc ggggaacatt    120
ggggaagatg gaacattgtc atgtacattt gagccagata tcaaactcaa tggaatagta    180
attcagtggc ttaaggaggg catcaagggc ctggtccacg aatttaagga ggggaaagac    240
gatctgtctc agcagcacga gatgttcagg ggcagaaccg ccgtcttcgc agaccaggtt    300
gtggtaggca acgccagttt gcggctgaaa aacgtgcagc tgactgacgc cggcacctac    360
acatgctata tccggacctc taagggcaag gggaacgcta atctcgagta caaaacaggc    420
gccttttcta tgccagagat caacgtggac tataacgcaa gctctgaaag tctgagatgc    480
gaggcgccaa ggtggttccc tcagcccacc gtcgcgtggg cttcccaggt ggatcaaggc    540
gccaactttt ctgaggtttc taacaccagc ttcgaactga acagcgaaaa tgtgacaatg    600
aaggtagtca gcgttctgta taacgtgacc atcaacaata cttactcctg tatgatagaa    660
aatgatatag ccaaggctac aggagatatt aaagtgacgg attcagaagt gaaaaggagg    720
agtcaactgc aactcttgaa tagcggc                                        747
```

(SEQ ID NO:23).

In another embodiment, the first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the murine amino acid sequence:

```
MEWSWVFLFF LSVTTGVHSG FGISGKHFIT VTTFTSAGNI GEDGTLSCTF EPDIKLNGIV     60
IQWLKEGIKG LVHEFKEGKD DLSQQHEMFR GRTAVFADQV VVGNASLRLK NVQLTDAGTY    120
TCYIRSSKGK GNANLEYKTG AFSMPEINVD YNASSESLRC EAPRWFPQPT VAWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTDSEVKRR    240
SQLQLLNSG                                                            249
```

(SEQ ID NO:24),

```
MEWSWVFLFF LSVTTGVHSG FGISGKHFIT VTTFTSAGNI GEDGTLSCTF EPDIKLNGIV     60
IQWLKEGIKG LVHEFKEGKD DLSQQHEMFR GRTAVFADQV VVGNASLRLK NVQLTDAGTY    120
TCYIRTSKGK GNANLEYKTG AFSMPEINVD YNASSESLRC EAPRWFPQPT VAWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTDSEVKRR    240
SQLQLLNSG                                                            249
```

(SEQ ID NO:25),

```
MASLGQIIFW SIINIIIILA GAIALIIGFG ISGKHFITVT TFTSAGNIGE DGTLSCTFEP     60
DIKLNGIVIQ WLKEGIKGLV HEFKEGKDDL SQQHEMFRGR TAVFADQVVV GNASLRLKNV    120
QLTDAGTYTC YIRSSKGKGN ANLEYKTGAF SMPEINVDYN ASSESLRCEA PRWFPQPTVA    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TDSEVKRRSQ LQLLNSG                                                   257
```

(SEQ ID NO:26), or

```
MASLGQIIFW SIINIIIILA GAIALIIGFG ISGKHFITVT TFTSAGNIGE DGTLSCTFEP     60
DIKLNGIVIQ WLKEGIKGLV HEFKEGKDDL SQQHEMFRGR TAVFADQVVV GNASLRLKNV    120
QLTDAGTYTC YIRTSKGKGN ANLEYKTGAF SMPEINVDYN ASSESLRCEA PRWFPQPTVA    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TDSEVKRRSQ LQLLNSG                                                   257
```

(SEQ ID NO:27).

The signal sequence is removed in the mature protein. Additionally, signal peptides from other polypeptides or organisms can be used to enhance the secretion of the fusion protein from a host during manufacture. SEQ ID NO:28 provides the murine amino acid sequence of SEQ ID NO:24 and SEQ ID NO:26 without the signal sequence:

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK     60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRSSKG KGNANLEYKT    120
GAFSMPEINV DYNASSESLR CEAPRWFPQP TVAWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTDSEVKR RSQLQLLNSG               230
```

(SEQ ID NO:28).

SEQ ID NO:29 provides the murine amino acid sequence of SEQ ID NO:25 and SEQ ID NO:27 without the signal sequence:

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK     60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRTSKG KGNANLEYKT    120
GAFSMPEINV DYNASSESLR CEAPRWFPQP TVAWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTDSEVKR RSQLQLLNSG               230
```

(SEQ ID NO:29)

In another embodiment, the first fusion partner of the fusion protein includes the IgV domain of murine B7-H4. In one embodiment, the IgV domain includes at least from the cysteine at position 56 of SEQ ID NO:2 or SEQ ID NO:5 to the cysteine at position 130 of SEQ ID NO:2 or SEQ ID NO:5. In another embodiment, the IgV domain contains a fragment of at least 25 or 50 amino acids of the polypeptide defined by this amino acid range.

The first fusion partner can be encoded by a nucleotide sequence having at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the following nucleotide sequence encoding an exemplary IgV domain:

```
ggattcggca taagcggcaa gcacttcatc acagttacaa cgtttacaag tgcggggaac     60
attggggaag atggaacatt gtcatgtaca tttgagccag atatcaaact caatggaata    120
gtaattcagt ggcttaagga gggcatcaag ggcctggtcc acgaatttaa ggaggggaaa    180
gacgatctgt ctcagcagca cgagatgttc aggggcagaa ccgccgtctt cgcagaccag    240
gttgtggtag gcaacgccag tttgcggctg aaaaacgtgc agctgactga cgccggcacc    300
tacacatgct atatccggtc ctctaagggc aaggggaacg ctaatctcga gtacaaaaca    360
ggcgcctttt ctatgccaga gatcaac                                        387
```

(SEQ ID NO:30) or

```
ggattcggca taagcggcaa gcacttcatc acagttacaa cgtttacaag tgcggggaac     60
attggggaag atggaacatt gtcatgtaca tttgagccag atatcaaact caatggaata    120
gtaattcagt ggcttaagga gggcatcaag ggcctggtcc acgaatttaa ggaggggaaa    180
gacgatctgt ctcagcagca cgagatgttc aggggcagaa ccgccgtctt cgcagaccag    240
gttgtggtag gcaacgccag tttgcggctg aaaaacgtgc agctgactga cgccggcacc    300
tacacatgct atatccggac ctctaagggc aaggggaacg ctaatctcga gtacaaaaca    360
ggcgcctttt ctatgccaga gatcaac                                        387
```

(SEQ ID NO:31).

The first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the murine amino acid sequence:

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK     60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRSSKG KGNANLEYKT    120
GAFSMPEIN                                                            129
```

(SEQ ID NO:32), or

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK     60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRTSKG KGNANLEYKT    120
GAFSMPEIN                                                            129
```

(SEQ ID NO:33).

b. Human extracellular domain fusion partners

In another embodiment, the first fusion partner of the fusion protein includes the extracellular domain of human B7-H4 or a fragment thereof. The first fusion partner can be encoded by a nucleotide sequence having at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

```
atggcttccc tggggcagat cctcttctgg agcataatta gcatcatcat tattctggct     60
ggagcaattg cactcatcat tggctttggt atttcaggga gacactccat cacagtcact    120
actgtcgcct cagctgggaa cattggggag gatggaatcc tgagctgcac ttttgaacct    180
gacatcaaac tttctgatat cgtgatacaa tggctgaagg aaggtgtttt aggcttggtc    240
catgagttca aagaaggcaa agatgagctg tcggagcagg atgaaatgtt cagaggccgg    300
acagcagtgt ttgctgatca agtgatagtt ggcaatgcct ctttgcggct gaaaaacgtg    360
```

caactcacag atgctggcac ctacaaatgt tatatcatca cttctaaagg caaggggaat 420
gctaaccttg agtataaaac tggagccttc agcatgccgg aagtgaatgt ggactataat 480
gccagctcag agaccttgcg gtgtgaggct ccccgatggt tcccccagcc cacagtggtc 540
tgggcatccc aagttgacca gggagccaac ttctcggaag tctccaatac cagctttgag 600
ctgaactctg agaatgtgac catgaaggtt gtgtctgtgc tctacaatgt tacgatcaac 660
aacacatact cctgtatgat tgaaaatgac attgccaaag caacagggga tatcaaagtg 720
acagaatcgg agatcaaaag gcggagt 747

(SEQ ID NO:34),

atggcttccc tggggcagat cctcttctgg agcataatta gcatcatcat tattctggct 60
ggagcaattg cactcatcat tggctttggt atttcaggga gacactccat cacagtcact 120
actgtcgcct cagctgggaa cattggggag gatggaatcc tgagctgcac ttttgaacct 180
gacatcaaac tttctgatat cgtgatacaa tggctgaagg aaggtgtttt aggcttggtc 240
catgagttca aagaaggcaa agatgagctg tcggagcagg atgaaatgtt cagaggccgg 300
acagcagtgt ttgctgatca agtgatagtt ggcaatgcct ctttgcggct gaaaaacgtg 360
caactcacag atgctggcac ctacaaatgt tatatcatca cttctaaagg caaggggaat 420
gctaaccttg agtataaaac tggagccttc agcatgccgg aagtgaatgt ggactataat 480
gccagctcag agaccttgcg gtgtgaggct ccccgatggt tcccccagcc cacagtggtc 540
tgggcatccc aagttgacca gggagccaac ttctcggaag tctccaatac cagctttgag 600
ctgaactctg agaatgtgac catgaaggtt gtgtctgtgc tctacaatgt tacgatcaac 660
aacacatact cctgtatgat tgaaaatgac attgccaaag caacagggga tatcaaagtg 720
acagaatcgg agatc 735

(SEQ ID NO:35),

atggcttccc tggggcagat cctcttctgg agcataatta gcatcatcat tattctggct 60
ggagcaattg cactcatcat tggctttggt atttcaggga gacactccat cacagtcact 120
actgtcgcct cagctgggaa cattggggag gatggaatcc tgagctgcac ttttgaacct 180
gacatcaaac tttctgatat cgtgatacaa tggctgaagg aaggtgtttt aggcttggtc 240
catgagttca aagaaggcaa agatgagctg tcggagcagg atgaaatgtt cagaggccgg 300
acagcagtgt ttgctgatca agtgatagtt ggcaatgcct ctttgcggct gaaaaacgtg 360
caactcacag atgctggcac ctacaaatgt tatatcatca cttctaaagg caaggggaat 420
gctaaccttg agtataaaac tggagccttc agcatgccgg aagtgaatgt ggactataat 480
gccagctcag agaccttgcg gtgtgaggct ccccgatggt tcccccagcc cacagtggtc 540
tgggcatccc aagttgacca gggagccaac ttctcggaag tctccaatac cagctttgag 600
ctgaactctg agaatgtgac catgaaggtt gtgtctgtgc tctacaatgt tacgatcaac 660
aacacatact cctgtatgat tgaaaatgac attgccaaag caacagggga tatcaaagtg 720
acagaatcgg agatcaaaag gcggagtcac ctacagctgc taaactcaaa ggcttct 777

(SEQ ID NO:36),

atggaatgga gctgggtatt tctgtttttc ctgtcagtaa cgactggcgt ccattcaggc 60
ttcggcatca gtggacggca cagtatcaca gtgaccaccg tcgcctccgc tggcaatata 120
ggtgaggatg gcatccagtc ctgtaccttt gagccggaca tcaaactgtc tgacatagtg 180
atacaatggc tgaaggaggg ggtgctcggt ctggtacatg agtttaagga agggaaggat 240
gaactgtccg agcaggatga gatgttccgg gggaggaccg ctgtgttcgc cgatcaggta 300
atcgtcggaa atgcaagtct cagattgaaa aatgtgcaac tgactgatgc tggcacgtat 360
aaatgctaca ttatcacaag taagggcaaa ggaaatgcta accttgagta taaaacaggc 420
gcattctcaa tgcccgaggt caatgtcgac tataatgcca gcagtgaaac attgcgctgt 480
gaagctcccc gctggttccc ccagccaacc gtggtctggg cctctcaggt tgatcagggg 540
gctaactttt ccgaggtgag caacaccagc ttcgaactca actctgagaa tgtgaccatg 600

aaagttgtgt ctgtcctgta taatgtaaca atcaacaaca cttattcatg catgattgaa 660
aacgacatcg ccaaggcaac aggtgatatt aaggtaactg aatccgagat caaacggcgg 720
tct 723

(SEQ ID NO:37),

atggaatgga gctgggtatt tctgtttttc ctgtcagtaa cgactggcgt ccattcaggc 60
ttcggcatca gtggacggca cagtatcaca gtgaccaccg tcgcctccgc tggcaatata 120
ggtgaggatg gcatccagtc ctgtaccttt gagccggaca tcaaactgtc tgacatagtg 180
atacaatggc tgaaggaggg ggtgctcggt ctggtacatg agtttaagga agggaaggat 240
gaactgtccg agcaggatga gatgttccgg gggaggaccg ctgtgttcgc cgatcaggta 300
atcgtcggaa atgcaagtct cagattgaaa aatgtgcaac tgactgatgc tggcacgtat 360
aaatgctaca ttatcacaag taagggcaaa ggaaatgcta accttgagta taaaacaggc 420
gcattctcaa tgcccgaggt caatgtcgac tataatgcca gcagtgaaac attgcgctgt 480
gaagctcccc gctggttccc ccagccaacc gtggtctggg cctctcaggt tgatcagggg 540
gctaactttt ccgaggtgag caacaccagc ttcgaactca actctgagaa tgtgaccatg 600
aaagttgtgt ctgtcctgta taatgtaaca atcaacaaca cttattcatg catgattgaa 660
aacgacatcg ccaaggcaac aggtgatatt aaggtaactg aatccgagat c 711

(SEQ ID NO:38),

atggaatgga gctgggtatt tctgtttttc ctgtcagtaa cgactggcgt ccattcaggc 60
ttcggcatca gtggacggca cagtatcaca gtgaccaccg tcgcctccgc tggcaatata 120
ggtgaggatg gcatccagtc ctgtaccttt gagccggaca tcaaactgtc tgacatagtg 180
atacaatggc tgaaggaggg ggtgctcggt ctggtacatg agtttaagga agggaaggat 240
gaactgtccg agcaggatga gatgttccgg gggaggaccg ctgtgttcgc cgatcaggta 300
atcgtcggaa atgcaagtct cagattgaaa aatgtgcaac tgactgatgc tggcacgtat 360
aaatgctaca ttatcacaag taagggcaaa ggaaatgcta accttgagta taaaacaggc 420
gcattctcaa tgcccgaggt caatgtcgac tataatgcca gcagtgaaac attgcgctgt 480
gaagctcccc gctggttccc ccagccaacc gtggtctggg cctctcaggt tgatcagggg 540
gctaactttt ccgaggtgag caacaccagc ttcgaactca actctgagaa tgtgaccatg 600
aaagttgtgt ctgtcctgta taatgtaaca atcaacaaca cttattcatg catgattgaa 660
aacgacatcg ccaaggcaac aggtgatatt aaggtaactg aatccgagat caaacggcgg 720
tctcacctac agctgctaaa ctcaaaggct tct 753

(SEQ ID NO:39),

atggaatgga gctgggtatt tctgtttttc ctgtcagtaa cgactggcgt ccattcaggc 60
ttcggcatca gtggacggca cagtatcaca gtgaccaccg tcgcctccgc tggcaatata 120
ggtgaggatg gcatcctgtc ctgtaccttt gagccggaca tcaaactgtc tgacatagtg 180
atacaatggc tgaaggaggg ggtgctcggt ctggtacatg agtttaagga agggaaggat 240
gaactgtccg agcaggatga gatgttccgg gggaggaccg ctgtgttcgc cgatcaggta 300
atcgtcggaa atgcaagtct cagattgaaa aatgtgcaac tgactgatgc tggcacgtat 360
aaatgctaca ttatcacaag taagggcaaa ggaaatgcta accttgagta taaaacaggc 420
gcattctcaa tgcccgaggt caatgtcgac tataatgcca gcagtgaaac attgcgctgt 480
gaagctcccc gctggttccc ccagccaacc gtggtctggg cctctcaggt tgatcagggg 540
gctaactttt ccgaggtgag caacaccagc ttcgaactca actctgagaa tgtgaccatg 600
aaagttgtgt ctgtcctgta taatgtaaca atcaacaaca cttattcatg catgattgaa 660
aacgacatcg ccaaggcaac aggtgatatt aaggtaactg aatccgagat caaacggcgg 720
tct 723

(SEQ ID NO:40),

```
atggaatgga gctgggtatt tctgtttttc ctgtcagtaa cgactggcgt ccattcaggc     60
ttcggcatca gtggacggca cagtatcaca gtgaccaccg tcgcctccgc tggcaatata    120
ggtgaggatg gcatcctgtc ctgtaccttt gagccggaca tcaaactgtc tgacatagtg    180
atacaatggc tgaaggaggg ggtgctcggt ctggtacatg agtttaagga agggaaggat    240
gaactgtccg agcaggatga gatgttccgg gggaggaccg ctgtgttcgc cgatcaggta    300
atcgtcggaa atgcaagtct cagattgaaa aatgtgcaac tgactgatgc tggcacgtat    360
aaatgctaca ttatcacaag taagggcaaa ggaaatgcta accttgagta taaaacaggc    420
gcattctcaa tgcccgaggt caatgtcgac tataatgcca gcagtgaaac attgcgctgt    480
gaagctcccc gctggttccc ccagccaacc gtggtctggg cctctcaggt tgatcagggg    540
gctaactttt ccgaggtgag caacaccagc ttcgaactca actctgagaa tgtgaccatg    600
aaagttgtgt ctgtcctgta taatgtaaca atcaacaaca cttattcatg catgattgaa    660
aacgacatcg ccaaggcaac aggtgatatt aaggtaactg aatccgagat c              711
```

(SEQ ID NO:41), or

```
atggaatgga gctgggtatt tctgtttttc ctgtcagtaa cgactggcgt ccattcaggc     60
ttcggcatca gtggacggca cagtatcaca gtgaccaccg tcgcctccgc tggcaatata    120
ggtgaggatg gcatcctgtc ctgtaccttt gagccggaca tcaaactgtc tgacatagtg    180
atacaatggc tgaaggaggg ggtgctcggt ctggtacatg agtttaagga agggaaggat    240
gaactgtccg agcaggatga gatgttccgg gggaggaccg ctgtgttcgc cgatcaggta    300
atcgtcggaa atgcaagtct cagattgaaa aatgtgcaac tgactgatgc tggcacgtat    360
aaatgctaca ttatcacaag taagggcaaa ggaaatgcta accttgagta taaaacaggc    420
gcattctcaa tgcccgaggt caatgtcgac tataatgcca gcagtgaaac attgcgctgt    480
gaagctcccc gctggttccc ccagccaacc gtggtctggg cctctcaggt tgatcagggg    540
gctaactttt ccgaggtgag caacaccagc ttcgaactca actctgagaa tgtgaccatg    600
aaagttgtgt ctgtcctgta taatgtaaca atcaacaaca cttattcatg catgattgaa    660
aacgacatcg ccaaggcaac aggtgatatt aaggtaactg aatccgagat caaacggcgg    720
tctcacctac agctgctaaa ctcaaaggct tct                                 753
```

(SEQ ID NO:42).

In another embodiment, the first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the human amino acid sequence:

```
MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV     60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY    120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR    240
S                                                                    241
```

(SEQ ID NO:43)

```
MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV     60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY    120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEI       237
```

(SEQ ID NO:44),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SHLQLLNSKA S 251

(SEQ ID NO:45),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
S 241

(SEQ ID NO:46),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEI 237

(SEQ ID NO:47),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SHLQLLNSKA S 251

(SEQ ID NO:48),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRS 249

(SEQ ID NO:49),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEI 245

(SEQ ID NO:50),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSH LQLLNSKAS 259

(SEQ ID NO:51),

```
MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRS                                                            249
```

(SEQ ID NO:52),

```
MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEI                                                                245
```

(SEQ ID NO:53), or

```
MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRSH LQLLNSKAS                                                 259
```

(SEQ ID NO:54).

The signal sequence will be removed in the mature protein. Additionally, signal peptides from other polypeptides or organisms can be used to enhance the secretion of the fusion protein from a host during manufacture. SEQ ID NO:55 provides the human amino acid sequence of SEQ ID NO:43 and SEQ ID NO:49 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RS                       222
```

(SEQ ID NO:55).

SEQ ID NO:56 provides the human amino acid sequence of SEQ ID NO:46 and SEQ ID NO:52 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RS                       222
```

(SEQ ID NO:56).

SEQ ID NO:57 provides the human amino acid sequence of SEQ ID NO:44 and SEQ ID NO:50 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEI                           218
```

(SEQ ID NO:57).

SEQ ID NO:58 provides the human amino acid sequence of SEQ ID NO:47 and SEQ ID NO:53 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEI                           218
```

(SEQ ID NO:58).

SEQ ID NO:59 provides the human amino acid sequence of SEQ ID NO:45 and SEQ ID NO:51 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK AS           232
```

(SEQ ID NO:59).

SEQ ID NO:60 provides the human amino acid sequence of SEQ ID NO:48 and SEQ ID NO:54 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK AS           232
```

(SEQ ID NO:60).

In other embodiments the final alanine and serine residues are removed from SEQ ID NOS: 45, 48, 51, 54, 59, and 60.

In another embodiment, the first fusion partner of the fusion protein includes the IgV domain of human B7-H4. In one embodiment, the IgV domain includes at least from the cysteine at position 56 of SEQ ID NO:9 or SEQ ID NO:12 to the cysteine at position 130 of SEQ ID NO:9 or SEQ ID NO:12. In another embodiment, the IgV domain contains a fragment of at least 25 or 50 amino acids of the polypeptide defined by this amino acid range.

The first fusion partner can be encoded by a nucleotide sequence having at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the following nucleotide sequence encoding an exemplary IgV domain:

```
ggcttcggca tcagtggacg gcacagtatc acagtgacca ccgtcgcctc cgctggcaat     60
ataggtgagg atggcatcca gtcctgtacc tttgagccgg acatcaaact gtctgacata    120
gtgatacaat ggctgaagga gggggtgctc ggtctggtac atgagtttaa ggaagggaag    180
gatgaactgt ccgagcagga tgagatgttc cgggggagga ccgctgtgtt cgccgatcag    240
gtaatcgtcg gaaatgcaag tctcagattg aaaaatgtgc aactgactga tgctggcacg    300
tataaatgct acattatcac aagtaagggc aaaggaaatg ctaaccttga gtataaaaca    360
ggcgcattct caatgcccga ggtcaat                                        387
```

(SEQ ID NO:61) or

```
ggcttcggca tcagtggacg gcacagtatc acagtgacca ccgtcgcctc cgctggcaat     60
ataggtgagg atggcatcct gtcctgtacc tttgagccgg acatcaaact gtctgacata    120
gtgatacaat ggctgaagga gggggtgctc ggtctggtac atgagtttaa ggaagggaag    180
gatgaactgt ccgagcagga tgagatgttc cgggggagga ccgctgtgtt cgccgatcag    240
gtaatcgtcg gaaatgcaag tctcagattg aaaaatgtgc aactgactga tgctggcacg    300
tataaatgct acattatcac aagtaagggc aaaggaaatg ctaaccttga gtataaaaca    360
ggcgcattct caatgcccga ggtcaat                                        387
```

(SEQ ID NO:62).

The first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the human amino acid sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVN                                                            129
```

(SEQ ID NO:63), or

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVN                                                            129
```

(SEQ ID NO:64).

c. Non-human primate extracellular domain fusion partners

In another embodiment, the first fusion partner of the fusion protein includes the extracellular domain of non-human primate B7-H4 or a fragment thereof. Exemplary non-human primates include, but are not limited to, chimapanzee (*Pan troglodytes*), rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*).

The first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the chimapanzee (*Pan troglodytes*) amino acid sequence:

```
MKPLTSRIIS IIIILAGAIA LIIGFGISGR HSITVTTVAS AGNIGEDGIL SCTFEPDIKL     60
SDIVIQWLKE GVLGLVHEFK EGKDELSEQD EMFRGRTAVF ADQVIVGNAS LRLKNVQLTD    120
AGTYKCYIIT SKGKGNANLE YKTGAFSMPE VNVDYNASSE TLRCEAPRWF PQPTVVWASQ    180
IDQGANFSEV SNTSFELNSE NVTMKVVSVL YNATINNTYS CMIENDIAKA TGDIKVTESE    240
IKRRS                                                                245
```

(SEQ ID NO:65),

```
MKPLTSRIIS IIIILAGAIA LIIGFGISGR HSITVTTVAS AGNIGEDGIL SCTFEPDIKL     60
SDIVIQWLKE GVLGLVHEFK EGKDELSEQD EMFRGRTAVF ADQVIVGNAS LRLKNVQLTD    120
AGTYKCYIIT SKGKGNANLE YKTGAFSMPE VNVDYNASSE TLRCEAPRWF PQPTVVWASQ    180
IDQGANFSEV SNTSFELNSE NVTMKVVSVL YNATINNTYS CMIENDIAKA TGDIKVTESE    240
I                                                                    241
```

(SEQ ID NO:66), or

```
MKPLTSRIIS IIIILAGAIA LIIGFGISGR HSITVTTVAS AGNIGEDGIL SCTFEPDIKL     60
SDIVIQWLKE GVLGLVHEFK EGKDELSEQD EMFRGRTAVF ADQVIVGNAS LRLKNVQLTD    120
AGTYKCYIIT SKGKGNANLE YKTGAFSMPE VNVDYNASSE TLRCEAPRWF PQPTVVWASQ    180
IDQGANFSEV SNTSFELNSE NVTMKVVSVL YNATINNTYS CMIENDIAKA TGDIKVTESE    240
IKRRSHLQLL NSKAS                                                     255
```

(SEQ ID NO:67).

The signal sequence will be removed in the mature protein. Additionally, signal peptides from other polypeptides or organisms can be used to enhance the secretion of the fusion protein from a host during manufacture.

SEQ ID NO:68 provides the chimapanzee amino acid sequence of SEQ ID NO:65 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQIDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNA TINNTYSCMI ENDIAKATGD IKVTESEIKR RS                       222
```

(SEQ ID NO:68).

SEQ ID NO:69 provides the chimapanzee amino acid sequence of SEQ ID NO:66 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQIDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNA TINNTYSCMI ENDIAKATGD IKVTESEI                            218
```

(SEQ ID NO:69).

SEQ ID NO:70 provides the chimapanzee amino acid sequence of SEQ ID NO:67 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQIDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNA TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK AS            232
```

(SEQ ID NO:70).

The first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the rhesus monkey (*Macaca mulatta*) amino acid sequence:

```
MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRS                                                            249
```

(SEQ ID NO:71),

```
MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEI                                                                245
```

(SEQ ID NO:72), or

```
MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRSH LQLLNSKAS                                                 259
```

(SEQ ID NO:73).

The signal sequence will be removed in the mature protein. Additionally, signal peptides from other polypeptides or organisms can be used to enhance the secretion of the fusion protein from a host during manufacture.

SEQ ID NO:74 provides the rhesus monkey amino acid sequence of SEQ ID NO:71 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RS                       222
```

(SEQ ID NO:74).

SEQ ID NO:75 provides the rhesus monkey amino acid sequence of SEQ ID NO:72 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEI                            218
```

(SEQ ID NO:75).

SEQ ID NO:76 provides the rhesus monkey amino acid sequence of SEQ ID NO:73 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK AS            232
```

(SEQ ID NO:76).

The first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the cynomolgus monkey (*Macaca fascicularis*) amino acid sequence:

```
MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRS                                                            249
```

(SEQ ID NO:77),

```
MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEI                                                                245
```

(SEQ ID NO:78), or

```
MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRSH LQLLNSKAS                                                 259
```

(SEQ ID NO:79).

The signal sequence will be removed in the mature protein. Additionally, signal peptides from other polypeptides or organisms can be used to enhance the secretion of the fusion protein from a host during manufacture.

SEQ ID NO:80 provides the cynomolgus monkey amino acid sequence of SEQ ID NO:77 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RS                       222
```

(SEQ ID NO:80).

SEQ ID NO:81 provides the cynomolgus monkey amino acid sequence of SEQ ID NO:78 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEI                            218
```

(SEQ ID NO:81).

SEQ ID NO:82 provides the cynomolgus monkey amino acid sequence of SEQ ID NO:79 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK AS            232
```

(SEQ ID NO:82).

In other embodiments the final alanine and serine residues are removed from SEQ ID NOS:67, 70, 73, 76, 79, and 82.

In another embodiment, the first fusion partner of the fusion protein includes the IgV domain of chimpanzee B7-H4. In another embodiment, the IgV domain includes at least from the cysteine at position 52 of SEQ ID NO:15 to the cysteine at position 126 of SEQ ID NO:15. In another embodiment, the IgV domain contains a fragment of at least 25 or 50 amino acids of the polypeptide defined by this amino acid range.

The first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the following chimapnzee amino acid sequence of the following exemplary IgV domain:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVN                                                            129
```

(SEQ ID NO:83).

In another embodiment, the first fusion partner of the fusion protein includes the IgV domain of rhesus monkey B7-H4. In one embodiment, the IgV domain includes at least from the cysteine at position 56 of SEQ ID

NO:17 to the cysteine at position 130 of SEQ ID NO:17. In another embodiment, the IgV domain contains a fragment of at least 25 or 50 amino acids of the polypeptide defined by this amino acid range.

The first fusion protein can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the rhesus monkey amino acid sequence of the following exemplary IgV domain:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVN                                                            129
```

(SEQ ID NO:84).

In another embodiment, the first fusion partner of the fusion protein includes the IgV domain of cynomolgus monkey B7-H4. In one embodiment, the IgV domain includes at least from the cysteine at position 56 of SEQ ID NO:19 to the cysteine at position 130 of SEQ ID NO:19. In another embodiment, the IgV domain contains a fragment of at least 25 or 50 amino acids of the polypeptide defined by this amino acid range.

The first fusion protein can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the cynomolgus monkey amino acid sequence of the following exemplary IgV domain:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVN                                                            129
```

(SEQ ID NO:85).

d. B7-H4 extracellular domain fragments

The B7-H4 extracellular domain can contain one or more amino acids from the signal peptide or the putative transmembrane domain of B7-H4. During secretion, the number of amino acids of the signal peptide that are cleaved can vary depending on the expression system and the host. Additionally, fragments of B7-H4 extracellular domain missing one or more amino acids from the C-terminus or the N-terminus that retain the ability to bind to the B7-H4 receptor can be used as a fusion partner for the disclosed fusion proteins.

For example, suitable fragments of murine B7-H4 that can be used as a first fusion partner include, but are not limited to, the following:

32-257, 32-256, 32-255, 32-254, 32-253, 32-252, 32-251, 32-250, 32-249,

31-257, 31-256, 31-255, 31-254, 31-253, 31-252, 31-251, 31-250, 31-249,
30-257, 30-256, 30-255, 30-254, 30-253, 30-252, 30-251, 30-250, 30-249,
29-257, 29-256, 29-255, 29-254, 29-253, 29-252, 29-251, 29-250, 29-249,
28-257, 28-256, 28-255, 28-254, 28-253, 28-252, 28-251, 28-250, 28-249,
27-257, 27-256, 27-255, 27-254, 27-253, 27-252, 27-251, 27-250, 27-249,
26-257, 26-256, 26-255, 26-254, 26-253, 26-252, 26-251, 26-250, 26-249,
25-257, 25-256, 25-255, 25-254, 25-253, 25-252, 25-251, 25-250, 25-249,
24-257, 24-256, 24-255, 24-254, 24-253, 24-252, 24-251, 24-250, 24-249,
of SEQ ID NO:26 or SEQ ID NO:27, or
24-249, 24-248, 24-247, 24-246, 24-245, 24-244, 24-243, 24-242, 24-241,
23-249, 23-248, 23-247, 23-246, 23-245, 23-244, 23-243, 23-242, 23-241,
22-249, 22-248, 22-247, 22-246, 22-245, 22-244, 22-243, 22-242, 22-241,
21-249, 21-248, 21-247, 21-246, 21-245, 21-244, 21-243, 21-242, 21-241,
20-249, 20-248, 20-247, 20-246, 20-245, 20-244, 20-243, 20-242, 20-241,
19-249, 19-248, 19-247, 19-246, 19-245, 19-244, 19-243, 19-242, 19-241,
18-249, 18-248, 18-247, 18-246, 18-245, 18-244, 18-243, 18-242, 18-241,
17-249, 17-248, 17-247, 17-246, 17-245, 17-244, 17-243, 17-242, 17-241,
16-249, 16-248, 16-247, 16-246, 16-245, 16-244, 16-243, 16-242, 16-241,
of SEQ ID NO:24 or SEQ ID NO:25.

Additional suitable fragments of murine B7-H4 include, but are not limited to, the following:

28-257, 28-258, 28-259, 28-260, 28-261, 28-262, 28-263,
29-257, 29-258, 29-259, 29-260, 29-261, 29-262, 29-263,
30-257, 30-258, 30-259, 30-260, 30-261, 30-262, 30-263,
31-257, 31-258, 31-259, 31-260, 31-261, 31-262, 31-263,
32-257, 32-258, 32-259, 32-260, 32-261, 32-262, 32-263,

of SEQ ID NO:2 or SEQ ID NO:5, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

Additional suitable fragments of murine B7-H4 include, but are not limited to, fragments containing at least 25, 20, 75, 100 or 125 amino acids

of the IgV domain as set forth in SEQ ID NO:32 or SEQ ID NO:33. Exemplary fragments include, but are not limited to:

16-144, 16-145, 16-146, 16-147, 16-148, 16-149, 16-150, 16-151, 16-152,
17-144, 17-145, 17-146, 17-147, 17-148, 17-149, 17-150, 17-151, 17-152,
18-144, 18-145, 18-146, 18-147, 18-148, 18-149, 18-150, 18-151, 18-152,
19-144, 19-145, 19-146, 19-147, 19-148, 19-149, 19-150, 19-151, 19-152,
20-144, 20-145, 20-146, 20-147, 20-148, 20-149, 20-150, 20-151, 20-152,
21-144, 21-145, 21-146, 21-147, 21-148, 21-149, 21-150, 21-151, 21-152,
22-144, 22-145, 22-146, 22-147, 22-148, 22-149, 22-150, 22-151, 22-152,
23-144, 23-145, 23-146, 23-147, 23-148, 23-149, 23-150, 23-151, 23-152,
24-144, 24-145, 24-146, 24-147, 24-148, 24-149, 24-150, 24-151, 24-152,

of SEQ ID NO:24 or SEQ ID NO:25, or

24-152, 24-153, 24-154, 24-155, 24-156, 24-157, 24-158, 24-159, 24-160,
25-152, 25-153, 25-154, 25-155, 25-156, 25-157, 25-158, 25-159, 25-160,
26-152, 26-153, 26-154, 26-155, 26-156, 26-157, 26-158, 26-159, 26-160,
27-152, 27-153, 27-154, 27-155, 27-156, 27-157, 27-158, 27-159, 27-160,
28-152, 28-153, 28-154, 28-155, 28-156, 28-157, 28-158, 28-159, 28-160,
29-152, 29-153, 29-154, 29-155, 29-156, 29-157, 29-158, 29-159, 29-160,
30-152, 30-153, 30-154, 30-155, 30-156, 30-157, 30-158, 30-159, 30-160,
31-152, 31-153, 31-154, 31-155, 31-156, 31-157, 31-158, 31-159, 31-160,
32-152, 32-153, 32-154, 32-155, 32-156, 32-157, 32-158, 32-159, 32-160,

of SEQ ID NO:26 or SEQ ID NO:27, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

Exemplary suitable fragments of human B7-H4 that can be used as a first fusion partner include, but are not limited to, the following:

32-249, 32-248, 32-247, 32-246, 32-245, 32-244, 32-243, 32-242, 32-241,
31-249, 31-248, 31-247, 31-246, 31-245, 31-244, 31-243, 31-242, 31-241,
30-249, 30-248, 30-247, 30-246, 30-245, 30-244, 30-243, 30-242, 30-241,
29-249, 29-248, 29-247, 29-246, 29-245, 29-244, 29-243, 29-242, 29-241,
28-249, 28-248, 28-247, 28-246, 28-245, 28-244, 28-243, 28-242, 28-241,

27-249, 27-248, 27-247, 27-246, 27-245, 27-244, 27-243, 27-242, 27-241,
26-249, 26-248, 26-247, 26-246, 26-245, 26-244, 26-243, 26-242, 26-241,
25-249, 25-248, 25-247, 25-246, 25-245, 25-244, 25-243, 25-242, 25-241,
24-249, 24-248, 24-247, 24-246, 24-245, 24-244, 24-243, 24-242, 24-241,
of SEQ ID NO:49, or SEQ ID NO:52, or
32-245, 32-244, 32-243, 32-242, 32-241, 32-240, 32-239, 32-238, 32-237,
31-245, 31-244, 31-243, 31-242, 31-241, 31-240, 31-239, 31-238, 31-237,
30-245, 30-244, 30-243, 30-242, 30-241, 30-240, 30-239, 30-238, 30-237,
29-245, 29-244, 29-243, 29-242, 29-241, 29-240, 29-239, 29-238, 29-237,
28-245, 28-244, 28-243, 28-242, 28-241, 28-240, 28-239, 28-238, 28-237,
27-245, 27-244, 27-243, 27-242, 27-241, 27-240, 27-239, 27-238, 27-237,
26-245, 26-244, 26-243, 26-242, 26-241, 26-240, 26-239, 26-238, 26-237,
25-245, 25-244, 25-243, 25-242, 25-241, 25-240, 25-239, 25-238, 25-237,
24-245, 24-244, 24-243, 24-242, 24-241, 24-240, 24-239, 24-238, 24-237,
of SEQ ID NO:50 or SEQ ID NO:53, or
32-259, 32-258, 32-257, 32-256, 32-255, 32-254, 32-253, 32-252, 32-251,
31-259, 31-258, 31-257, 31-256, 31-255, 31-254, 31-253, 31-252, 31-251,
30-259, 30-258, 30-257, 30-256, 30-255, 30-254, 30-253, 30-252, 30-251,
29-259, 29-258, 29-257, 29-256, 29-255, 29-254, 29-253, 29-252, 29-251,
28-259, 28-258, 28-257, 28-256, 28-255, 28-254, 28-253, 28-252, 28-251,
27-259, 27-258, 27-257, 27-256, 27-255, 27-254, 27-253, 27-252, 27-251,
26-259, 26-258, 26-257, 26-256, 26-255, 26-254, 26-253, 26-252, 26-251,
25-259, 25-258, 25-257, 25-256, 25-255, 25-254, 25-253, 25-252, 25-251,
24-259, 24-258, 24-257, 24-256, 24-255, 24-254, 24-253, 24-252, 24-251,
of SEQ ID NO:51 or SEQ ID NO:54, or
24-241, 24-240, 24-239, 24-238, 24-237, 24-236, 24-235, 24-234, 24-233,
23-241, 23-240, 23-239, 23-238, 23-237, 23-236, 23-235, 23-234, 23-233,
22-241, 22-240, 22-239, 22-238, 22-237, 22-236, 22-235, 22-234, 22-233,
21-241, 21-240, 21-239, 21-238, 21-237, 21-236, 21-235, 21-234, 21-233,
20-241, 20-240, 20-239, 20-238, 20-237, 20-236, 20-235, 20-234, 20-233,
19-241, 19-240, 19-239, 19-238, 19-237, 19-236, 19-235, 19-234, 19-233,
18-241, 18-240, 18-239, 18-238, 18-237, 18-236, 18-235, 18-234, 18-233,
17-241, 17-240, 17-239, 17-238, 17-237, 17-236, 17-235, 17-234, 17-233,

16-241, 16-240, 16-239, 16-238, 16-237, 16-236, 16-235, 16-234, 16-233, of SEQ ID NO:43 or SEQ ID NO:46, or

24-237, 24-236, 24-235, 24-234, 24-233, 24-232, 24-231, 24-230, 24-229, 23-237, 23-236, 23-235, 23-234, 23-233, 23-232, 23-231, 23-230, 23-229, 22-237, 22-236, 22-235, 22-234, 22-233, 22-232, 22-231, 22-230, 22-229, 21-237, 21-236, 21-235, 21-234, 21-233, 21-232, 21-231, 21-230, 21-229, 20-237, 20-236, 20-235, 20-234, 20-233, 20-232, 20-231, 20-230, 20-229, 19-237, 19-236, 19-235, 19-234, 19-233, 19-232, 19-231, 19-230, 19-229, 18-237, 18-236, 18-235, 18-234, 18-233, 18-232, 18-231, 18-230, 18-229, 17-237, 17-236, 17-235, 17-234, 17-233, 17-232, 17-231, 17-230, 17-229, 16-237, 16-236, 16-235, 16-234, 16-233, 16-232, 16-231, 16-230, 16-229, of SEQ ID NO:44 or SEQ ID NO:47, or

24-251, 24-250, 24-249, 24-248, 24-247, 24-246, 24-245, 24-244, 24-243, 23-251, 23-250, 23-249, 23-248, 23-247, 23-246, 23-245, 23-244, 23-243, 22-251, 22-250, 22-249, 22-248, 22-247, 22-246, 22-245, 22-244, 22-243, 21-251, 21-250, 21-249, 21-248, 21-247, 21-246, 21-245, 21-244, 21-243, 20-251, 20-250, 20-249, 20-248, 20-247, 20-246, 20-245, 20-244, 20-243, 19-251, 19-250, 19-249, 19-248, 19-247, 19-246, 19-245, 19-244, 19-243, 18-251, 18-250, 18-249, 18-248, 18-247, 18-246, 18-245, 18-244, 18-243, 17-251, 17-250, 17-249, 17-248, 17-247, 17-246, 17-245, 17-244, 17-243, 16-251, 16-250, 16-249, 16-248, 16-247, 16-246, 16-245, 16-244, 16-243, of SEQ ID NO:45 or SEQ ID NO:48.

Additional suitable fragments of human B7-H4 include, but are not limited to, the following:

27-249, 27-250, 27-251, 27-252, 27-253, 27-254, 27-255, 27-256, 27-257, 27-259, 27-260,

28-249, 28-250, 28-251, 28-252, 28-253, 28-254, 28-255, 28-256, 28-257, 28-259, 28-260,

29-249, 29-250, 29-251, 29-252, 29-253, 29-254, 29-255, 29-256, 29-257, 29-259, 29-260,

30-249, 30-250, 30-251, 30-252, 30-253, 30-254, 30-255, 30-256, 30-257, 30-259, 30-260,

31-249, 31-250, 31-251, 31-252, 31-253, 31-254, 31-255, 31-256, 31-257, 31-259, 31-260,

32-249, 32-250, 32-251, 32-252, 32-253, 32-254, 32-255, 32-256, 32-257, 32-259, 32-260

of SEQ ID NO:9 or SEQ ID NO:12, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

Additional suitable fragments of human B7-H4 include, but are not limited to, fragments containing at least 25, 20, 75, 100 or 125 amino acids of the IgV domain as set forth in SEQ ID NO:63 or SEQ ID NO:64. Exemplary fragments include, but are not limited to:

16-144, 16-145, 16-146, 16-147, 16-148, 16-149, 16-150, 16-151, 16-152,
17-144, 17-145, 17-146, 17-147, 17-148, 17-149, 17-150, 17-151, 17-152,
18-144, 18-145, 18-146, 18-147, 18-148, 18-149, 18-150, 18-151, 18-152,
19-144, 19-145, 19-146, 19-147, 19-148, 19-149, 19-150, 19-151, 19-152,
20-144, 20-145, 20-146, 20-147, 20-148, 20-149, 20-150, 20-151, 20-152,
21-144, 21-145, 21-146, 21-147, 21-148, 21-149, 21-150, 21-151, 21-152,
22-144, 22-145, 22-146, 22-147, 22-148, 22-149, 22-150, 22-151, 22-152,
23-144, 23-145, 23-146, 23-147, 23-148, 23-149, 23-150, 23-151, 23-152,
24-144, 24-145, 24-146, 24-147, 24-148, 24-149, 24-150, 24-151, 24-152,

of any of SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, or SEQ ID NO:48, or

24-152, 24-153, 24-154, 24-155, 24-156, 24-157, 24-158, 24-159, 24-160,
25-152, 25-153, 25-154, 25-155, 25-156, 25-157, 25-158, 25-159, 25-160,
26-152, 26-153, 26-154, 26-155, 26-156, 26-157, 26-158, 26-159, 26-160,
27-152, 27-153, 27-154, 27-155, 27-156, 27-157, 27-158, 27-159, 27-160,
28-152, 28-153, 28-154, 28-155, 28-156, 28-157, 28-158, 28-159, 28-160,
29-152, 29-153, 29-154, 29-155, 29-156, 29-157, 29-158, 29-159, 29-160,
30-152, 30-153, 30-154, 30-155, 30-156, 30-157, 30-158, 30-159, 30-160,
31-152, 31-153, 31-154, 31-155, 31-156, 31-157, 31-158, 31-159, 31-160,
32-152, 32-153, 32-154, 32-155, 32-156, 32-157, 32-158, 32-159, 32-160,

of any of SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, or SEQ ID NO:54, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

Exemplary suitable fragments of non-human primate B7-H4 that can be used as a first fusion partner include, but are not limited to, the following:
32-249, 32-248, 32-247, 32-246, 32-245, 32-244, 32-243, 32-242, 32-241,
31-249, 31-248, 31-247, 31-246, 31-245, 31-244, 31-243, 31-242, 31-241,
30-249, 30-248, 30-247, 30-246, 30-245, 30-244, 30-243, 30-242, 30-241,
29-249, 29-248, 29-247, 29-246, 29-245, 29-244, 29-243, 29-242, 29-241,
28-249, 28-248, 28-247, 28-246, 28-245, 28-244, 28-243, 28-242, 28-241,
27-249, 27-248, 27-247, 27-246, 27-245, 27-244, 27-243, 27-242, 27-241,
26-249, 26-248, 26-247, 26-246, 26-245, 26-244, 26-243, 26-242, 26-241,
25-249, 25-248, 25-247, 25-246, 25-245, 25-244, 25-243, 25-242, 25-241,
24-249, 24-248, 24-247, 24-246, 24-245, 24-244, 24-243, 24-242, 24-241,
of SEQ ID NO:71, or SEQ ID NO:77, or
32-245, 32-244, 32-243, 32-242, 32-241, 32-240, 32-239, 32-238, 32-237,
31-245, 31-244, 31-243, 31-242, 31-241, 31-240, 31-239, 31-238, 31-237,
30-245, 30-244, 30-243, 30-242, 30-241, 30-240, 30-239, 30-238, 30-237,
29-245, 29-244, 29-243, 29-242, 29-241, 29-240, 29-239, 29-238, 29-237,
28-245, 28-244, 28-243, 28-242, 28-241, 28-240, 28-239, 28-238, 28-237,
27-245, 27-244, 27-243, 27-242, 27-241, 27-240, 27-239, 27-238, 27-237,
26-245, 26-244, 26-243, 26-242, 26-241, 26-240, 26-239, 26-238, 26-237,
25-245, 25-244, 25-243, 25-242, 25-241, 25-240, 25-239, 25-238, 25-237,
24-245, 24-244, 24-243, 24-242, 24-241, 24-240, 24-239, 24-238, 24-237,
of SEQ ID NO:72 or SEQ ID NO:78, or
32-259, 32-258, 32-257, 32-256, 32-255, 32-254, 32-253, 32-252, 32-251,
31-259, 31-258, 31-257, 31-256, 31-255, 31-254, 31-253, 31-252, 31-251,
30-259, 30-258, 30-257, 30-256, 30-255, 30-254, 30-253, 30-252, 30-251,
29-259, 29-258, 29-257, 29-256, 29-255, 29-254, 29-253, 29-252, 29-251,
28-259, 28-258, 28-257, 28-256, 28-255, 28-254, 28-253, 28-252, 28-251,

27-259, 27-258, 27-257, 27-256, 27-255, 27-254, 27-253, 27-252, 27-251,
26-259, 26-258, 26-257, 26-256, 26-255, 26-254, 26-253, 26-252, 26-251,
25-259, 25-258, 25-257, 25-256, 25-255, 25-254, 25-253, 25-252, 25-251,
24-259, 24-258, 24-257, 24-256, 24-255, 24-254, 24-253, 24-252, 24-251,
of SEQ ID NO:73 or SEQ ID NO:79, or
28-245, 28-244, 28-243, 28-242, 28-241, 28-240, 28-239, 28-238, 28-237,
27-245, 27-244, 27-243, 27-242, 27-241, 27-240, 27-239, 27-238, 27-237,
26-245, 26-244, 26-243, 26-242, 26-241, 26-240, 26-239, 26-238, 26-237,
25-245, 25-244, 25-243, 25-242, 25-241, 25-240, 25-239, 25-238, 25-237,
24-245, 24-244, 24-243, 24-242, 24-241, 24-240, 24-239, 24-238, 24-237,
23-245, 23-244, 23-243, 23-242, 23-241, 23-240, 23-239, 23-238, 23-237,
22-245, 22-244, 22-243, 22-242, 22-241, 22-240, 22-239, 22-238, 22-237,
21-245, 21-244, 21-243, 21-242, 21-241, 21-240, 21-239, 21-238, 21-237,
20-245, 20-244, 20-243, 20-242, 20-241, 20-240, 20-239, 20-238, 20-237,
of SEQ ID NO:65, or
28-241, 28-240, 28-239, 28-238, 28-237, 28-236, 28-235, 28-234, 28-233,
27-241, 27-240, 27-239, 27-238, 27-237, 27-236, 27-235, 27-234, 27-233,
26-241, 26-240, 26-239, 26-238, 26-237, 26-236, 26-235, 26-234, 26-233,
25-241, 25-240, 25-239, 25-238, 25-237, 25-236, 25-235, 25-234, 25-233,
24-241, 24-240, 24-239, 24-238, 24-237, 24-236, 24-235, 24-234, 24-233,
23-241, 23-240, 23-239, 23-238, 23-237, 23-236, 23-235, 23-234, 23-233,
22-241, 22-240, 22-239, 22-238, 22-237, 22-236, 22-235, 22-234, 22-233,
21-241, 21-240, 21-239, 21-238, 21-237, 21-236, 21-235, 21-234, 21-233,
20-241, 20-240, 20-239, 20-238, 20-237, 20-236, 20-235, 20-234, 20-233,
of SEQ ID NO:66, or
28-255, 28-254, 28-253, 28-252, 28-251, 28-250, 28-249, 28-248, 28-247,
27-255, 27-254, 27-253, 27-252, 27-251, 27-250, 27-249, 27-248, 27-247,
26-255, 26-254, 26-253, 26-252, 26-251, 26-250, 26-249, 26-248, 26-247,
25-255, 25-254, 25-253, 25-252, 25-251, 25-250, 25-249, 25-248, 25-247,
24-255, 24-254, 24-253, 24-252, 24-251, 24-250, 24-249, 24-248, 24-247,
23-255, 23-254, 23-253, 23-252, 23-251, 23-250, 23-249, 23-248, 23-247,
22-255, 22-254, 22-253, 22-252, 22-251, 22-250, 22-249, 22-248, 22-247,
21-255, 21-254, 21-253, 21-252, 21-251, 21-250, 21-249, 21-248, 21-247,

20-255, 20-254, 20-253, 20-252, 20-251, 20-250, 20-249, 20-248, 20-247, of SEQ ID NO:67.

Additional suitable fragments of non-human primate B7-H4 include, but are not limited to, the following:

27-249, 27-250, 27-251, 27-252, 27-253, 27-254, 27-255, 27-256, 27-257, 27-259, 27-260,

28-249, 28-250, 28-251, 28-252, 28-253, 28-254, 28-255, 28-256, 28-257, 28-259, 28-260,

29-249, 29-250, 29-251, 29-252, 29-253, 29-254, 29-255, 29-256, 29-257, 29-259, 29-260,

30-249, 30-250, 30-251, 30-252, 30-253, 30-254, 30-255, 30-256, 30-257, 30-259, 30-260,

31-249, 31-250, 31-251, 31-252, 31-253, 31-254, 31-255, 31-256, 31-257, 31-259, 31-260,

32-249, 32-250, 32-251, 32-252, 32-253, 32-254, 32-255, 32-256, 32-257, 32-259, 32-260

of SEQ ID NO:17 or SEQ ID NO:19, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

Additional suitable fragments of non-human primate B7-H4 include, but are not limited to, the following:

23-245, 23-246, 23-247, 23-248, 23-249, 23-250, 23-251, 23-252, 23-253, 23-254, 23-255,

24-245, 24-246, 24-247, 24-248, 24-249, 24-250, 24-251, 24-252, 24-253, 24-254, 24-255,

25-245, 25-246, 25-247, 25-248, 25-249, 25-250, 25-251, 25-252, 25-253, 25-254, 25-255,

26-245, 26-246, 26-247, 26-248, 26-249, 26-250, 26-251, 26-252, 26-253, 26-254, 26-255,

27-245, 27-246, 27-247, 27-248, 27-249, 27-250, 27-251, 27-252, 27-253, 27-254, 27-255,

28-245, 28-246, 28-247, 28-248, 28-249, 28-250, 28-251, 28-252, 28-253, 28-254, 28-255

of SEQ ID NO:15, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

Additional suitable fragments of non-human primate B7-H4 include, but are not limited to, fragments containing at least 25, 20, 75, 100 or 125 amino acids of the IgV domain as set forth in SEQ ID NO:83, SEQ ID NO:84 or SEQ ID NO:85. Exemplary fragments include, but are not limited to:

20-148, 20-149, 20-150, 20-151, 20-152, 20-153, 20-154, 20-155, 20-156,
21-148, 21-149, 21-150, 21-151, 21-152, 21-153, 21-154, 21-155, 21-156,
22-148, 22-149, 22-150, 22-151, 22-152, 22-153, 22-154, 22-155, 22-156,
23-148, 23-149, 23-150, 23-151, 23-152, 23-153, 23-154, 23-155, 23-156,
24-148, 24-149, 24-150, 24-151, 24-152, 24-153, 24-154, 24-155, 20-156,
25-148, 25-149, 25-150, 25-151, 25-152, 25-153, 25-154, 25-155, 25-156,
26-148, 26-149, 26-150, 26-151, 26-152, 26-153, 26-154, 26-155, 26-156,
27-148, 27-149, 27-150, 27-151, 27-152, 27-153, 27-154, 27-155, 27-156,
28-148, 28-149, 28-150, 28-151, 28-152, 28-153, 28-154, 28-155, 28-156,

of any of SEQ ID NO:65, SEQ ID NO:66, or SEQ ID NO:67, or

24-152, 24-153, 24-154, 24-155, 24-156, 24-157, 24-158, 24-159, 24-160,
25-152, 25-153, 25-154, 25-155, 25-156, 25-157, 25-158, 25-159, 25-160,
26-152, 26-153, 26-154, 26-155, 26-156, 26-157, 26-158, 26-159, 26-160,
27-152, 27-153, 27-154, 27-155, 27-156, 27-157, 27-158, 27-159, 27-160,
28-152, 28-153, 28-154, 28-155, 28-156, 28-157, 28-158, 28-159, 28-160,
29-152, 29-153, 29-154, 29-155, 29-156, 29-157, 29-158, 29-159, 29-160,
30-152, 30-153, 30-154, 30-155, 30-156, 30-157, 30-158, 30-159, 30-160,
31-152, 31-153, 31-154, 31-155, 31-156, 31-157, 31-158, 31-159, 31-160,
32-152, 32-153, 32-154, 32-155, 32-156, 32-157, 32-158, 32-159, 32-160,

of any of SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:77, SEQ ID NO:78, or SEQ ID NO:79, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal

peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

2. Variants of B7-H4 polypeptides

Useful variants include those that increase biological activity, as indicated by any of the assays described herein, or that increase half life or stability of the protein. The B7-H4 polypeptides and B7-H4 fragments, or fusions thereof having B7-H4 activity, can be engineered to increase biological activity. In a preferred embodiment, the B7-H4 polypeptide or fusion protein has been modified with at least one amino acid substitution, deletion, or insertion that increases the binding of the molecule to an immune cell, for example a T cell, and transmits an inhibitory signal into the T cell.

Other preferred variants are those B7-H4 polypetpides that are engineered to selectively bind to one type of T cell versus other immune cells. For example, the B7-H4 polypeptide can be engineered to bind preferentially to Tregs, Th0, Th1, Th17, or Th22 cells. Preferential binding refers to binding that is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or greater for one type of cell over another type of cell.

Still other variants of B7-H4 can be engineered to have reduced binding to immune cells relative to wildtype B7-H4. These variants can be used in combination with variants having stronger binding properties to modulate the immune response with a moderate impact.

Finally, variant B7-H4 polypeptides can be engineered to have an increased half-life relative to wildtype. These variants typically are modified to resist enzymatic degradation. Exemplary modifications include modified amino acid residues and modified peptide bonds that resist enzymatic degradation. Various modifications to achieve this are known in the art. For example, the juxtamembrane region of B7-H4 includes a dibasic motif, KRRS, which could potentially be recognized and cleaved, for example by a member of the proprotein convertase family of proteases.This motif (KRRS) can be removed to increase half life. The variants can be modified to adjust for effects of affinity for the receptor on the half life of B7-H4 polypeptides, fragments, or fusions thereof at serum and endosomal pH.

B. Second polypeptide

The B7-H4 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 B7-H4 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.

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. SEQ ID NOS: 88 and 89 provide exemplary sequences for the hinge, C_H2 and C_H3 regions of a human immunoglobulin Cγ1.

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 are referred to as B7-H4-Ig.

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 B7-H4 polypeptides, fusion proteins, or fragments thereof. Suitable amino acid substitutions include conservative and non-conservative substitutions, as described above.

In another embodiment the second polypeptide may have a conjugation domain through which additional molecules can be bound to the B7-H4 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 B7-H4 fusion protein. In another embodiment the conjugated molecule is Polyethylene Glycol (PEG).

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. Modications 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.

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).

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.

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.

C. Peptide or polypeptide linker domain

The disclosed B7-H4 fusion proteins optionally contain a peptide or polypeptide linker domain that separates the B7-H4 polypeptide from the second polypeptide.

1. Hinge region of antibodies

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.

In one embodiment, B7-H4 fusion polypeptides contain the hinge, C_H2 and C_H3 regions of a human immunoglobulin Cγ1 chain encoded by a

nucleic acid having at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

```
gagcctaagt catgtgacaa gacccatacg tgcccaccct gtcccgctcc agaactgctg     60
gggggaccta gcgttttctt gttcccccca aagcccaagg acaccctcat gatctcacgg    120
actcccgaag taacatgcgt agtagtcgac gtgagccacg aggatcctga agtgaagttt    180
aattggtacg tggacggagt cgaggtgcat aatgccaaaa ctaaacctcg ggaggagcag    240
tataacagta cctaccgcgt ggtatccgtc ttgacagtgc tccaccagga ctggctgaat    300
ggtaaggagt ataaatgcaa ggtcagcaac aaagctcttc ccgccccaat tgaaaagact    360
atcagcaagg ccaagggaca accccgcgag ccccaggttt acacccttcc accttcacga    420
gacgagctga ccaagaacca ggtgtctctg acttgtctgg tcaaaggttt ctatccttcc    480
gacatcgcag tggagtggga gtcaaacggg cagcctgaga ataactacaa gaccacaccc    540
ccagtgcttg atagcgatgg gagcttttte ctctacagta agctgactgt ggacaaatcc    600
cgctggcagc agggaaacgt tttctcttgt agcgtcatgc atgaggccct ccacaaccat    660
tatactcaga aaagcctgag tctgagtccc ggcaaa                               696
```

(SEQ ID NO:86), or

```
gacaagaccc atacgtgccc accctgtccc gctccagaac tgctgggggg acctagcgtt     60
ttcttgttcc ccccaaagcc caaggacacc ctcatgatct cacggactcc cgaagtaaca    120
tgcgtagtag tcgacgtgag ccacgaggat cctgaagtga agtttaattg gtacgtggac    180
ggagtcgagg tgcataatgc caaaactaaa cctcgggagg agcagtataa cagtacctac    240
cgcgtggtat ccgtcttgac agtgctccac caggactggc tgaatggtaa ggagtataaa    300
tgcaaggtca gcaacaaagc tcttcccgcc ccaattgaaa agactatcag caaggccaag    360
ggacaacccc gcgagcccca ggtttacacc cttccacctt cacgagacga gctgaccaag    420
aaccaggtgt ctctgacttg tctggtcaaa ggtttctatc cttccgacat cgcagtggag    480
tgggagtcaa acgggcagcc tgagaataac tacaagacca cacccccagt gcttgatagc    540
gatgggagct ttttcctcta cagtaagctg actgtggaca aatcccgctg gcagcaggga    600
aacgttttct cttgtagcgt catgcatgag gccctccaca accattatac tcagaaaagc    660
ctgagtctga gtcccggcaa a                                              681
```

(SEQ ID NO:87).

The hinge, C_H2 and C_H3 regions of a human immunoglobulin Cγ1 chain encoded by SEQ ID NO:86 has the following amino acid sequence:

```
EPKSCDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF     60
NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT    120
ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP    180
PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK            232
```

(SEQ ID NO:88).

The hinge, CH2 and CH3 regions of a human immunoglobulin Cγ1 chain encoded by SEQ ID NO:87 has the following amino acid sequence:

```
DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD     60
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK    120
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS    180
DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK                  227
```

(SEQ ID NO:89).

The hinge can be further shortened to remove amino acids 1, 2, 3, 4, 5, or combinations thereof of SEQ ID NO:89. In one embodiment, amino acids 1 and 2 of SEQ ID NO:89 are deleted.

In another embodiment, the B7-H4 fusion polypeptides contain the hinge, C_H2 and C_H3 regions of a murine immunoglobulin Cγ2a chain encoded by a nucleic acid having at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

```
gagccaagag gtcctacgat caagccctgc ccgccttgta aatgcccagc tccaaatttg     60
ctgggtggac cgtcagtctt tatcttcccg ccaaagataa aggacgtctt gatgattagt    120
ctgagcccca tcgtgacatg cgttgtggtg gatgtttcag aggatgaccc cgacgtgcaa    180
atcagttggt tcgttaacaa cgtggaggtg cataccgctc aaacccagac ccacagagag    240
gattataaca gcaccctgcg ggtagtgtcc gccctgccga tccagcatca ggattggatg    300
agcgggaaag agttcaagtg taaggtaaac aacaaagatc tgccagcgcc gattgaacga    360
accattagca agccgaaagg gagcgtgcgc gcacctcagg tttacgtcct tcctccacca    420
gaagaggaga tgacgaaaaa gcaggtgacc ctgacatgca tggtaactga ctttatgcca    480
gaagatattt acgtggaatg gactaataac ggaaagacag agctcaatta caagaacact    540
gagcctgttc tggattctga tggcagctac tttatgtact ccaaattgag ggtcgagaag    600
aagaattggg tcgagagaaa cagttatagt tgctcagtgg tgcatgaggg cctccataat    660
catcacacca caaagtcctt cagccgaacg cccgggaaa                            699
```

(SEQ ID NO:90)

The hinge, C_H2 and C_H3 regions of a murine immunoglobulin Cγ2a chain encoded by SEQ ID NO:90 has the following amino acid sequence:

```
EPRGPTIKPC PPCKCPAPNL LGGPSVFIFP PKIKDVLMIS LSPIVTCVVV DVSEDDPDVQ     60
ISWFVNNVEV HTAQTQTHRE DYNSTLRVVS ALPIQHQDWM SGKEFKCKVN NKDLPAPIER    120
TISKPKGSVR APQVYVLPPP EEEMTKKQVT LTCMVTDFMP EDIYVEWTNN GKTELNYKNT    180
EPVLDSDGSY FMYSKLRVEK KNWVERNSYS CSVVHEGLHN HHTTKSFSRT PGK           233
```

(SEQ ID NO:91)

In another embodiment, the linker domain contains a hinge region of an immunoglobulin as described above, and further includes one or more additional immunoglobulin domains.

2. Other peptide/polypeptide linker domains

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:92), Ala-Ser, Gly-Gly-Gly-Ser (SEQ ID NO:93), $(Gly_4\text{-}Ser)_3$ (SEQ ID NO:94) and $(Gly_4\text{-}Ser)_4$ (SEQ ID NO:95). Additional flexible peptide/polypeptide sequences are well known in the art.

D. Dimerization, multimerization and targeting domains

The fusion proteins disclosed herein optionally contain a dimerization or multimerization domain that functions to dimerize or multimerize two or more fusion proteins. The domain that functions to dimerize or multimerize the fusion proteins can either be a separate domain, or alternatively can be contained within one of the other domains (B7-H4 polypeptide, second polypeptide, or peptide/polypeptide linker domain) of the fusion protein.

1. Dimerization domains

A "dimerization domain" is formed by the association of at least two amino acid residues or of at least two peptides or polypeptides (which may have the same, or different, amino acid sequences). The peptides or polypeptides may interact with each other through covalent and/or non-covalent association(s). Preferred dimerization domains contain at least one cysteine that is capable of forming an intermolecular disulfide bond with a cysteine on the partner fusion protein. The dimerization domain can contain one or more cysteine residues such that disulfide bond(s) can form between the partner fusion proteins. In one embodiment, dimerization domains contain one, two or three to about ten cysteine residues. In a preferred embodiment, the dimerization domain is the hinge region of an immunoglobulin.

Additional exemplary dimerization domain can be any known in the art and include, but not limited to, coiled coils, acid patches, zinc fingers, calcium hands, a C_H1-C_L pair, an "interface" with an engineered "knob" and/or "protruberance" as described in U.S. Patent No. 5,821,333, leucine zippers (e.g., from jun and/or fos) (U.S. Patent No. 5,932,448), SH2 (src

homology 2), SH3 (src Homology 3) (Vidal, et al., *Biochemistry*, 43, 7336-44 ((2004)), phosphotyrosine binding (PTB) (Zhou, et al., *Nature*, 378:584-592 (1995)), WW (Sudol, *Prog. Biochys. Mol. Bio.*, 65:113-132 (1996)), PDZ (Kim, et al., *Nature*, 378: 85-88 (1995); Komau, et al., *Science*, 269:1737-1740 (1995)) 14-3-3, WD40 (Hu, et al., *J Biol Chem.*, 273, 33489-33494 (1998)) EH, Lim, an isoleucine zipper, a receptor dimer pair (e.g., interleukin-8 receptor (IL-8R); and integrin heterodimers such as LFA-1 and GPIIIb/IIIa), or the dimerization region(s) thereof, dimeric ligand polypeptides (e.g. nerve growth factor (NGF), neurotrophin-3 (NT-3), interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), VEGF-C, VEGF-D, PDGF members, and brain-derived neurotrophic factor (BDNF) (Arakawa, et al., *J. Biol. Chem.*, 269(45): 27833-27839 (1994) and Radziejewski, et al., *Biochem.*, 32(48): 1350 (1993)) and can also be variants of these domains in which the affinity is altered. The polypeptide pairs can be identified by methods known in the art, including yeast two hybrid screens. Yeast two hybrid screens are described in U.S. Pat. Nos. 5,283,173 and 6,562,576. Affinities between a pair of interacting domains can be determined using methods known in the art, including as described in Katahira, et al., *J. Biol. Chem.*, 277, 9242-9246 (2002)). Alternatively, a library of peptide sequences can be screened for heterodimerization, for example, using the methods described in WO 01/00814. Useful methods for protein-protein interactions are also described in U.S. Patent No. 6,790,624.

2. Multimerization domains

A "multimerization domain" is a domain that causes three or more peptides or polypeptides to interact with each other through covalent and/or non-covalent association(s). Suitable multimerization domains include, but are not limited to, coiled-coil domains. A coiled-coil is a peptide sequence with a contiguous pattern of mainly hydrophobic residues spaced 3 and 4 residues apart, usually in a sequence of seven amino acids (heptad repeat) or eleven amino acids (undecad repeat), which assembles (folds) to form a multimeric bundle of helices. Coiled-coils with sequences including some irregular distribution of the 3 and 4 residues spacing are also contemplated. Hydrophobic residues are in particular the hydrophobic amino acids Val, Ile,

Leu, Met, Tyr, Phe and Trp. "Mainly hydrophobic" means that at least 50% of the residues must be selected from the mentioned hydrophobic amino acids.

The coiled coil domain may be derived from laminin. In the extracellular space, the heterotrimeric coiled coil protein laminin plays an important role in the formation of basement membranes. Apparently, the multifunctional oligomeric structure is required for laminin function. Coiled coil domains may also be derived from the thrombospondins in which three (TSP-1 and TSP-2) or five (TSP-3, TSP-4 and TSP-5) chains are connected, or from COMP (COMPcc) (Guo, et at., *EMBO J.*, 1998, 17: 5265-5272) which folds into a parallel five-stranded coiled coil (Malashkevich, et al., *Science*, 274: 761-765 (1996)).

Additional coiled-coil domains derived from other proteins, and other domains that mediate polypeptide multimerization are known in the art and are suitable for use in the disclosed fusion proteins.

In another embodiment, B7-H4 polypeptides, fusion proteins, or fragments thereof can be induced to form multimers by binding to a second multivalent polypeptide, such as an antibody. Antibodies suitable for use to multimerize B7-H4 polypeptides, fusion proteins, or fragments thereof include, but are not limited to, IgM antibodies and cross-linked, multivalent IgG, IgA, IgD, or IgE complexes.

3. Targeting Domains

The B7-H4 polypeptides and fusion proteins can contain a targeting domain to target the molecule to specific sites in the body. Preferred targeting domains target the molecule to areas of inflammation. Exemplary targeting domains are antibodies, or antigen binding fragments thereof that are specific for inflamed tissue or to a proinflammatory cytokine including but not limited to IL17, IL-4, IL-6, IL-12, IL-21, IL-22, and IL-23. In the case of neurological disorders such as Multiple Sclerosis, the targeting domain may target the molecule to the CNS or may bind to VCAM-1 on the vascular epithelium. Additional targeting domains can be peptide aptamers specific for a proinflammatory molecule. In other embodiments, the B7-H4 fusion protein can include a binding partner specific for a polypeptide

displayed on the surface of an immune cell, for example a T cell. In still other embodiments, the targeting domain specifically targets activated immune cells. Preferred immune cells that are targeted include Th0, Th1, Th17 and Th22 T cells, other cells that secrete, or cause other cells to secrete inflammatory molecules including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs, and Tregs. For example, a targeting domain for Tregs may bind specifically to CD25.

E. Exemplary fusion proteins

A representative murine B7-H4 fusion protein is encoded by a nucleic acid having at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

```
atggcttcct tggggcagat catcttttgg agtattatta acatcatcat catcctggct     60
ggggccatcg cactcatcat tggctttggc atttcaggca agcacttcat cacggtcacg    120
accttcacct cagctggaaa cattggagag gacgggaccc tgagctgcac ttttgaacct    180
gacatcaaac tcaacggcat cgtcatccag tggctgaaag aaggcatcaa aggtttggtc    240
cacgagttca aagaaggcaa agacgacctc tcacagcagc atgagatgtt cagaggccgc    300
acagcagtgt ttgctgatca ggtggtagtt ggcaatgctt ccctgagact gaaaaacgtg    360
cagctcacgg atgctggcac ctacacatgt tacatccgca cctcaaaagg caaagggaat    420
gcaaaccttg agtataagac cggagccttc agtatgccag agataaatgt ggactataat    480
gccagttcag agagtttacg ctgcgaggct cctcggtggt tcccccagcc cacagtggcc    540
tgggcatctc aagtcgacca aggagccaat ttctcagaag tctccaacac cagctttgag    600
ttgaactctg agaatgtgac catgaaggtc gtatctgtgc tctacaatgt cacaatcaac    660
aacacatact cctgtatgat tgaaaacgac attgccaaag ccaccgggga catcaaagtg    720
acagattcag aggtcaaaag gcgaagtcag ctgcagttgc tgaactctgg ggagccaaga    780
ggtcctacga tcaagccctg cccgccttgt aaatgcccag ctccaaattt gctgggtgga    840
ccgtcagtct ttatcttccc gccaaagata aaggacgtct tgatgattag tctgagcccc    900
atcgtgacat gcgttgtggt ggatgtttca gaggatgacc ccgacgtgca aatcagttgg    960
ttcgttaaca acgtggaggt gcataccgct caaacccaga cccacagaga ggattataac   1020
agcaccctgc gggtagtgtc cgccctgccg atccagcatc aggattggat gagcgggaaa   1080
gagttcaagt gtaaggtaaa caacaaagat ctgccagcgc cgattgaacg aaccattagc   1140
aagccgaaag ggagcgtgcg cgcacctcag gtttacgtcc ttcctccacc agaagaggag   1200
atgacgaaaa agcaggtgac cctgacatgc atggtaactg actttatgcc agaagatatt   1260
tacgtggaat ggactaataa cggaaagaca gagctcaatt acaagaacac tgagcctgtt   1320
ctggattctg atggcagcta ctttatgtac tccaaattga gggtcgagaa gaagaattgg   1380
gtcgagagaa acagttatag ttgctcagtg gtgcatgagg gcctccataa tcatcacacc   1440
acaaagtcct tcagccgaac gcccgggaaa                                    1470
```

(SEQ ID NO:96),

atggagtggt catgggtttt tctgttcttt cttagcgtga ctacaggcgt ccattcagga 60
ttcggcataa gcggcaagca cttcatcaca gttacaacgt ttacaagtgc ggggaacatt 120
ggggaagatg gaacattgtc atgtacattt gagccagata tcaaactcaa tggaatagta 180
attcagtggc ttaaggaggg catcaagggc ctggtccacg aatttaagga ggggaaagac 240
gatctgtctc agcagcacga gatgttcagg ggcagaaccg ccgtcttcgc agaccaggtt 300
gtggtaggca acgccagttt gcggctgaaa aacgtgcagc tgactgacgc cggcacctac 360
acatgctata tccggtcctc taagggcaag gggaacgcta atctcgagta caaaacaggc 420
gccttttcta tgccagagat caacgtggac tataacgcaa gctctgaaag tctgagatgc 480
gaggcgccaa ggtggttccc tcagcccacc gtcgcgtggg cttcccaggt ggatcaaggc 540
gccaactttt ctgaggtttc taacaccagc ttcgaactga acagcgaaaa tgtgacaatg 600
aaggtagtca gcgttctgta taacgtgacc atcaacaata cttactcctg tatgatagaa 660
aatgatatag ccaaggctac aggagatatt aaagtgacgg attcagaagt gaaaaggagg 720
agtcaactgc aactcttgaa tagcggcgag ccaagaggtc ctacgatcaa gccctgcccg 780
ccttgtaaat gcccagctcc aaatttgctg ggtggaccgt cagtctttat cttcccgcca 840
aagataaagg acgtcttgat gattagtctg agccccatcg tgacatgcgt tgtggtggat 900
gtttcagagg atgaccccga cgtgcaaatc agttggttcg ttaacaacgt ggaggtgcat 960
accgctcaaa cccagaccca cagagaggat tataacagca ccctgcgggt agtgtccgcc 1020
ctgccgatcc agcatcagga ttggatgagc gggaaagagt tcaagtgtaa ggtaaacaac 1080
aaagatctgc cagcgccgat tgaacgaacc attagcaagc cgaaagggag cgtgcgcgca 1140
cctcaggttt acgtccttcc tccaccagaa gaggagatga cgaaaaagca ggtgaccctg 1200
acatgcatgg taactgactt tatgccagaa gatatttacg tggaatggac taataacgga 1260
aagacagagc tcaattacaa gaacactgag cctgttctgg attctgatgg cagctacttt 1320
atgtactcca aattgagggt cgagaagaag aattgggtcg agagaaacag ttatagttgc 1380
tcagtggtgc atgagggcct ccataatcat cacaccacaa agtccttcag ccgaacgccc 1440
gggaaa 1446

(SEQ ID NO:97) or

atggagtggt catgggtttt tctgttcttt cttagcgtga ctacaggcgt ccattcagga 60
ttcggcataa gcggcaagca cttcatcaca gttacaacgt ttacaagtgc ggggaacatt 120
ggggaagatg gaacattgtc atgtacattt gagccagata tcaaactcaa tggaatagta 180
attcagtggc ttaaggaggg catcaagggc ctggtccacg aatttaagga ggggaaagac 240
gatctgtctc agcagcacga gatgttcagg ggcagaaccg ccgtcttcgc agaccaggtt 300
gtggtaggca acgccagttt gcggctgaaa aacgtgcagc tgactgacgc cggcacctac 360
acatgctata tccggacctc taagggcaag gggaacgcta atctcgagta caaaacaggc 420
gccttttcta tgccagagat caacgtggac tataacgcaa gctctgaaag tctgagatgc 480
gaggcgccaa ggtggttccc tcagcccacc gtcgcgtggg cttcccaggt ggatcaaggc 540
gccaactttt ctgaggtttc taacaccagc ttcgaactga acagcgaaaa tgtgacaatg 600
aaggtagtca gcgttctgta taacgtgacc atcaacaata cttactcctg tatgatagaa 660
aatgatatag ccaaggctac aggagatatt aaagtgacgg attcagaagt gaaaaggagg 720
agtcaactgc aactcttgaa tagcggcgag ccaagaggtc ctacgatcaa gccctgcccg 780
ccttgtaaat gcccagctcc aaatttgctg ggtggaccgt cagtctttat cttcccgcca 840
aagataaagg acgtcttgat gattagtctg agccccatcg tgacatgcgt tgtggtggat 900
gtttcagagg atgaccccga cgtgcaaatc agttggttcg ttaacaacgt ggaggtgcat 960
accgctcaaa cccagaccca cagagaggat tataacagca ccctgcgggt agtgtccgcc 1020
ctgccgatcc agcatcagga ttggatgagc gggaaagagt tcaagtgtaa ggtaaacaac 1080
aaagatctgc cagcgccgat tgaacgaacc attagcaagc cgaaagggag cgtgcgcgca 1140
cctcaggttt acgtccttcc tccaccagaa gaggagatga cgaaaaagca ggtgaccctg 1200
acatgcatgg taactgactt tatgccagaa gatatttacg tggaatggac taataacgga 1260

```
aagacagagc tcaattacaa gaacactgag cctgttctgg attctgatgg cagctacttt   1320
atgtactcca aattgagggt cgagaagaag aattgggtcg agagaaacag ttatagttgc   1380
tcagtggtgc atgagggcct ccataatcat cacaccacaa agtccttcag ccgaacgccc   1440
gggaaa                                                              1446
```

(SEQ ID NO:98)

In another embodiment, a representative murine B7-H4 fusion protein has at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

```
MASLGQIIFW SIINIIIILA GAIALIIGFG ISGKHFITVT TFTSAGNIGE DGTLSCTFEP     60
DIKLNGIVIQ WLKEGIKGLV HEFKEGKDDL SQQHEMFRGR TAVFADQVVV GNASLRLKNV    120
QLTDAGTYTC YIRSSKGKGN ANLEYKTGAF SMPEINVDYN ASSESLRCEA PRWFPQPTVA    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TDSEVKRRSQ LQLLNSGEPR GPTIKPCPPC KCPAPNLLGG PSVFIFPPKI KDVLMISLSP    300
IVTCVVVDVS EDDPDVQISW FVNNVEVHTA QTQTHREDYN STLRVVSALP IQHQDWMSGK    360
EFKCKVNNKD LPAPIERTIS KPKGSVRAPQ VYVLPPPEEE MTKKQVTLTC MVTDFMPEDI    420
YVEWTNNGKT ELNYKNTEPV LDSDGSYFMY SKLRVEKKNW VERNSYSCSV VHEGLHNHHT    480
TKSFSRTPGK                                                           490
```

(SEQ ID NO:99),

```
MASLGQIIFW SIINIIIILA GAIALIIGFG ISGKHFITVT TFTSAGNIGE DGTLSCTFEP     60
DIKLNGIVIQ WLKEGIKGLV HEFKEGKDDL SQQHEMFRGR TAVFADQVVV GNASLRLKNV    120
QLTDAGTYTC YIRTSKGKGN ANLEYKTGAF SMPEINVDYN ASSESLRCEA PRWFPQPTVA    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TDSEVKRRSQ LQLLNSGEPR GPTIKPCPPC KCPAPNLLGG PSVFIFPPKI KDVLMISLSP    300
IVTCVVVDVS EDDPDVQISW FVNNVEVHTA QTQTHREDYN STLRVVSALP IQHQDWMSGK    360
EFKCKVNNKD LPAPIERTIS KPKGSVRAPQ VYVLPPPEEE MTKKQVTLTC MVTDFMPEDI    420
YVEWTNNGKT ELNYKNTEPV LDSDGSYFMY SKLRVEKKNW VERNSYSCSV VHEGLHNHHT    480
TKSFSRTPGK                                                           490
```

(SEQ ID NO:100),

```
MEWSWVFLFF LSVTTGVHSG FGISGKHFIT VTTFTSAGNI GEDGTLSCTF EPDIKLNGIV     60
IQWLKEGIKG LVHEFKEGKD DLSQQHEMFR GRTAVFADQV VVGNASLRLK NVQLTDAGTY    120
TCYIRSSKGK GNANLEYKTG AFSMPEINVD YNASSESLRC EAPRWFPQPT VAWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTDSEVKRR    240
SQLQLLNSGE PRGPTIKPCP PCKCPAPNLL GGPSVFIFPP KIKDVLMISL SPIVTCVVVD    300
VSEDDPDVQI SWFVNNVEVH TAQTQTHRED YNSTLRVVSA LPIQHQDWMS GKEFKCKVNN    360
KDLPAPIERT ISKPKGSVRA PQVYVLPPPE EEMTKKQVTL TCMVTDFMPE DIYVEWTNNG    420
KTELNYKNTE PVLDSDGSYF MYSKLRVEKK NWVERNSYSC SVVHEGLHNH HTTKSFSRTP    480
GK                                                                   482
```

(SEQ ID NO:101) or

```
MEWSWVFLFF LSVTTGVHSG FGISGKHFIT VTTFTSAGNI GEDGTLSCTF EPDIKLNGIV     60
IQWLKEGIKG LVHEFKEGKD DLSQQHEMFR GRTAVFADQV VVGNASLRLK NVQLTDAGTY    120
TCYIRTSKGK GNANLEYKTG AFSMPEINVD YNASSESLRC EAPRWFPQPT VAWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTDSEVKRR    240
SQLQLLNSGE PRGPTIKPCP PCKCPAPNLL GGPSVFIFPP KIKDVLMISL SPIVTCVVVD    300
VSEDDPDVQI SWFVNNVEVH TAQTQTHRED YNSTLRVVSA LPIQHQDWMS GKEFKCKVNN    360
KDLPAPIERT ISKPKGSVRA PQVYVLPPPE EEMTKKQVTL TCMVTDFMPE DIYVEWTNNG    420
KTELNYKNTE PVLDSDGSYF MYSKLRVEKK NWVERNSYSC SVVHEGLHNH HTTKSFSRTP    480
GK                                                                   482
```

(SEQ ID NO:102).

The amino acid sequence of the murine B7-H4 fusion protein of SEQ ID NO:99 and SEQ ID NO:101 without the signal sequence is:

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK     60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRSSKG KGNANLEYKT    120
GAFSMPEINV DYNASSESLR CEAPRWFPQP TVAWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTDSEVKR RSQLQLLNSG EPRGPTIKPC    240
PPCKCPAPNL LGGPSVFIFP PKIKDVLMIS LSPIVTCVVV DVSEDDPDVQ ISWFVNNVEV    300
HTAQTQTHRE DYNSTLRVVS ALPIQHQDWM SGKEFKCKVN NKDLPAPIER TISKPKGSVR    360
APQVYVLPPP EEEMTKKQVT LTCMVTDFMP EDIYVEWTNN GKTELNYKNT EPVLDSDGSY    420
FMYSKLRVEK KNWVERNSYS CSVVHEGLHN HHTTKSFSRT PGK                      463
```

(SEQ ID NO:103).

The amino acid sequence of the murine B7-H4 fusion protein of SEQ ID NO:100 and SEQ ID NO:102 without the signal sequence is:

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK     60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRTSKG KGNANLEYKT    120
GAFSMPEINV DYNASSESLR CEAPRWFPQP TVAWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTDSEVKR RSQLQLLNSG EPRGPTIKPC    240
PPCKCPAPNL LGGPSVFIFP PKIKDVLMIS LSPIVTCVVV DVSEDDPDVQ ISWFVNNVEV    300
HTAQTQTHRE DYNSTLRVVS ALPIQHQDWM SGKEFKCKVN NKDLPAPIER TISKPKGSVR    360
APQVYVLPPP EEEMTKKQVT LTCMVTDFMP EDIYVEWTNN GKTELNYKNT EPVLDSDGSY    420
FMYSKLRVEK KNWVERNSYS CSVVHEGLHN HHTTKSFSRT PGK                      463
```

(SEQ ID NO:104).

A representative human B7-H4 fusion protein is encoded by a nucleic acid having at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

```
atggcttccc tggggcagat cctcttctgg agcataatta gcatcatcat tattctggct     60
ggagcaattg cactcatcat tggctttggt atttcaggga gacactccat cacagtcact    120
actgtcgcct cagctgggaa cattggggag gatggaatcc tgagctgcac ttttgaacct    180
gacatcaaac tttctgatat cgtgatacaa tggctgaagg aaggtgtttt aggcttggtc    240
catgagttca aagaaggcaa agatgagctg tcggagcagg atgaaatgtt cagaggccgg    300
acagcagtgt ttgctgatca agtgatagtt ggcaatgcct ctttgcggct gaaaaacgtg    360
caactcacag atgctggcac ctacaaatgt tatatcatca cttctaaagg caaggggaat    420
gctaaccttg agtataaaac tggagccttc agcatgccgg aagtgaatgt ggactataat    480
```

gccagctcag agaccttgcg gtgtgaggct ccccgatggt tcccccagcc cacagtggtc 540
tgggcatccc aagttgacca gggagccaac ttctcggaag tctccaatac cagctttgag 600
ctgaactctg agaatgtgac catgaaggtt gtgtctgtgc tctacaatgt tacgatcaac 660
aacacatact cctgtatgat tgaaaatgac attgccaaag caacagggga tatcaaagtg 720
acagaatcgg agatcaaaag gcggagtgag cctaagtcat gtgacaagac ccatacgtgc 780
ccaccctgtc ccgctccaga actgctgggg ggacctagcg ttttcttgtt ccccccaaag 840
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(SEQ ID NO:112), or

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(SEQ ID NO:113).

In another embodiment, a representative human B7-H4 fusion protein has at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

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MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP     60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRSE PKSCDKTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV    300
SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK    360
ALPAPIEKTI SKAKGQPREP QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ    420
PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG    480
K                                                                     481
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(SEQ ID NO:114).

In another embodiment, a representative human B7-H4 fusion protein has at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

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MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP     60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRSD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP    300
EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP    360
IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY    420
KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK        476
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(SEQ ID NO:115),

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WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF    300
NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT    360
ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP    420
PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK            472
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(SEQ ID NO:116),

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MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP     60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRSH LQLLNSKDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV    300
VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV    360
SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES    420
NGQPENNYKT TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL    480
SPGK                                                                  484
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(SEQ ID NO:117),

```
MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRSE PKSCDKTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV    300
```

SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK 360
ALPAPIEKTI SKAKGQPREP QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ 420
PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG 480
K 481

(SEQ ID NO:118),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP 300
EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP 360
IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY 420
KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK 476

(SEQ ID NO:119),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF 300
NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT 360
ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP 420
PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK 472

(SEQ ID NO:120),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSH LQLLNSKDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV 300
VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV 360
SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES 420
NGQPENNYKT TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL 480
SPGK 484

(SEQ ID NO:121),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK 300
FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK 360
TISKAKGQPR EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT 420
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK 473

(SEQ ID NO:122),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180

ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV 300
DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA 360
KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD 420
SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK 468

(SEQ ID NO:123),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIDKT 240
HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE 300
VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP 360
REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS 420
FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK 464

(SEQ ID NO:124),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SHLQLLNSKD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP 300
EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP 360
IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY 420
KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK 476

(SEQ ID NO:125),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK 300
FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK 360
TISKAKGQPR EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT 420
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK 473

(SEQ ID NO:126),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV 300
DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA 360
KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD 420
SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK 468

(SEQ ID NO:127),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180

ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIDKT 240
HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE 300
VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP 360
REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS 420
FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK 464

(SEQ ID NO:128), or

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SHLQLLNSKD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP 300
EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP 360
IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY 420
KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK 476

(SEQ ID NO:129).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:114 and SEQ ID NO:122 without the signal sequence is:

GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK 60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT 120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT 180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSEPKSCDKT HTCPPCPAPE 240
LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE 300
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP 360
SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD 420
KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK 454

(SEQ ID NO:130).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:115 and SEQ ID NO:123 without the signal sequence is:

GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK 60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT 120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT 180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSDKTHTCPP CPAPELLGGP 240
SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS 300
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL 360
TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ 420
QGNVFSCSVM HEALHNHYTQ KSLSLSPGK 449

(SEQ ID NO:131).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:116 and SEQ ID NO:124 without the signal sequence is:

GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK 60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT 120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT 180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIDK THTCPPCPAP ELLGGPSVFL 240

```
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV    300
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ    360
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV    420
FSCSVMHEAL HNHYTQKSLS LSPGK                                          445
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(SEQ ID NO:132).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:117 and SEQ ID NO:125 without the signal sequence is:

```
GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK DKTHTCPPCP    240
APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK    300
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT    360
LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL    420
TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK                             457
```

(SEQ ID NO:133).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:118 and SEQ ID NO:126 without the signal sequence is:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSEPKSCDKT HTCPPCPAPE    240
LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE    300
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP    360
SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD    420
KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK                                454
```

(SEQ ID NO:134).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:119 and SEQ ID NO:127 without the signal sequence is:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSDKTHTCPP CPAPELLGGP    240
SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS    300
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL    360
TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ    420
QGNVFSCSVM HEALHNHYTQ KSLSLSPGK                                      449
```

(SEQ ID NO:135).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:120 and SEQ ID NO:128 without the signal sequence is:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
```

```
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT   180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIDK THTCPPCPAP ELLGGPSVFL   240
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV   300
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ   360
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV   420
FSCSVMHEAL HNHYTQKSLS LSPGK                                        445
```

(SEQ ID NO:136).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:121 and SEQ ID NO:129 without the signal sequence is:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK    60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT   120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT   180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK DKTHTCPPCP   240
APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK   300
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT   360
LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL   420
TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK                           457
```

(SEQ ID NO:137).

The aforementioned exemplary fusion proteins can incorporate any combination of the variants described herein. In another embodiment the terminal lysine of the aforementioned exemplary fusion proteins is deleted.

The disclosed fusion proteins can be isolated using standard molecular biology techniques. For example, an expression vector containing a DNA sequence encoding a B7-H4-Ig fusion protein is transfected into 293 cells by calcium phosphate precipitation and cultured in serum-free DMEM. The supernatant is collected at 72 h and the fusion protein is purified by Protein G, or preferably Protein A SEPHAROSE® columns (Pharmacia, Uppsala, Sweden).

F. Fusion protein dimers and multimers

B7-H4 fusion polypeptides can be dimerized or multimerized. Dimerization or multimerization can occur between or among two or more fusion proteins through dimerization or multimerization domains, including those described above. Alternatively, dimerization or multimerization of fusion proteins can occur by chemical crosslinking. Fusion protein dimers can be homodimers or heterodimers. Fusion protein multimers can be homomultimers or heteromultimers.

Fusion protein dimers as disclosed herein are of formula II:

$$N\text{-}R_1\text{-}R_2\text{-}R_3\text{-}C$$
$$N\text{-}R_4\text{-}R_5\text{-}R_6\text{-}C$$

or, alternatively, are of formula III:

$$N\text{-}R_1\text{-}R_2\text{-}R_3\text{-}C$$
$$C\text{-}R_4\text{-}R_5\text{-}R_6\text{-}N$$

wherein the fusion proteins of the dimer provided by formula II are defined as being in a parallel orientation and the fusion proteins of the dimer provided by formula III are defined as being in an antiparallel orientation. Parallel and antiparallel dimers are also referred to as cis and trans dimers, respectively. "N" and "C" represent the N- and C-termini of the fusion protein, respectively. The fusion protein constituents "R_1", "R_2" and "R_3" are as defined above with respect to formula I. With respect to both formula II and formula III, "R_4" is a B7-H4 polypeptide or a second polypeptide, "R_5" is an optional peptide/polypeptide linker domain, and "R_6" is a B7-H4 polypeptide or a second polypeptide, wherein "R_6" is a B7-H4 polypeptide when "R_4" is a second polypeptide, and "R_6" is a second polypeptide when "R_4" is a B7-H4 polypeptide. In one embodiment, "R_1" is a B7-H4 polypeptide, "R_4" is also a B7-H4 polypeptide, and "R_3" and "R_6" are both second polypeptides.

Fusion protein dimers of formula II are defined as homodimers when "R_1" = "R_4", "R_2" = "R_5" and "R_3" = "R_6". Similarly, fusion protein dimers of formula III are defined as homodimers when "R_1" = "R_6", "R_2" = "R_5" and "R_3" = "R_4". Fusion protein dimers are defined as heterodimers when these conditions are not met for any reason. For example, heterodimers may contain domain orientations that meet these conditions (i.e., for a dimer according to formula II, "R_1" and "R_4" are both B7-H4 polypeptides, "R_2" and "R_5" are both peptide/polypeptide liker domains and "R_3" and "R_6" are both second polypeptides), however the species of one or more of these

domains is not identical. For example, although "R_3" and "R_6" may both be B7-H4 polypeptides, one polypeptide may contain a wild-type B7-H4 amino acid sequence while the other polypeptide may be a variant B7-H4 polypeptide. An exemplary variant B7-H4 polypeptide is B7-H4 polypeptide that has been modified to have increased or decreased binding to a target cell, increased activity on immune cells, increased or decreased half life or stability. Dimers of fusion proteins that contain either a C_H1 or C_L region of an immunoglobulin as part of the polypeptide linker domain preferably form heterodimers wherein one fusion protein of the dimer contains a C_H1 region and the other fusion protein of the dimer contains a C_L region.

Fusion proteins can also be used to form multimers. As with dimers, multimers may be parallel multimers, in which all fusion proteins of the multimer are aligned in the same orientation with respect to their N- and C-termini. Multimers may be antiparallel multimers, in which the fusion proteins of the multimer are alternatively aligned in opposite orientations with respect to their N- and C-termini. Multimers (parallel or antiparallel) can be either homomultimers or heteromultimers.

G. Peptide and polypeptide modifications

The fusion proteins may be modified by chemical moieties that may be present in polypeptides in a normal cellular environment, for example, phosphorylation, methylation, amidation, sulfation, acylation, glycosylation, sumoylation and ubiquitylation. Fusion proteins may also be modified with a label capable of providing a detectable signal, either directly or indirectly, including, but not limited to, radioisotopes and fluorescent compounds.

The fusion proteins may also be modified by chemical moieties that are not normally added to polypeptides in a cellular environment. For example, the disclosed fusion proteins may also be modified by covalent attachment of polymer chains, including, but not limited to, polyethylene glycol polymer (PEG) chains (i.e. pegylation). Conjugation of macromolecules to PEG has emerged recently as an effective strategy to alter the pharmacokinetic (PK) profiles of a variety of drugs, and thereby to improve their therapeutic potential. PEG conjugation increases retention of drugs in the circulation by protecting against enzymatic digestion, slowing

filtration by the kidneys and reducing the generation of neutralizing antibodies. In addition, PEG conjugates can be used to allow multimerization of the fusion proteins.

Modifications may be introduced into the molecule by reacting targeted amino acid residues of the polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. Another modification is cyclization of the protein.

Examples of chemical derivatives of the polypeptides include lysinyl and amino terminal residues derivatized with succinic or other carboxylic acid anhydrides. Derivatization with a cyclic carboxylic anhydride has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; *O*-methylisourea; 2,4 pentanedione; and transaminase-catalyzed reaction with glyoxylate. Carboxyl side groups, aspartyl or glutamyl, may be selectively modified by reaction with carbodiimides (R—N=C=N--R') such as 1-cyclohexyl-3-(2-morpholinyl-(4-ethyl)carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues can be converted to asparaginyl and glutaminyl residues by reaction with ammonia. Fusion proteins may also include one or more D-amino acids that are substituted for one or more L-amino acids.

H. Modified Binding Properties

Binding properties of the B7-H4 polypeptides, fragments and fusions thereof (collectively referred to as B7-H4 polypeptides) are relevant to the dose and dose regimen to be administered. In one embodiment the disclosed B7-H4 polypeptides have binding properties to at least one receptor on a T cell that demonstrate a higher term, or higher percentage, of occupancy of receptor molecules on immune cells relative to other ligands of the receptor molecules. In other embodiments, the disclosed B7-H4 polypeptides have reduced binding affinity to a receptor on T cells relative to wildtype B7-H4, allowing the protein to dissociate in a period of less than three months, two

months, one month, three weeks, two weeks, one week, or a few days after administration.

In some embodiments the B7-H4 polypeptides, or fragments, or fusions thereof have a relatively high affinity for its receptor, and may therefore have a relatively slow off rate. In other embodiments, the B7-H4 polypeptides are administered intermittently over a period of days, weeks or months to dampen immune responses which are allowed to recover prior to the next administration, which may serve to reduce the immune response without completely turning the immune response off and may avoid long term side effects.

III. Isolated nucleic acid molecules

Isolated nucleic acid sequences encoding B7-H4 polypeptides, fragments and fusions thereof are disclosed herein. Useful murine B7-H4 nucleic acids have at least about 80, 85, 90, 95 or 100% sequence identity to the B7-H4 nucleic acid having GenBank Accession Number NM_178594 or AY280973. Useful human B7-H4 nucleic acids have at least about 80, 85, 90, 95 or 100% sequence identity to the B7-H4 nucleic acid having GenBank Accession Number AK026071. As used herein, "isolated nucleic acid" refers to a nucleic acid that is separated from other nucleic acid molecules that are present in a mammalian genome, including nucleic acids that normally flank one or both sides of the nucleic acid in a mammalian genome (e.g., nucleic acids that encode non-B7-H4 proteins). The term "isolated" as used herein with respect to nucleic acids also includes the combination with any non-naturally-occurring nucleic acid sequence, since such non-naturally-occurring sequences are not found in nature and do not have immediately contiguous sequences in a naturally-occurring genome.

An isolated nucleic acid can be, for example, a DNA molecule, provided one of the nucleic acid sequences normally found immediately flanking that DNA molecule in a naturally-occurring genome is removed or absent. Thus, an isolated nucleic acid includes, without limitation, a DNA molecule that exists as a separate molecule independent of other sequences (e.g., a chemically synthesized nucleic acid, or a cDNA or genomic DNA fragment produced by PCR or restriction endonuclease treatment), as well as

recombinant DNA that is incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, lentivirus, adenovirus, or herpes virus), or into the genomic DNA of a prokaryote or eukaryote. In addition, an isolated nucleic acid can include an engineered nucleic acid such as a recombinant DNA molecule that is part of a hybrid or fusion nucleic acid. A nucleic acid existing among hundreds to millions of other nucleic acids within, for example, a cDNA library or a genomic library, or a gel slice containing a genomic DNA restriction digest, is not to be considered an isolated nucleic acid.

Nucleic acids encoding B7-H4 fusion polypeptides may be optimized for expression in the expression host of choice. Codons may be substituted with alternative codons encoding the same amino acid to account for differences in codon usage between the mammal from which the B7-H4 nucleic acid sequence is derived and the expression host. In this manner, the nucleic acids may be synthesized using expression host-preferred codons.

Nucleic acids can be in sense or antisense orientation, or can be complementary to a reference sequence encoding a B7-H4 polypeptide. Nucleic acids can be DNA, RNA, or nucleic acid analogs. Nucleic acid analogs can be modified at the base moiety, sugar moiety, or phosphate backbone. Such modification can improve, for example, stability, hybridization, or solubility of the nucleic acid. Modifications at the base moiety can include deoxyuridine for deoxythymidine, and 5-methyl-2'-deoxycytidine or 5-bromo-2'-deoxycytidine for deoxycytidine. Modifications of the sugar moiety can include modification of the 2' hydroxyl of the ribose sugar to form 2'-O-methyl or 2'-O-allyl sugars. The deoxyribose phosphate backbone can be modified to produce morpholino nucleic acids, in which each base moiety is linked to a six membered, morpholino ring, or peptide nucleic acids, in which the deoxyphosphate backbone is replaced by a pseudopeptide backbone and the four bases are retained. See, for example, Summerton and Weller (1997) *Antisense Nucleic Acid Drug Dev.* 7:187-195; and Hyrup *et al.* (1996) *Bioorgan. Med. Chem.* 4:5-23. In addition, the deoxyphosphate backbone can be replaced with, for

example, a phosphorothioate or phosphorodithioate backbone, a phosphoroamidite, or an alkyl phosphotriester backbone.

Nucleic acids encoding polypeptides can be administered to subjects in need thereof. Nucleic delivery involves introduction of "foreign" nucleic acids into a cell and ultimately, into a live animal. Compositions and methods for delivering nucleic acids to a subject are known in the art (see Understanding Gene Therapy, Lemoine, N.R., ed., BIOS Scientific Publishers, Oxford, 2008).

IV. Vectors and host cells

Vectors encoding B7-H4 polypeptides, fragments and fusions thereof are also provided. Nucleic acids, such as those described above, can be inserted into vectors for expression in cells. As used herein, a "vector" is a replicon, such as a plasmid, phage, virus or cosmid, into which another DNA segment may be inserted so as to bring about the replication of the inserted segment. Vectors can be expression vectors. An "expression vector" is a vector that includes one or more expression control sequences, and an "expression control sequence" is a DNA sequence that controls and regulates the transcription and/or translation of another DNA sequence.

Nucleic acids in vectors can be operably linked to one or more expression control sequences. As used herein, "operably linked" means incorporated into a genetic construct so that expression control sequences effectively control expression of a coding sequence of interest. Examples of expression control sequences include promoters, enhancers, and transcription terminating regions. A promoter is an expression control sequence composed of a region of a DNA molecule, typically within 100 nucleotides upstream of the point at which transcription starts (generally near the initiation site for RNA polymerase II). To bring a coding sequence under the control of a promoter, it is necessary to position the translation initiation site of the translational reading frame of the polypeptide between one and about fifty nucleotides downstream of the promoter. Enhancers provide expression specificity in terms of time, location, and level. Unlike promoters, enhancers can function when located at various distances from the transcription site.

An enhancer also can be located downstream from the transcription initiation site. A coding sequence is "operably linked" and "under the control" of expression control sequences in a cell when RNA polymerase is able to transcribe the coding sequence into mRNA, which then can be translated into the protein encoded by the coding sequence.

Suitable expression vectors include, without limitation, plasmids and viral vectors derived from, for example, bacteriophage, baculoviruses, tobacco mosaic virus, herpes viruses, cytomegalo virus, retroviruses, vaccinia viruses, adenoviruses, and adeno-associated viruses. Numerous vectors and expression systems are commercially available from such corporations as Novagen (Madison, WI), Clontech (Palo Alto, CA), Stratagene (La Jolla, CA), and Invitrogen Life Technologies (Carlsbad, CA).

An expression vector can include a tag sequence. Tag sequences, are typically expressed as a fusion with the encoded polypeptide. Such tags can be inserted anywhere within the polypeptide including at either the carboxyl or amino terminus. Examples of useful tags include, but are not limited to, green fluorescent protein (GFP), glutathione S-transferase (GST), polyhistidine, c-myc, hemagglutinin, Flag™ tag (Kodak, New Haven, CT), maltose E binding protein and protein A. In one embodiment, a nucleic acid molecule encoding a B7-H4 fusion polypeptide is present in a vector containing nucleic acids that encode one or more domains of an Ig 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.

Vectors containing nucleic acids to be expressed can be transferred into host cells. The term "host cell" is intended to include prokaryotic and eukaryotic cells into which a recombinant expression vector can be introduced. As used herein, "transformed" and "transfected" encompass the introduction of a nucleic acid molecule (e.g., a vector) into a cell by one of a number of techniques. Although not limited to a particular technique, a number of these techniques are well established within the art. Prokaryotic cells can be transformed with nucleic acids by, for example, electroporation or calcium chloride mediated transformation. Nucleic acids can be

transfected into mammalian cells by techniques including, for example, calcium phosphate co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, or microinjection. Host cells (e.g., a prokaryotic cell or a eukaryotic cell such as a CHO cell) can be used to, for example, produce the B7-H4 fusion polypeptides described herein.

The vectors described can be used to express B7-H4 in cells, for example, cells for transplantation such as islet cells. An exemplary vector includes, but is not limited to, an adenoviral vector. One approach includes nucleic acid transfer into primary cells in culture followed by autologous transplantation of the *ex vivo* transformed cells into the host, either systemically or into a particular organ or tissue. *Ex vivo* methods can include, for example, the steps of harvesting cells from a subject, culturing the cells, transducing them with an expression vector, and maintaining the cells under conditions suitable for expression of the encoded polypeptides. These methods are known in the art of molecular biology. The transduction step can be accomplished by any standard means used for *ex vivo* gene therapy, including, for example, calcium phosphate, lipofection, electroporation, viral infection, and biolistic gene transfer. Alternatively, liposomes or polymeric microparticles can be used. Cells that have been successfully transduced then can be selected, for example, for expression of the coding sequence or of a drug resistance gene. The cells then can be lethally irradiated (if desired) and injected or implanted into the subject. In one embodiment, expression vectors containing nucleic acids encoding fusion proteins are transfected into cells that are administered to a subject in need thereof.

In vivo nucleic acid therapy can be accomplished by direct transfer of a functionally active DNA into mammalian somatic tissue or organ *in vivo*. For example, nucleic acids encoding polypeptides disclosed herein can be administered directly to lymphoid tissues or tumors. Alternatively, lymphoid tissue specific targeting can be achieved using lymphoid tissue-specific transcriptional regulatory elements (TREs) such as a B lymphocyte-, T lymphocyte-, or dendritic cell-specific TRE. Lymphoid tissue specific TREs are known in the art.

Nucleic acids may also be administered *in vivo* by viral means. Nucleic acid molecules encoding fusion proteins may be packaged into retrovirus vectors using packaging cell lines that produce replication-defective retroviruses, as is well-known in the art. Other virus vectors may also be used, including recombinant adenoviruses and vaccinia virus, which can be rendered non-replicating. In addition to naked DNA or RNA, or viral vectors, engineered bacteria may be used as vectors.

Nucleic acids may also be delivered by other carriers, including liposomes, polymeric micro- and nanoparticles and polycations such as asialoglycoprotein/polylysine.

In addition to virus- and carrier-mediated gene transfer *in vivo*, physical means well-known in the art can be used for direct transfer of DNA, including administration of plasmid DNA and particle-bombardment mediated gene transfer.

V. Pharmaceutical compositions

Pharmaceutical compositions including B7-H4 polypeptides, fragments, fusion polypeptides, nucleic acids, and vectors disclosed herein are provided. Pharmaceutical compositions containing peptides or polypeptides may be for administration by parenteral (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), transdermal (either passively or using iontophoresis or electroporation), or transmucosal (nasal, vaginal, rectal, or sublingual) routes of administration or using bioerodible inserts and can be formulated in dosage forms appropriate for each route of administration.

In some *in vivo* approaches, the compositions disclosed herein are administered to a subject in a therapeutically effective amount. As used herein the term "effective amount" or "therapeutically effective amount" means a dosage sufficient to treat, inhibit, or alleviate one or more symptoms of the disorder being treated or to otherwise provide a desired pharmacologic and/or physiologic effect. The precise dosage will vary according to a variety of factors such as subject-dependent variables (e.g., age, immune system health, etc.), the disease, and the treatment being effected.

For the polypeptide compositions disclosed herein and nucleic acids encoding the same, as further studies are conducted, information will emerge regarding appropriate dosage levels for treatment of various conditions in various patients, and the ordinary skilled worker, considering the therapeutic context, age, and general health of the recipient, will be able to ascertain proper dosing. The selected dosage depends upon the desired therapeutic effect, on the route of administration, and on the duration of the treatment desired. For polypeptide compositions, generally dosage levels of 0.001 to 20 mg/kg of body weight daily are administered to mammals. Generally, for intravenous injection or infusion, dosage may be lower.

In certain embodiments, the polypeptide compositions are administered locally, for example by injection directly into a site to be treated. Typcially, the injection causes an increased localized concentration of the polypeptide compositions which is greater than that which can be achieved by systemic administration. For example, in the case of a neurological disorder like Multiple Sclerosis, the protein may be administered locally to a site near the CNS. The polypeptide compositions can be combined with a matrix as described above to assist in creating a increased localized concentration of the polypeptide compositions by reducing the passive diffusion of the polypeptides out of the site to be treated.

A. Formulations for parenteral administration

In a preferred embodiment, compositions disclosed herein, including those containing peptides and polypeptides, are administered in an aqueous solution, by parenteral injection. The formulation may also be in the form of a suspension or emulsion. In general, pharmaceutical compositions are provided including effective amounts of a peptide or polypeptide, and optionally include pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions optionally include one or more for the following: diluents, sterile water, buffered saline of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; and additives such as detergents and solubilizing agents (e.g., TWEEN 20 (polysorbate-20), TWEEN 80 (polysorbate-80)),

anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), and preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol). Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. The formulations may be lyophilized and redissolved/resuspended immediately before use. The formulation may be sterilized by, for example, filtration through a bacteria retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions.

B. Formulations for topical administration

Fusion proteins disclosed herein can be applied topically. Topical administration does not work well for most peptide formulations, although it can be effective especially if applied to the lungs, nasal, oral (sublingual, buccal), vaginal, or rectal mucosa.

Compositions can be delivered to the lungs while inhaling and traverse across the lung epithelial lining to the blood stream when delivered either as an aerosol or spray dried particles having an aerodynamic diameter of less than about 5 microns.

A wide range of mechanical devices designed for pulmonary delivery of therapeutic products can be used, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art. Some specific examples of commercially available devices are the Ultravent nebulizer (Mallinckrodt Inc., St. Louis, Mo.); the Acorn II nebulizer (Marquest Medical Products, Englewood, Colo.); the Ventolin metered dose inhaler (Glaxo Inc., Research Triangle Park, N.C.); and the Spinhaler powder inhaler (Fisons Corp., Bedford, Mass.). Nektar, Alkermes and Mannkind all have inhalable insulin powder preparations approved or in clinical trials where the technology could be applied to the formulations described herein.

Formulations for administration to the mucosa will typically be spray dried drug particles, which may be incorporated into a tablet, gel, capsule, suspension or emulsion. Standard pharmaceutical excipients are available

from any formulator. Oral formulations may be in the form of chewing gum, gel strips, tablets or lozenges.

Transdermal formulations may also be prepared. These will typically be ointments, lotions, sprays, or patches, all of which can be prepared using standard technology. Transdermal formulations will require the inclusion of penetration enhancers.

C. Controlled delivery polymeric matrices

Fusion proteins disclosed herein may also be administered in controlled release formulations. Controlled release polymeric devices can be made for long term release systemically following implantation of a polymeric device (rod, cylinder, film, disk) or injection (microparticles). The matrix can be in the form of microparticles such as microspheres, where peptides are dispersed within a solid polymeric matrix or microcapsules, where the core is of a different material than the polymeric shell, and the peptide is dispersed or suspended in the core, which may be liquid or solid in nature. Unless specifically defined herein, microparticles, microspheres, and microcapsules are used interchangeably. Alternatively, the polymer may be cast as a thin slab or film, ranging from nanometers to four centimeters, a powder produced by grinding or other standard techniques, or even a gel such as a hydrogel.

Either non-biodegradable or biodegradable matrices can be used for delivery of fusion polypeptides or nucleic acids encoding the fusion polypeptides, although biodegradable matrices are preferred. These may be natural or synthetic polymers, although synthetic polymers are preferred due to the better characterization of degradation and release profiles. The polymer is selected based on the period over which release is desired. In some cases linear release may be most useful, although in others a pulse release or "bulk release" may provide more effective results. The polymer may be in the form of a hydrogel (typically in absorbing up to about 90% by weight of water), and can optionally be crosslinked with multivalent ions or polymers.

The matrices can be formed by solvent evaporation, spray drying, solvent extraction and other methods known to those skilled in the art.

Bioerodible microspheres can be prepared using any of the methods developed for making microspheres for drug delivery, for example, as described by Mathiowitz and Langer, *J. Controlled Release*, 5:13-22 (1987); Mathiowitz, et al., *Reactive Polymers*, 6:275-283 (1987); and Mathiowitz, et al., *J. Appl. Polymer Sci.*, 35:755-774 (1988).

The devices can be formulated for local release to treat the area of implantation or injection – which will typically deliver a dosage that is much less than the dosage for treatment of an entire body – or systemic delivery. These can be implanted or injected subcutaneously, into the muscle, fat, or swallowed.

VI. Methods of manufacture

A. Methods for producing fusion proteins

The disclosed fusion proteins can be manufactured using conventional techniques that are known in the art. Isolated fusion proteins can be obtained by, for example, chemical synthesis or by recombinant production in a host cell. To recombinantly produce a fusion protein, a nucleic acid containing a nucleotide sequence encoding the fusion protein can be used to transform, transduce, or transfect a bacterial or eukaryotic host cell (e.g., an insect, yeast, or mammalian cell). In general, nucleic acid constructs include a regulatory sequence operably linked to a nucleotide sequence encoding the fusion protein. Regulatory sequences (also referred to herein as expression control sequences) typically do not encode a gene product, but instead affect the expression of the nucleic acid sequences to which they are operably linked.

Useful prokaryotic and eukaryotic systems for expressing and producing polypeptides are well known in the art include, for example, *Escherichia coli* strains such as BL-21, and cultured mammalian cells such as CHO cells.

In eukaryotic host cells, a number of viral-based expression systems can be utilized to express fusion proteins. Viral based expression systems are well known in the art and include, but are not limited to, baculoviral, SV40, retroviral, or vaccinia based viral vectors.

Mammalian cell lines that stably express variant fusion proteins can be produced using expression vectors with appropriate control elements and a selectable marker. For example, the eukaryotic expression vectors pCR3.1 (Invitrogen Life Technologies) and p91023(B) (see Wong *et al.* (1985) *Science* 228:810-815) are suitable for expression of variant polypeptides in, for example, Chinese hamster ovary (CHO) cells, COS-1 cells, human embryonic kidney 293 cells, NIH3T3 cells, BHK21 cells, MDCK cells, and human vascular endothelial cells (HUVEC). Additional suitable expression systems include the GS Gene Expression System™ available through Lonza Group Ltd.

Following introduction of an expression vector by electroporation, lipofection, calcium phosphate, or calcium chloride co-precipitation, DEAE dextran, or other suitable transfection method, stable cell lines can be selected (e.g., by metabolic selection, or antibiotic resistance to G418, kanamycin, or hygromycin). The transfected cells can be cultured such that the polypeptide of interest is expressed, and the polypeptide can be recovered from, for example, the cell culture supernatant or from lysed cells. Alternatively, a fusion protein can be produced by (a) ligating amplified sequences into a mammalian expression vector such as pcDNA3 (Invitrogen Life Technologies), and (b) transcribing and translating *in vitro* using wheat germ extract or rabbit reticulocyte lysate.

Fusion proteins can be isolated using, for example, chromatographic methods such as affinity chromatography, ion exhange chromatography, hydrophobic interaction chromatography, DEAE ion exchange, gel filtration, and hydroxylapatite chromatography. In some embodiments, fusion proteins can be engineered to contain an additional domain containing amino acid sequence that allows the polypeptides to be captured onto an affinity matrix. For example, an Fc-fusion polypeptide in a cell culture supernatant or a cytoplasmic extract can be isolated using a protein A column. In addition, a tag such as c-myc, hemagglutinin, polyhistidine, or Flag™ (Kodak) can be used to aid polypeptide purification. Such tags can be inserted anywhere within the polypeptide, including at either the carboxyl or amino terminus. Other fusions that can be useful include enzymes that aid in the detection of

the polypeptide, such as alkaline phosphatase. Immunoaffinity chromatography also can be used to purify polypeptides. Fusion proteins can additionally be engineered to contain a secretory signal (if there is not a secretory signal already present) that causes the fusion protein to be secreted by the cells in which it is produced. The secreted fusion proteins can then conveniently be isolated from the cell media.

B. Methods for producing isolated nucleic acid molecules

Isolated nucleic acid molecules can be produced by standard techniques, including, without limitation, common molecular cloning and chemical nucleic acid synthesis techniques. For example, polymerase chain reaction (PCR) techniques can be used to obtain an isolated nucleic acid encoding a variant polypeptide. PCR is a technique in which target nucleic acids are enzymatically amplified. Typically, sequence information from the ends of the region of interest or beyond can be employed to design oligonucleotide primers that are identical in sequence to opposite strands of the template to be amplified. PCR can be used to amplify specific sequences from DNA as well as RNA, including sequences from total genomic DNA or total cellular RNA. Primers typically are 14 to 40 nucleotides in length, but can range from 10 nucleotides to hundreds of nucleotides in length. General PCR techniques are described, for example in PCR Primer: A Laboratory Manual, ed. by Dieffenbach and Dveksler, Cold Spring Harbor Laboratory Press, 1995. When using RNA as a source of template, reverse transcriptase can be used to synthesize a complementary DNA (cDNA) strand. Ligase chain reaction, strand displacement amplification, self-sustained sequence replication or nucleic acid sequence-based amplification also can be used to obtain isolated nucleic acids. See, for example, Lewis (1992) *Genetic Engineering News* 12:1; Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878; and Weiss (1991) *Science* 254:1292-1293.

Isolated nucleic acids can be chemically synthesized, either as a single nucleic acid molecule or as a series of oligonucleotides (e.g., using phosphoramidite technology for automated DNA synthesis in the 3' to 5' direction). For example, one or more pairs of long oligonucleotides (e.g., >100 nucleotides) can be synthesized that contain the desired sequence, with

each pair containing a short segment of complementarity (e.g., about 15 nucleotides) such that a duplex is formed when the oligonucleotide pair is annealed. DNA polymerase can be used to extend the oligonucleotides, resulting in a single, double-stranded nucleic acid molecule per oligonucleotide pair, which then can be ligated into a vector. Isolated nucleic acids can also obtained by mutagenesis. Fusion protein-encoding nucleic acids can be mutated using standard techniques, including oligonucleotide-directed mutagenesis and/or site-directed mutagenesis through PCR. See, Short Protocols in Molecular Biology. Chapter 8, Green Publishing Associates and John Wiley & Sons, edited by Ausubel *et al,* 1992. Examples of amino acid positions that can be modified include those described herein.

VII. Methods of Therapeutic Use

The B7-H4 polypeptides, or fragments, or fusions thereof disclosed herein are useful as therapeutic agents. Immune cells, preferably T cells, can be contacted *in vivo* or *ex vivo* with B7-H4 fusion polypeptides to decrease or inhibit immune responses including, but not limited to inflammation. The T cells contacted with B7-H4 fusion polypeptides can be any cell which express the T cell receptor, including α/β and γ/δ T cell receptors. T-cells include all cells which express CD3, including T-cell subsets which also express CD4 and CD8. T-cells include both naive and memory cells and effector cells such as CTL. T-cells also include regulatory cells such as Th1, Tc1, Th2, Tc2, Th3, Th17, Th22, Treg, and Tr1 cells. T-cells also include NKT-cells and similar unique classes of the T-cell lineage. For example the compositions can be used to modulate Th1, Th17, Th22, or other cells that secrete, or cause other cells to secrete, inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs. The compositions can also be used to increase or promote the activity of Tregs, increase the production of cytokines such as IL-10 from Tregs, increase the differentiation of Tregs, increase the number of Tregs, or increase the survival of Tregs.

In some embodiments, the disclosed B7-H4 polypeptides, or fragments, or fusions thereof are administered in combination with a second

therapeutic. Combination therapies may be useful in immune modulation. In some embodiments, B7-H4 polypeptides, or fragments, or fusions can be used to attenuate or reverse the activity of a pro-inflammatory drug, and/or limit the adverse effects of such drugs.

Other immune cells that can be treated with the disclosed B7-H4 polypeptides, fragments or fusion thereof include T cell precursors, antigen presenting cells such as dendritic cells and monocytes or their precursors, B cells or combinations thereof. The B7-H4 compositions can be used to modulate the production of antibodies by B cells by contacting the B cells with an effective amount of the B7-H4 composition to inhibit or reduce antibody production by the B cell relative to a control. The B7-H4 compositions can also modulate the production of cytokines by the B cells.

A. Methods of treating inflammatory responses

A preferred embodiment provides methods for treating or alleviating one or more symptoms of inflammation. In a more preferred embodiment, the compositions and methods disclosed are useful for treating chronic and persistent inflammation. Inflammation in general can be treated using the disclosed B7-H4 polypeptides or fragment or fusions thereof.

An immune response including inflammation can be inhibited or reduced in a subject, preferably a human, by administering an effective amount of B7-H4 polypeptide or fragment, or fusion thereof to inhibit or reduce the biological activity of an immune cell or to reduce the amounts of proinflammatory molecules at a site of inflammation. Exemplary proinflammatory molecules include, but are not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs.

Th1 and Th17 are exemplary T cells that can be targeted for inhibition by B7-H4 polypeptides, fusion proteins or fragments thereof to inhibit or reduce inflammation. The B7-H4 fusion proteins are useful for treating inflammation by any or all of the following: inhibiting or reducing differentiation of Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs; inhibiting or reducing activity of Th1, Th17, Th22, and/or other cells

that secrete, or cause other cells to secrete, inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs; inhibiting or reducing the Th1 and/or Th17 pathways; inhibiting or reducing cytokine production and/or secretion by Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs; inhibiting or reducing proliferation of Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs.

Additionally, B7-H4-Ig can cause Tregs to have an enhanced suppressive effect on an immune response. Tregs can suppress differentiation, proliferation, activity, and/or cytokine production and/or secretion by Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs. For example, B7-H4-Ig can cause Tregs to have an enhanced suppressive effect on Th1 and/or Th17 cells to reduce the level of IFN-γ and IL-17 produced, respectively. B7-H4-Ig can also act directly on Tregs to promote or enhance production of IL-10 to suppress the Th1 and Th17 pathway, or to increase the number of Tregs.

Figures 1A and 1B show two proposed modes of action of B7-H4 fusion proteins for inhibiting inflammation. Figure 1A shows B7-H4-Ig acts at multiple points in multiple T cell pathways. For example, B7-H4-Ig can inhibit the differentiation of naïve T cells into either Th1 or Th17 cells. Alternatively, B7-H4-Ig can interact with Th1 cells or Th17 cells, or both to inhibit or reduce the production of proinflammatory molecules. Additionally, B7-H4-Ig can increase the differentiation of and/or cause Tregs to have an enhanced suppressive effect on the Th1 and Th17 pathways to reduce the level of INF-γ and/or IL-17 produced. B7-H4-Ig enhances the production of IL-10 from Tregs and this inhibits the activity of Th1 and Th17 cells.

Figure 1B shows a second model in which B7-H4-Ig targets immature antigen presenting cells, such as dentritic cells (DCs), and inhibits cell maturation. Immature, antigen-capturing, dentritic cells have the capacity to mature into antigen-presenting, T cell-priming cells; converting antigens into immunogens and expressing molecules such as cytokines, chemokines, costimulatory molecules and proteases to initiate an immune response. The ability of DCs to regulate immunity is dependent on DC maturation. A variety of factors can induce maturation following antigen uptake and processing within DCs, including: whole bacteria or bacterial-derived antigens (*e.g.* lipopolysaccharide, LPS), inflammatory molecules, ligation of select cell surface receptors (*e.g.* CD40) and viral products (*e.g.* double-stranded RNA). The maturation process involves a signal to the DC that leads to increased surface expression of MHC class I and II molecules and the production of costimulatory molecules.

In the model presented in Figure 1B, B7-H4-Ig blocks DC maturation, which prevents the development of naïve T cells into either Th1 or Th17 cells, and reduces the production of proinflammatory molecules. Additionally, B7-H4-Ig can increase the pool of immature DC favoring the differentiation of and/or causing Tregs to have an enhanced suppressive effect on the Th1 and Th17 pathways to reduce the level of INF-γ and/or IL-17 produced. In this way, B7-H4-Ig enhances the production of IL-10 from Tregs and this inhibits the activity of Th1 and Th17 cells.

1. Inhibition of the Th1 Pathway

a. Inhibition of Th1 Development

One method for inhibiting or reducing inflammation includes administering an effective amount of a B7-H4 polypeptide, fusion protein, variants thereof, or fragments thereof to inhibit Th1 development in a subject in need thereof. It has been discovered that inflammation can be inhibited or reduced by blocking naïve T cells from differentiating into Th1 cells by administering B7-H4 polypeptides, fusion proteins, fragments thereof or variants thereof. In one embodiment, the B7-H4 polypeptides or fusion protein thereof increases the suppressive ability of Tregs on naïve T cells to inhibit or reduce naïve T cells from differentiating into Th1 cells and thereby

reduce the number of Th1 cells in a subject. Alternatively, the B7-H4 polypeptides or fusion protein thereof inhibits or reduces proliferation of TH1 cells. B7-H4 polypeptides, fragments or fusions proteins thereof may also reduce naïve T cells from differentiating into Th1 cells, by blocking antigen presenting cell maturation. By restricting the number of Th1 cells that can develop in the subject, the amount of proinflammatory molecules such as INF-γ can be reduced or contained. INF-γ stimulates the production or release of other proinflammatory molecules including IL-1β, TNF-α, and MMPs. Thus, by controlling the number of Th1 cells in a subject, the levels of these other proinflammatory molecules can be controlled, thereby reducing inflammatory responses.

b. Inhibition of Proinflammatory molecules

Another embodiment provides a method of inhibiting or reducing inflammation in a subject by administering to the subject an effective amount of a B7-H4 polypeptide, fusion protein thereof, or fragment thereof to inhibit or reduce production of proinflammatory molecules by Th1 cells. Exemplary proinflammatory molecules produced by Th1 cells includes IFN-γ. In this embodiment the B7-H4 polypeptide, fusion protein thereof, or fragment thereof can interact directly with the Th1 cell and inhibit or reduce IFN-γ production by the Th1 cells. In this embodiment, the amount of proinflammatory molecules is regulated rather than the population of Th1 cells.

2. Inhibition of the Th17 Pathway

a. Inhibition of Th17 Development

Inflammation can also be inhibited or reduced in a subject by administering an effective amount of a B7-H4 polypeptide, fragment or fusion thereof, to inhibit or block naïve T cells from developing into Th17 cells. In one embodiment, the B7-H4 polypeptide or fusion protein increases the suppressive activity of Tregs on the differentiation of naïve T cells into Th17 cells by an amount sufficient to reduce the number of Th17 cells in a subject. Alternatively, the B7-H4 polypeptide or fusion protein thereof inhibits or reduces proliferation of TH17 cells. B7-H4 polypeptides or fusions proteins thereof may also reduce naïve T cells from differentiating

into Th17 cells, by blocking antigen presenting cell maturation. By reducing the population of Th17 cells in a subject, the amount of IL-17 can be reduced, as well as IL-22 and IL-21. IL-17 is a proinflammatory cytokine that causes increases in other proinflammatory molecules such as IL-1β, TNF-α, and MMPs. Thus, by reducing the amount of IL-17 these other proinflammatory molecules can be reduced, thereby reducing or inhibiting inflammation.

b. Inhibition of IL-17 Production

Still another embodiment provides a method for treating inflammation in a subject by administering an effective amount of B7-H4 polypeptide, fusion protein thereof, or fragments thereof, to inhibit production of IL-17 by Th17 cells, as well as IL-22 and IL-21. In this embodiment, the B7-H4 polypeptide or fusion protein can act directly on Th17 cells, for example by binding to Th17 cells resulting in inhibition of IL-17 (or IL-22 and IL-21) production by those Th17 cells. As noted above, inhibition or reduction of IL-17 (and IL-22 or IL-21) leads to the reduction of other proinflammatory molecules, thereby reducing or inhibitng inflammation.

3. Inhibiting Th1 and Th17 Pathways

The disclosed B7-H4 polypeptides, fusion proteins, and fragments thereof can be used to inhibit both the Th1 and Th17 pathways simultaneously. Using one anti-inflammatory agent to inhibit two separate pathways provides more robust inhibition or reduction of the immune response.

4. Tregs

Inflammation can also be treated by administering B7-H4 polypeptides, fusion proteins thereof, or fragments thereof to a subject in an amount effective to enhance the suppressive activity of IL-10 producing Tregs to enhance suppressive activity on the Th1 and/or Th17 pathways. In this embodiment the disclosed B7-H4 polypeptides and fusion proteins cause an increased suppressive effect on IFN-γ and/or IL-17 production relative to Tregs alone.

Another embodiment provides a method for treating inflammation by administering an effective amount of B7-H4 polypeptide, fusion proteins thereof, or fragments thereof to increase production of IL-10 by Tregs. Increased production of IL-10 results in the descreased production of IL-17 by Th17 cells and deceased production of IFN- α by Th1 cells. In this embodiment, the B7-H4 polypeptides, fusion proteins, and fragments thereof can interact directly with Tregs to increase IL-10 production by the Tregs.

Still another embodiment provides a method for treating inflammation by administering an effective amount of B7-H4 polypeptides, fusion proteins thereof, and fragments thereof to inhibit or interfere with the Th1 pathway, Th17 pathway and to enhance the suppressive effect on the Th1 and Th17 pathway by Tregs (see Figure 1A). B7-H4 polypeptides or fusions proteins thereof may also increase the pool of Tregs by blocking antigen presenting cell maturation (see Figure 1B).

The B7-H4 polypeptides, fusion proteins thereof and fragments thereof can also be administered to a subject in an amount effective to increase Treg cell populations or numbers.

IL-10 and TGF-β production by Tregs can be increased relative to a control by contacting the Tregs with an effective amount of B7-H4 polypeptides, B7-H4 fusion proteins, or fragments thereof having B7-H4 activity. The increase can occur *in vitro* or *in vivo*.

5. Soluble B7-H4

Soluble B7-H4 (sH4) acts as a decoy molecule that competes with the cell surface B7-H4 for binding to the B7-H4 receptor and does not result in an inhibitory signal to the T cell. B7-H4 inhibits cell cycle progression of T cells in the presence of antigen stimulation. B7-H4 can inhibit innate immunity by suppressing proliferation of neutrophil progenitors. It is believed that elevated levels of sH4 block the inhibitory effect of endogenous B7-H4.

Therefore, an inflammatory response can be treated by interfering with the biological activity of sH4 *in vivo*, for example, by administering to an individual in need thereof an effective amount of an agent that inhibits or decreases the ability of sH4 to bind to the B7-H4 receptor, or augments the

activity of the endogenous inhibitory B7-H4 molecules. Interference of sH4 biological activity can be accomplished by administering B7-H4 fusion polypeptides disclosed herein.

Administration is not limited to the treatment of existing conditions, diseases or disorders (i.e. an existing inflammatory or autoimmune disease or disorder) but can also be used to prevent or lower the risk of developing such diseases in an individual, i.e., for prophylactic use. Potential candidates for prophylactic vaccination include individuals with a high risk of developing an inflammatory or autoimmune disease or disorder, i.e., with a personal or familial history of certain types of autoimmune disorders.

B. Inflammatory Disease to Be Treated

Representative inflammatory or autoimmune diseases and disorders that may be treated using B7-H4 fusion polypeptides include, but are not limited to, rheumatoid arthritis, systemic lupus erythematosus, alopecia areata, anklosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, autoimmune lymphoproliferative syndrome (alps), autoimmune thrombocytopenic purpura (ATP), Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac sprue-dermatitis, chronic fatigue syndrome immune deficiency, syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, cicatricial pemphigoid, cold agglutinin disease, Crest syndrome, Crohn's disease, Dego's disease, dermatomyositis, dermatomyositis - juvenile, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia – fibromyositis, grave's disease, guillain-barre, hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), Iga nephropathy, insulin dependent diabetes (Type I), juvenile arthritis, Meniere's disease, mixed connective tissue disease, multiple sclerosis, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychondritis, polyglancular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, Raynaud's phenomenon, Reiter's syndrome, rheumatic fever, sarcoidosis, scleroderma, Sjogren's syndrome, stiff-man syndrome, Takayasu arteritis,

temporal arteritis/giant cell arteritis, ulcerative colitis, uveitis, vasculitis, vitiligo, and Wegener's granulomatosis.

B7-H4 acts at multiple points in the inflammatory pathway and at a higher level whereby it acts as a master regulator to control to influence the expression and/or activity of effectory cytokines such as TNF-α. Therefore, the B7-H4 compositions described herein are particularly useful for treating patients that do not respond to TNF-α blockers such as Enbrel, Remicade, Cimzia and Humira, or where TNF-α blockers are not safe or effective. In addition, because of its activity as a master regulator in the inflammatory pathway, the B7-H4 compositions disclosed are particularly useful for treating chronic and persistent inflammation. In a preferred embodiment, the B7-H4 compositions described herein are used to treat relapsing and/or remitting multiple sclerosis.

C. Inhibition of Epitope Spreading

Epitope spreading refers to the ability of B and T cell immune response to diversify both at the level of specificity, from a single determinant to many sites on an auto antigen, and at the level of V gene usage (Monneaux, F. *et al.*, *Arthritis & Rheumatism*, 46(6): 1430-1438 (2002). Epitope spreading is not restricted to systemic autoimmune disease. It has been described in T cell dependent organ specific diseases such as IDDM and multiple sclerosis in humans and EAE induced experimental animals with a variety of myelin proteins.

Epitope spreading involves the acquired recognition of new epitopes in the same self molecule as well as epitopes residing in proteins that are associated in the same macromolecular complex. Epitope spreading can be assessed by measuring delayed-type hypersensitivity (DTH) responses, methods of which are known in the art.

One embodiment provides a method for inhibiting or reducing epitope spreading in a subject by administering to the subject an effective amount of B7-H4 polypeptide, fragment or fusion protein thereof. In a preferred embodiment the B7-H4 polypeptide, fragment or fusion protein thereof inhibits epitope spreading in individuals with multiple sclerosis. Preferably, the B7-H4 polypeptide or fusion thereof inhibits or blocks

multiple points of the inflammation pathway.

Yet another embodiment provides a method for inhibiting or reducing epitope spreading in subjects with multiple sclerosis by administering to a subject an effective amount of B7-H4 polypeptide, fragment or fusion protein thereof to inhibit or reduce differentiation of, proliferation of, activity of, and/or cytokine production and/or secretion by Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs. Another embodiment provides a method for treating multiple sclerosis by administering to a subject an effective amount of B7-H4 polypeptide, fragment or fusion protein thereof to interact with Tregs, enhance Treg activity, promote or enhances IL-10 secretion by Tregs, increase the number of Tregs, increase the suppressive capacity of Tregs, or combinations thereof.

D. Combination therapy

B7-H4 fusion polypeptides can be used alone or in combination with additional therapeutic agents. The additional therapeutic agents include, but are not limited to, immunosuppressive agents (e.g., antibodies against other lymphocyte surface markers (e.g., CD40, alpha-4 integrin) or against cytokines), other fusion proteins (e.g., CTLA-4-Ig (Orencia®), TNFR-Ig (Enbrel®)), TNF-α blockers such as Enbrel, Remicade, Cimzia and Humira, cyclophosphamide (CTX) (i.e. Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune™), methotrexate (MTX) (i.e. Rheumatrex®, Trexall®), belimumab (i.e. Benlysta®), or other immunosuppressive drugs (e.g., cyclosporin A, FK506-like compounds, rapamycin compounds, or steroids), anti-proliferatives, cytotoxic agents, or other compounds that may assist in immunosuppression.

In a preferred embodiment, the additional therapeutic agent functions to inhibit or reduce T cell activation through a separate pathway. In one such embodiment, the additional therapeutic agent is a CTLA-4 fusion protein, such as CTLA-4-Ig (abatacept). CTLA-4-Ig fusion proteins compete with the co-stimulatory receptor, CD28, on T cells for binding to CD80/CD86 (B7-1/B7-2) on antigen presenting cells, and thus function to inhibit T cell

activation. In another embodiment, the additional therapeutic agent is a CTLA-4-Ig fusion protein known as belatacept. Belatacept contains two amino acid substuitutions (L104E and A29Y) that markedly increase its avidity to CD86 *in vivo*. In another embodiment, the additional therapeutic agent is Maxy-4.

In another embodiment, the second therapeutic agent is cyclophosphamide (CTX). Cyclophosphamide (the generic name for Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune™), also known as cytophosphane, is a nitrogen mustard alkylating agent from the oxazophorines group. It is used to treat various types of cancer and some autoimmune disorders. In a preferred embodiment, B7-H4-Ig and CTX are coadministered in effective amount to prevent or treat a chronic autoimmune disease or disorder such as Systemic lupus erythematosus (SLE). Cyclophosphamide (CTX) is the primary drug used for diffuse proliferative glomerulonephritis in patients with renal lupus. As described in detail in Example 18 below, it has been discovered that a combination treatment with a low dose of cyclophosphamide (50 mg/kg, once every 2 weeks), the current treatment modality in humans, plus B7-H4-Ig resulted in prevention of lupus disease progression in the MRL/*lpr* lupus model. In some embodiments the combination therapy is administered in an effective amount to reduce the blood or serum levels of anti-double stranded DNA (anti-ds DNA) auto antibodies and/or to reduce proteinuria in a patient in need thereof.

In another embodiment, the second therapeutic agent increases the amount of adenosine in the serum, see, for example, WO 08/147482. In a preferred embodiment, the second therapeutic is CD73-Ig, recombinant CD73, or another agent (e.g. a cytokine or monoclonal antibody or small moelcule) that increases the expression of CD73, see for example WO 04/084933. In another embodiment the second therapeutic agent is Interferon-beta.

In another embodiment, the second therapeutic is Tysabri or another therapeutic for MS. In a preferred embodiment, B7-H4-Ig is cycled with Tysabri or used during a drug holiday in order to allow less frequent dosing

with the second therapeutic and reduce the risk of side effects such as PML and to prevent resistance to the second therapeutic.

In another embodiment, the second therapeutic agent preferentially treats chronic inflammation, whereby the treatment regimen targets both acute and chronic inflammation. In a preferred embodiment the second therapeutic is a TNF-α blocker.

In another embodiment, the second therapeutic agent is a small molecule that inhibits or reduces differentiation, proliferation, activity, and/or cytokine production and/or secretion by Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs. In another embodiment, the second therapeutic agent is a small molecule that interacts with Tregs, enhances Treg activity, promotes or enhances IL-10 secretion by Tregs, increases the number of Tregs, increases the suppressive capacity of Tregs, or combinations thereof.

Typically useful small molecules are organic molecules, preferably small organic compounds having a molecular weight of more than 100 and less than about 2,500 daltons, more preferably between 100 and 2000, more preferably between about 100 and about 1250, more preferably between about 100 and about 1000, more preferably between about 100 and about 750, more preferably between about 200 and about 500 daltons. Small molecules comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The small molecules often comprise cyclical carbon or heterocyclic structures andlor aromatic or polyaromatic structures substituted with one or more of the above functional groups. Small molecules also include biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. In one embodiment, the small molecule is retinoic acid or a derivative thereof. The examples below demonstrate that retinoic acid inhibits or reduces differentiation and/or activity of Th17 cells.

In a preferred embodiment, the compositions are used in combination or succession with compounds that increase Treg activity or production. Exemplary Treg enhancing agents include but are not limited to glucocorticoid fluticasone, salmeteroal, antibodies to IL-12, IFN-γ, and IL-4; vitamin D3, and dexamethasone, and combinations thereof. Antibodies to other proinflammatory molecules can also be used in combination or alternation with the disclosed B7-H4 polypeptides, fusion proteins, or fragments thereof. Preferred antibodies bind to IL-6, IL-23, IL-22 or IL-21.

As used herein the term "rapamycin compound" includes the neutral tricyclic compound rapamycin, rapamycin derivatives, rapamycin analogs, and other macrolide compounds which are thought to have the same mechanism of action as rapamycin (e.g., inhibition of cytokine function). The language "rapamycin compounds" includes compounds with structural similarity to rapamycin, e.g., compounds with a similar macrocyclic structure, which have been modified to enhance their therapeutic effectiveness. Exemplary Rapamycin compounds are known in the art (See, e.g. WO95122972, WO 95116691, WO 95104738, U.S. Patent No. 6,015,809; 5,989,591; U.S. Patent No. 5,567,709; 5,559,112; 5,530,006; 5,484,790; 5,385,908; 5,202,332; 5,162,333; 5,780,462; 5,120,727).

The language "FK506-like compounds" includes FK506, and FK506 derivatives and analogs, e.g., compounds with structural similarity to FK506, e.g., compounds with a similar macrocyclic structure which have been modified to enhance their therapeutic effectiveness. Examples of FK506-like compounds include, for example, those described in WO 00101385. Preferably, the language "rapamycin compound" as used herein does not include FK506-like compounds.

Other suitable therapeutics include, but are not limited to, anti-inflammatory agents. The anti-inflammatory agent can be non-steroidal, steroidal, or a combination thereof. One embodiment provides oral compositions containing about 1% (w/w) to about 5% (w/w), typically about 2.5 % (w/w) or an anti-inflammatory agent. Representative examples of non-steroidal anti-inflammatory agents include, without limitation, oxicams, such as piroxicam, isoxicam, tenoxicam, sudoxicam; salicylates, such as

aspirin, disalcid, benorylate, trilisate, safapryn, solprin, diflunisal, and fendosal; acetic acid derivatives, such as diclofenac, fenclofenac, indomethacin, sulindac, tolmetin, isoxepac, furofenac, tiopinac, zidometacin, acematacin, fentiazac, zomepirac, clindanac, oxepinac, felbinac, and ketorolac; fenamates, such as mefenamic, meclofenamic, flufenamic, niflumic, and tolfenamic acids; propionic acid derivatives, such as ibuprofen, naproxen, benoxaprofen, flurbiprofen, ketoprofen, fenoprofen, fenbufen, indopropfen, pirprofen, carprofen, oxaprozin, pranoprofen, miroprofen, tioxaprofen, suprofen, alminoprofen, and tiaprofenic; pyrazoles, such as phenylbutazone, oxyphenbutazone, feprazone, azapropazone, and trimethazone. Mixtures of these non-steroidal anti-inflammatory agents may also be employed.

Representative examples of steroidal anti-inflammatory drugs include, without limitation, corticosteroids such as hydrocortisone, hydroxyl-triamcinolone, alpha-methyl dexamethasone, dexamethasone-phosphate, beclomethasone dipropionates, clobetasol valerate, desonide, desoxymethasone, desoxycorticosterone acetate, dexamethasone, dichlorisone, diflorasone diacetate, diflucortolone valerate, fluadrenolone, fluclorolone acetonide, fludrocortisone, flumethasone pivalate, fluosinolone acetonide, fluocinonide, flucortine butylesters, fluocortolone, fluprednidene (fluprednylidene) acetate, flurandrenolone, halcinonide, hydrocortisone acetate, hydrocortisone butyrate, methylprednisolone, triamcinolone acetonide, cortisone, cortodoxone, flucetonide, fludrocortisone, difluorosone diacetate, fluradrenolone, fludrocortisone, diflurosone diacetate, fluradrenolone acetonide, medrysone, amcinafel, amcinafide, betamethasone and the balance of its esters, chloroprednisone, chlorprednisone acetate, clocortelone, clescinolone, dichlorisone, diflurprednate, flucloronide, flunisolide, fluoromethalone, fluperolone, fluprednisolone, hydrocortisone valerate, hydrocortisone cyclopentylpropionate, hydrocortamate, meprednisone, paramethasone, prednisolone, prednisone, beclomethasone dipropionate, triamcinolone, and mixtures thereof.

In another embodiment, the additional therapeutic agents include compositions that inhibit or interfere with sH4 activity, to treat inflammatory

disorders in subjects. In one embodiment, B7-H4 fusion polypeptides are administered to a subject for the treatment of an inflammatory disease wherein the subject has little or non-detectable amounts of sH4. In another embodiment, B7-H4 fusion polypeptides are administered to treat one or more symptoms of an inflammatory disease in subjects having elevated levels of sH4. Elevated levels of sH4 can be determined by comparing levels of sH4 is subjects known to have an inflammatory disorder with levels of sH4 in subjects that do not have an inflammatory disorder.

E. Pharmacodynamic Markers

The effectiveness of treatments using the B7-H4 polypeptides, fragments thereof, or fusion proteins thereof can be determined by assaying a sample obtained from a subject receiving treatment with B7-H4 polypeptides or fusion proteins thereof for changes in levels of biomarkers such as serum proteins, preferably pro-inflammatory cytokines, chemokines, acute phase markers, and/or antibodies, such as total IgG, or specific disease-related IgG, or other serum proteins for example sH4. For example, baseline levels of biomarkers in a serum sample obtained from a subject can be determined prior to treatment with B7-H4 polypeptides or fusion proteins. After or during treatment with B7-H4 polypeptides or fusion proteins thereof, biomarker levels in blood samples from the subject can be monitored. A change in biomarker level, for example a decline in cytokine levels, relative to baseline levels indicates that the treatment is effective in reducing one or more symptoms of an inflammatory disorder. Alternatively, the cytokine levels in blood samples from a subject undergoing treatment with B7-H4 polypeptides or fusion proteins thereof can be compared to predetermined levels of biomarkers determined from subjects without inflammatory disorders. In some embodiments the level of only one biomarker is monitored. In other embodiments, the levels of 2, 3, 4, 5 or more biomarkers are monitored.

The effectiveness of treatments using the B7-H4 polypeptides, fragments thereof, or fusion proteins can also be determined by assaying a sample obtained from a subject receiving treatment with B7-H4 polypeptides or fusion proteins thereof for changes in levels lymphocyte populations, such

as increased numbers of Treg, or decreased numbers of activated Th1 or Th17 cells compared to a control.

In some embodiments, the effectiveness of treatments using the B7-H4 polypeptides, fragaments thereof, or fusion proteins are determined by monitoring disease specific markers or symptoms, using methods known in the art. For example imaging can be employed to assess effectiveness of treatment for Multiple Sclerosis, or delayed-type hypersensitive (DTH) can be monitored to assess effectiveness of treatment for lupus.

The effectiveness of treatments using the B7-H4 polypeptides, fragments thereof, or fusion proteins thereof can also be determined by assaying a sample obtained from a subject receiving treatment with B7-H4 polypeptides or fusion proteins thereof for changes in the expression levels of genes, including, but not limited to, those encoding serum proteins, preferably pro-inflammatory cytokines and/or chemokines, as well as secreted factors, cell surface receptors, and transcription factors that are characteristic of Th1, Th17, and Treg cells. Methods of measuring gene expression are well known in the art and include, but are not limited to, quantitative RT-PCR and microarray analysis.

Exemplary markers that can be monitored to determine the effectiveness of treatment with B7-H4 polypeptides, fragments and fusion proteins thereof, can be found throughout the examples below, particularly in Figures 7, 8, 9, 57 and 58, and include, but are not limited to, one or more of IL-1β, TNF-α, TGF-beta, IFN-γ, IL-10, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs. Biomarkers particularly useful for monitoring arthritis are described in Example 3 and Figures 7, 8, and 9 below, and preferably include, but are not limited to, CRP, ET-1, IL-6, MCP-1, MCP-3, MIP-2 and TNF-α. Another marker useful for monitoring the effectiveness of treatment with B7-H4 polypeptides, fragments and fusions thereof, and combination therapies incorporating these proteins, is the level of CD73 in a tissue fluid of a patient, see for example WO 09/05352.

F. Patient Selection

The effectiveness of the B7-H4 polypeptides, fragments and fusion proteins thereof described herein can be predicted by pre-screening target

patients for levels of biomarkers, or gene expression as described above, or polymorphisms within the genes encoding downstream effector genes.

In a non-limiting example, patients that have elevated levels of one or more inflammatory cytokines or chemokines relative to a subject that does not have an inflammatory disorder can be selected for treatment with a B7-H4 polypeptide, fragment or fusion protein. Alternatively, patients that have a polymorphism in or more inflammatory cytokine or chemokine genes can be selected for treatment with a B7-H4 polypeptide or fusion protein. For example, patients with particular polymorphisms within the IL-10 gene may be expected to respond more or less well to treatment with B7-H4 compositions, depending on the nature of the polymorphism. Exemplary molecules and their respective genes that can be screened to determine if B7-H4 composition treatment will be effective include, but are not limited to, one or more of IL-1β, TNF-α, TGF-beta, IFN-γ, IL-10, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs. Another marker useful for selecting patients for treatment with B7-H4 polypeptides, fragments and fusions thereof, and combination therapies incorporating these proteins, is the level of CD73 in a tissue fluid of a patient. Inflammatory molecule levels can be measured by known methods including, but not limited to, quantitative RT-PCR and ELISA. Methods of identifying gene polymorphisms are well known in the art and include, but are not limited to, DNA sequencing and DNA microarrays.

Patients can also be monitored for the efficacy of a treatment with a B7-H4 polypeptide or fusion protein for an inflammatory disorder by screening the patients for levels of one or more inflammatory molecules during the course of treatment and increasing the amount of B7-H4 administered to the subject if the levels of one or more cytokines is elevated in the subject compared to levels in a control subject that does not have an inflammatory disorder, or decreasing the amount of B7-H4 administered to the subject if the levels of one or more cytokines is reduced in the subject compared to levels in a control subject that does not have an inflammatory disorder.

EXAMPLES

Example 1: B7-H4-Ig (murine) in the Collagen-Induced Arthritis (CIA) Prophylactic Model

Methods and Materials

CIA is a well-characterized mouse model for human RA, in which injection of collagen II (CII) into DBA/1J mice induces swelling and progressive inflammation in joints resulting in arthritis. As shown in Figure 2, DBA/1J mice (Jackson Labs) were administrated intraperitoneally (IP), 0.5 mg of B7-H4-Ig (20 mg/kg) on the same day as the CII immunization. B7-H4-Ig treatment continued 2 times each week, up to 6 weeks. On day 21, mice were rechallenged with CII emulsified in IFA. Figure 2 outlines a brief experimental design. Day 40 was the last B7-H4-Ig treatment. Mouse paws/joints were monitored 2 times every week starting on day 26 using the arthritis scoring system displayed on Table 1.

Table 1: Scoring system for subjective evaluation of arthritis severity.

Disease Score	Degree of Inflammation
0	No evidence of erythema and swelling
1	Erythema and mild swelling confined to the tarsals or ankle joint
2	Erythema and mild swelling extending from the ankle to the tarsals
3	Erythema and moderate swelling extending from the ankle to metatarsal joints
4	Erythema and severe swelling encompass the ankle, foot, and digits, or ankylosis.

Results

The overall arthritis scores of the B7-H4-Ig treated CIA mice were significantly lower than the scores for vehicle-injected CIA mice on days 33 and beyond as shown in Figure 3.

Example 2: Therapeutic CIA Model

Methods and Materials

Figure 4 outlines a brief experimental design of the CIA therapeutic study. DBA/1J mice were first immunized with chicken CII emulsified in CFA. Twenty one days later, the mice were rechallenged with CII emulsified in IFA and randomized into 2 groups. Group 1 (n=10) were IP injected with 300 μg of B7-H4-Ig, 3 times a week for 4 weeks. Group 2 (n=10) were injected with vehicle. Mouse paws/joints were monitored and scored 3 times a week.

Results

As shown in Figure 5, B7-H4-Ig could significantly suppress arthritis development. The overall arthritis scores of the B7-H4-Ig treated CIA mice were significantly lower than the ones of vehicle injected CIA mice on days 38 and beyond.

Example 3: Treatment of CIA using human B7-H4-Ig

Materials and Methods

Animals

DBA/1 mice (Taconic Farms, Inc. DBA1B0)

Ear tag (National Band 1005-1)

Electric clipper (Oster)

Induction of CIA

Hooke Kit™ Chicken Collagen/CFA Emulsion (Hooke Laboratories EK-0210)

Hooke Kit™ Chicken Collagen/IFA Emulsion (Hooke Laboratories EK-0211)

Treatment

B7-H4-Ig

RPA110010

Synagis® (palivizumab, MedImmune NDC# 60574-4112-1); used as a human IgG_1 isotype control

Murine B7-H4-Ig

Murine IgG_{2a} isotype control (BioXCell Cl.18)

Syringe, 3 mL with Luer-Lok tip (BD 309585)

Needle, 27 gauge (BD 305109)

Amplimmune formulation buffer (10 mM sodium phosphate, pH 7.5, 8% w/w sucrose, 0.01% polysorbate-80)

Serum Collection

Lancet, (Medipoint Goldenrod 4mm)

Microtainer® Serum Separator Tubes (BD 365956)

Induction of Arthritis

DBA/1 mice (10 per group) aged 7-9 weeks were used for the CIA model. Mice from several vendors (Jackson Laboratories, Harlan Laboratories, and Taconic Farms) were tested and it was determined that Taconic mice are most appropriate for the CIA studies. Taconic mice develop more consistent and severe disease symptoms and show continued disease progression, while mice from the other vendors often show stable, less severe disease, even in the absence of treatment. female (F) mice in AA#79 and male (M) mice in AA#80, were tested to determine which gender to use in future experiments.

On the day before the study is initiated, the hair on the right flank of each mouse was removed using an electric clipper. A metal identification tag was placed on the right ear of each mouse. On Day 0, mice were immunized with 100 μL of chicken collagen type II/Complete Freund's Adjuvant (CII/CFA) emulsion in the right flank. On Day 20, the hair on the left flank of each mouse was removed using an electric clipper, and on Day 21, mice were immunized with 100 μL of chicken collagen type II/Incomplete Freund's Adjuvant (CII/IFA) emulsion in the left flank. Pre-filled syringes of CII/CFA and CII/IFA emulsion purchased from Hooke Laboratories were used to ensure consistent dosing with and potency of the immunogen.

Disease Monitoring

Early arthritis symptoms, such as erythema and mild swelling, usually appear on Day 21, and on Day 28, more severe symptoms such as swelling in many digits and inflammation extending to the tarsal joint are typically present. Each limb is evaluated for severity of arthritis symptoms three times a week using a widely accepted arthritis severity score system,

shown in Table 2. The scores from each of the 4 limbs are summed to yield the disease score of each mouse.

Table 2 Scoring system for evaluation of arthritis severity in each limb

Disease Score	**Degree of Inflammation**
0	Normal paw with no evidence of erythema or swelling
1	Erythema of the paw
2	Erythema of the paw and mild swelling in one toe
3	Entire paw inflamed and swollen
4	Erythema and severe swelling encompass the ankle, foot, and digits, or ankylosis. If the paw is ankylosed, the mouse cannot grip the wire top of the cage

Treatment

A representative timeline for induction of disease and treatment in a therapeutic murine CIA model is shown in Figure 6. This treatment regimen is used for proof-of-concept studies. Regimens using fewer doses, less frequent administration, and/or lower dosages may also be effective. Treatment was initiated when the average disease score for the study animals was greater than four. This corresponded to Day 29 in AA#79 and Day 24 in AA#80. By waiting until animals are symptomatic, it was ensured that the model is performed in a therapeutic rather than prophylactic mode. Treatment groups are shown in Table 3.

Table 3 AA#79 and AA#80 treatment groups.

Group	# Mice	Treatment	Dosing Regimen	Dosing Level
A	10	Vehicle	3× weekly, 8 doses	
B	10	Murine IgG2a control	1× weekly, 3 doses	20 mg/kg
C	10	Murine B7-H4-Ig	3× weekly, 8 doses	20 mg/kg
D	10	Synagis® (human IgG1 control)	1× weekly, 3 doses	20 mg/kg
E	10	B7-H4-Ig	3× weekly, 8 doses	20 mg/kg
F	10	RPA110010	3× weekly, 8 doses	20 mg/kg

B7-H4-Ig, RPA110010, and murine B7-H4-Ig proteins were administered by intraperitoneal (IP) injection 3 times a week for a total of 8 doses. RPA110010 is an extracellular domain variant of B7-H4-Ig, SEQ ID NO:126. Control murine and human IgG proteins were given once a week for a total of 3 doses. B7-H4-Ig is cleared from circulation more rapidly than control IgG, so different dosing schedules are used to compensate for this difference. All proteins were diluted to the desired concentration (500 μg in 500 μL, or 1 mg/mL) with sterile PBS immediately before injection. Vehicle control mice receive Amplimmune formulation buffer diluted 1:10 in PBS, with an injection volume of 500 μL.

Serum Biomarker Analysis

In AA#80, serum samples were collected on Day 27, Day 34, Day 41 and Day 55 via the submandibular vein. Approximately 200 μL of blood per mouse was collected in Microtainer® serum separator tubes. Serum was removed following centrifugation and stored at <-65 °C until analysis.

Selected serum samples were sent to Rules-Based Medicine (Austin, TX) for quantitative immunoassay multi-analyte profiling of serum samples,

with the goal of identifying biomarkers that could be used to monitor disease progression and response to B7-H4-Ig treatment. Day 27, Day 34, and Day 41 sera from the three representative mice in the B7-H4-Ig group (tag # 3332, 3334, and 3338) and three representative mice in the Synagis® group (tag # 3324, 3329, and 3330) were analyzed for levels of 58 analytes using the RodentMAP v2.9 Testing Service.

Data from the Rules-Based Medicine analysis was analyzed using Microsoft Excel and GraphPad Prism software. T-tests were performed to compare the levels of each analyte in the B7-H4-Ig versus Synagis® treated samples. No correction was made for multiple comparisons. Additionally, correlation coefficients were calculated for each analyte versus disease score.

Results

Efficacy of B7-H4-Ig in the CIA Model

As shown in Figures 7 and 8 and Tables 4A, 4B, 5A, 5B, 6, and 7, in both AA#79 and AA#80, mice treated with B7-H4-Ig and murine B7-H4-Ig had lower disease scores than mice in the control groups, consistent with the results of earlier studies. B7-H4-Ig shows superior efficacy in both studies, and in AA#79 disease stabilization in the B7-H4-Ig group is sustained after cessation of treatment. The RPA110010 variant was not effective in either study.

Table 4A: AA#79 CIA disease scores

		Study Day																				
	Tag#	12	18	22	25	27	29	32	34	36	39	41	43	46	48	50	54	57
A:Vehicle	3041	0	0	2	4	4	6	7	7	9	11	13	15	15	14	14	13	15
	3042	0	2	3	2	4	6	7	8	9	10	15	14	15	15	15	13	12
	3043	0	0	2	2	6	8	12	13	13	13	13	12	15	14	15	16	14
	3044	1	3	3	4	4	4	10	10	14	11	13	12	12	13	11	12	11
	3045	1	2	3	3	4	6	6	7	7	6	10	11	10	13	12	12	13
	3046	1	2	2	3	4	6	7	7	8	10	10	10	10	10	8	11	13
	3047	0	3	4	4	5	6	5	6	6	5	6	6	8	7	6	6	8
	3048	0	1	2	3	4	7	8	6	8	7	6	10	11	9	8	11	11
	3049	1	4	4	4	6	8	10	9	11	14	6	14	14	14	13	13	13
	3050	0	2	2	4	4	6	8	10	12	12	6	12	14	13	14	14	14
B: Murine IgG Control	3051	0	2	2	4	4	6	7	7	7	8	6	9	10	9	10	11	11
	3052	0	2	3	4	4	7	6	6	6	5	6	6	8	8	6	6	9
	3053	0	2	2	3	7	9	10	12	11	11	6	12	14	15	14	14	13
	3054	0	1	2	4	4	6	12	12	11	12	6	13	12	12	13	15	13
	3055	1	0	3	4	6	7	8	7	7	8	6	7	9	9	8	10	11
	3056	0	2	3	4	3	7	8	8	8	7	6	12	12	11	13	13	12
	3057	1	1	2	4	4	6	7	9	8	8	6	9	9	10	9	9	10
	3058	0	0	2	3	5	7	10	10	10	11	6	14	15	15	15	14	13
	3059	0	2	3	3	4	5	7	6	7	6	6	8	10	12	15	12	13
	3060	0	2	3	4	6	7	9	12	12	10	6	13	14	12	16	16	15

Table 4B: AA#79 CIA disease scores

C: Murine B7-H4-Ig

	Study Day																					
Tag#	12	18	22	25	27	29	32	34	36	39	41	43	46	48	50	54	57
3061	1	2	4	4	5	5	5	2	2	5	6	6	6	6	5	4	8
3062	0	2	2	3	4	5	5	3	6	7	6	9	12	13	12	11	13
3063	2	3	3	4	6	7	3	2	3	7	6	11	14	13	14	14	12
3064	0	2	2	3	4	7	8	8	7	8	6	13	15	14	12	14	13
3065	1	2	2	3	4	5	3	2	2	2	6	9	9	10	9	9	10
3066	0	2	4	3	4	7	9	10	9	9	6	11	15	14	14	14	14
3067	0	1	2	4	4	6	6	3	2	1	6	4	6	10	11	10	10
3068	1	0	2	4	3	6	5	3	1	4	6	8	12	12	14	15	15
3069	0	3	2	3	5	6	3	4	2	6	6	10	12	13	12	10	10
3070	1	2	3	4	4	5	8	8	8	8	6	9	12	14	15	15	13

Table 5A: AA#79 CIA disease scores

		Study Day																			
	Tag#	12	18	22	25	27	29	32	34	36	39	41	43	46	48	50	54	57
D: Syngis	3071	1	0	3	2	4	5	6	6	7	9	10	8	9	9	7	8	8
	3072	0	0	3	2	3	5	7	7	9	9	9	9	8	9	11	11	13
	3073	2	1	2	2	4	6	6	7	8	12	11	11	15	14	14	14	15
	3074	0	1	2	3	5	5	5	5	5	6	7	6	9	5	4	6	6
	3075	0	2	4	2	5	6	10	10	11	11	12	12	14	13	11	13	13
	3076	1	2	2	5	7	6	9	9	9	8	10	11	11	11	11	12	14
	3077	2	1	2	2	4	5	9	10	10	11	13	11	12	13	12	12	13
	3078	1	1	2	2	4	4	7	6	8	6	6	6	6	5	9	8	7
	3079	1	2	2	3	5	6	8	9	9	9	10	10	10	12	12	13	12
	3080	0	2	3	2	6	10	10	11	11	12	11	12	14	13	12	12	15
E: Human B7-H4-Ig	3081	1	2	2	2	3	6	5	4	4	5	5	3	4	5	6	3	5
	3082	0	2	2	2	5	5	4	2	5	12	12	10	9	10	9	10	11
	3083	1	1	2	3	4	4	4	4	1	6	7	6	10	10	9	10	10
	3084	0	1	2	2	3	4	5	5	6	9	10	9	11	10	10	13	10
	3085	0	1	2	3	4	5	2	5	3	3	5	3	3	6	6	5	5
	3086	0	0	1	4	4	7	9	12	12	11	10	10	10	11	12	13	12
	3087	1	1	1	3	5	6	6	5	3	1	6	6	5	5	4	4	6
	3088	2	3	2	2	3	5	4	4	2	2	3	4	3	2	3	2	5
	3089	2	2	2	3	4	5	6	5	3	5	7	8	10	7	8	7	7
	3090	1	1	2	3	5	4	4	3	1	2	7	7	9	5	5	8	8

F: RPA110010

Table 5B: AA#79 CIA disease scores

	Study Day																					
Tag#	12	18	22	25	27	29	32	34	36	39	41	43	46	48	50	54	57
3091	0	2	2	4	4	6	6	5	6	7	6	7	10	8	10	7	9
3092	1	1	3	4	5	5	6	6	5	6	9	11	12	10	12	10	12
3093	0	2	2	3	4	5	6	6	5	9	8	7	12	8	9	11	10
3094	1	1	3	3	5	8	10	11	10	11	11	10	14	12	12	13	12
3095	1	1	2	4	4	7	9	11	12	13	13	14	14	14	14	13	16
3096	0	2	4	3	4	5	4	6	7	7	7	4	11	13	13	12	12
3097	0	2	2	2	4	5	4	3	6	4	4	3	9	5	3	5	6
3098	2	3	3	3	5	4	5	5	7	6	8	5	8	10	11	12	13
3099	1	1	3	3	5	5	5	6	7	6	8	9	9	10	11	11	12
3100	1	1	2	4	4	5	9	12	12	15	16	16	16	16	16	16	14

Table 6: AA#80 CIA disease scores

		Study Day										
	Tag #	20	22	24	27	29	31	34	36	38	41	44
A: Vehicle	3291	4	3	5	5	7	10	12	14	14	14	13
	3292	2	2	4	3	5	4	9	10	9	10	11
	3293	7	5	7	6	8	6	8	10	10	10	10
	3294	3	3	4	5	5	6	6	6	6	5	6
	3295	5	4	5	6	7	8	11	11	11	14	12
	3296	4	5	7	6	7	9	9	10	12	12	13
	3297	6	5	7	9	12	11	12	11	13	12	11
	3298	3	4	5	6	7	8	7	7	9	8	9
	3299	3	3	3	6	6	7	8	7	10	9	12
	3300	1	3	2	5	6	6	7	7	7	9	9
B: Murine IgG Control	3301	5	5	6	5	8	11	13	15	15	16	16
	3302	5	2	5	5	5	5	6	8	10	10	11
	3303	6	4	5	6	9	10	9	10	10	10	10
	3304	7	4	6	7	7	7	10	10	10	9	10
	3305	3	3	6	6	6	7	9	11	12	12	12
	3306	1	4	5	6	5	7	6	7	8	9	9
	3307	4	2	5	7	11	12	13	15	16	16	15
	3308	2	1	6	6	6	5	6	7	9	13	13
	3309	4	3	3	7	4	6	5	7	6	9	8
	3310	5	4	5	5	6	6	8	7	7	10	8
C: Murine B7-H4-Ig	3311	3	4	5	3	3	9	12	11	12	12	11
	3312	3	6	4	3	3	1	3	3	8	9	9
	3313	3	5	6	4	5	5	6	6	9	8	4
	3314	8	8	10	7	7	8	10	11	13	11	12
	3315	2	3	3	2	1	3	2	6	8	6	8
	3316	3	5	8	3	4	7	6	8	8	9	7
	3317	4	6	6	7	7	7	11	12	12	13	12
	3318	1	3	5	3	4	4	1	1	7	12	11
	3319	0	2	2	2	2	1	1	0	9	10	7
	3320	3	5	6	4	4	7	8	7	9	9	8

Table 7: AA#80 CIA disease scores

		Study Day										
	Tag #	20	22	24	27	29	31	34	36	38	41	44
D: Syngis	3321	2	5	3	7	4	8	8	11	10	14	12
	3322	4	4	5	6	7	8	7	8	7	10	7
	3323	6	8	8	8	11	11	11	13	12	13	13
	3324	4	4	5	2	4	7	10	11	10	12	12
	3325	4	5	5	6	6	8	10	10	11	12	10
	3326	1	2	6	4	5	5	7	8	11	13	14
	3327	2	4	5	6	5	6	8	7	6	7	7
	3328	5	5	6	6	5	6	6	8	8	8	8
	3329	3	2	5	4	6	3	8	8	6	9	7
	3330	4	5	6	5	5	6	8	8	7	10	11
E: Human B7-H4-Ig	3331	4	4	4	3	2	2	2	3	3	4	3
	3332	2	4	5	2	2	3	5	7	7	9	11
	3333	2	4	2	2	3	2	1	4	9	9	11
	3334	3	4	5	3	2	2	4	5	5	6	8
	3335	3	3	4	2	1	1	2	6	3	9	9
	3336	4	5	5	3	5	4	5	7	6	10	12
	3337	5	5	4	4	5	4	6	7	10	13	14
	3338	5	5	5	2	2	5	4	4	3	9	4
	3339	2	5	5	2	2	2	5	4	3	9	11
	3340	3	2	6	3	7	7	8	11	12	13	13
F: PRA110010	3341	6	5	5	6	5	7	9	12	10	12	12
	3342	6	6	7	7	6	11	13	12	12	12	12
	3343	3	4	5	4	5	10	13	12	13	13	13
	3344	4	4	5	5	7	12	16	16	16	16	16
	3345	4	3	5	3	2	4	6	4	4	7	9
	3346	1	3	5	3	2	2	3	5	9	11	12
	3347	4	5	5	5	4	7	5	7	11	13	12
	3348	3	3	5	5	5	6	5	7	6	9	7
	3349	3	4	4	2	2	4	11	11	11	11	14
	3350	1	3	5	5	9	9	8	9	7	8	8

By Day 21 following CII immunization, most animals had begun to develop inflammatory symptoms and the severity of disease continued to progress through Day 45. B7-H4-Ig treated mice show a prolonged period of stable disease scores, and in AA#79 this effect is maintained after treatment is stopped. Disease scores are transiently stabilized in the murine B7-H4-Ig treated animals during treatment (Day 29- Day 46 in AA#79 and Day 24-Day 41 in AA#80) but quickly rebound. Disease progression in the control treatment groups and RPA110010 all display a similar profile.

Serum Biomarker Analysis

Serum levels of several proteins were found to correlate with disease progression and/or differ between B7-H4-Ig and Synagis® treated mice, as shown in Figures 9, 10, and 11, and described below. Figures 9 and 10 show the Rules Based Medicine complete data set. Figure 11 shows the Rules Based Medicine data analysis summary. Analytes are shaded if there is a potentially significant difference (p less than 0.05) between Synagis® and B7-H4-Ig treated groups at Day 27, Day 34, Day 41, or overall. Analytes are also shaded if the correlation coefficient between the analyte and the clinical score is less than 0.3. These markers may serve as objective measures of disease severity and/or biomarkers for response to B7-H4-Ig treatment.

C-Reactive Protein (CRP)

CRP protein levels correlate with clinical score, as shown in Figure 12, however CRP levels are not significantly different between the two groups. CRP is a commonly used clinical biomarker for inflammation and is well accepted as a marker for RA disease activity. High concentrations of CRP predict joint erosion. CRP has been used as a biomarker in a rhesus CIA model, but is not commonly used as a biomarker in mouse. SAP, described below, rather than CRP is considered the dominant acute phase protein in mouse.

Endothelin 1 (ET-1)

ET-1 levels are significantly lower in B7-H4-Ig treated mice at Day 41 and overall, as shown in Figure 13. Endothelins act as potent vasoconstrictors. Elevated plasma levels have been reported in RA patients. ET-1 may also play a role in recruiting neutrophils to the synovium.

Glutathione S-Transferase alpha (GST-α)

GST-α levels are undetectable in 7/9 serum samples from Synagis®-treated mice, but detectable in 5/9 serum samples from B7-H4-Ig treated mice, including 2/3 Day 34 samples and 3/3 Day 41 samples. GST-α is involved in the detoxification of small molecules and elevated levels can indicate liver toxicity. In all cases the levels detected were only slightly above the limit of detection.

Interleukin-6 (IL-6)

IL-6 levels increase with disease score, as shown in Figure 14. The highest levels are all in the Synagis® treated group, but the difference between Synagis® and B7-H4-Ig groups did not reach statistical significance in this study. An earlier study showed that treatment with murine B7-H4-Ig decreases IL-6 levels in the CIA model.

Growth-related alpha protein (Gro- α)

Levels of Gro-α (also called chemokine (C-X-C motif) ligand 1, CXCL1) increase with disease score. The highest levels are all in the Synagis® treated group, but this does not reach statistical significance in this study. Gro-α is involved in neutrophil chemotaxis and activation.

Monocyte Chemotactic Protein-1 (MCP-1)

Levels of MCP-1 (also called chemokine (C-C motif) ligand 2, CCL2) are significantly lower in B7-H4-Ig treated mice at Day 41 and overall, as shown in Figure 15. MCP-1 recruits monocytes, memory T cells, and dendritic cells to sites of injury or inflammation. MCP-1 promotes macrophage recruitment to the joints in RA, perpetuating inflammation. An earlier study showed that murine B7-H4-Ig also decreases MCP-1 levels in the CIA model.

Monocyte Chemotactic Protein-3 (MCP-3)

Levels of MCP-3 (also called chemokine (C-C motif) ligand 7, CCL7) increase with disease score, and are significantly lower in B7-H4-Ig treated mice at Day 41 and overall, as shown in Figures 16 and 17. MCP-3 is 74% identical to MCP-1. MCP-3 is a monocyte and T cell chemoattractant.

Macrophage Inflammatory Protein-2 (MIP-2)

MIP-2 (also called chemokine (C-X-C motif) ligand 14, CXCL14) levels increase with disease score. Levels are significantly lower in B7-H4-Ig treated mice than Synagis® treated mice at Day 41, as shown in Figure 18. MIP-2 is potent chemoattractant for neutrophils.

Serum amyloid protein (SAP)

SAP levels increase with disease score, but not significantly different between B7-H4-Ig and Synagis treated groups. SAP is considered the major acute phase protein in mice and a general marker of inflammation.

Tumor Necrosis Factor Alpha (TNF-α)

Levels are above the limit of detection in 5/9 serum samples from Synagis® treated mice, but none of the sera from B7-H4-Ig treated mice, as shown in Figure 19. TNF-α is a key mediator of RA disease activity. An earlier study showed that treatment with murine B7-H4-Ig decreases TNF-α levels in the CIA model.

In the data presented in Example 3, B7-H4-Ig shows strong efficacy in murine models of CIA, and is able to stabilize disease scores compared to vehicle and control IgG treated animals. B7-H4-Ig appears to be more potent than murine B7-H4-Ig in female and male DBA/1 mice, and induced a long term protective effect in the female mice. The course of disease is slightly different in the male and female mice. Human B7-H4-Ig is more potent than its murine analog in the CIA model, while RPA110010 was not active in AA#79 or AA#80. In the context of the murine T_H17 differentiation assay, B7-H4-Ig and RPA110010 have similar activity and murine B7-H4-Ig is more potent. It is possible that the pharmacokinetic properties of these three molecules differ. Further studies will be performed to assess the relative activity of these molecules *in vitro* and *in vivo*.

Serum protein analysis performed by Rules-Based Medicine confirmed earlier studies and provided new readouts for CIA model disease progression and response to B7-H4-Ig treatment. The most promising markers include CRP, ET-1, IL-6, MCP-1, MCP-3, MIP-2 and TNF-α. The results of the serum biomarker study, as well as previously published results suggest that B7-H4-Ig may affect neutrophil maturation and migration

into the synovium (Zhu,G. *et al., Blood* 113, 1759-1767 (2009), Azuma,T. *et al., PLoS. Med.* 6, e1000166 (2009)).

Example 4: B7-H4-Ig Reduces Proinflammatory molecules

Methods and Materials

Plasma was collected on day 33 from mice treated as described in Example 3 and analyzed for proinflammatory molecules, e.g. TNFα, IL6, and chemokine (MCP-1) using the BD™ Cytometric Bead Array (CBA) Flex Sets.

Results

Data presented in Figures 20A, 20B, and 20C, respectively, demonstrate B7-H4-Ig significantly reduced TNFα, IL6 and MCP-1 production in the treated CIA mice.

Example 5: B7-H4-Ig affects cytokine production and T cell differentiation

Materials and Methods

Mice

BALB/c mice, DO11.10, and C57BL/6 mice were purchased from Jackson Laboratory. SJL mice were purchased from Harlan. All mice were maintained according to NIH guidelines. Mice used in the study were between 6 and 9 weeks of age.

Other reagents

Mouse Control IgG: Rockland, #010-0102; $CD4^+$ T cell negative isolation kit: Miltenyi, #130-090-860; $CD62L^+$ positive selection magnetic beads: Miltenyi, **#130-049-701; $CD25^+$ T cell depletion:** anti-mCD25-PE: Miltenyi, #120-000-900, and anti-PE magnetic beads, Miltenyi: #120-000-294 ; Dynabeads® Mouse CD3/CD28 T Cell Expander beads: Invitrogen, #11452D; Mouse Cytokine Kit: Millipore, MPXMCYTO-70K; 96-well flat bottom: Costar, #3596; β-mercaptoethanol: Invitrogen, #21985-023; HL-1 media: Lonza #344017; $OVA_{323-339}$: ISQAVHAAHAEINEAGR (SEQ ID NO:138) ; $PLP_{130-151}$: HSLGKWLGHPDKF (SEQ ID NO: 139); $PLP_{130-151}$: NTWTTCQSIAFPSK (SEQ ID NO:140); MOG_{35-55}: MEVGWYRSPFSRVVHLYRNGK (SEQ ID NO:141); were synthesized; MILLIPLEX™MAP: Millipore

Cytokines and Antibodies

Table 8 lists detail information on cytokines and antibodies used for *in vitro* T helper cell polarization.

Table 8 Cytokines and antibodies for *in vitro* T helper cell polarization

	Reagent	Vendor (Cat#)	Desired (per well)	100x Stock
Th1 cells	rIL-2 (human)	NCI	1.066 ng/mL (128 U/mL)	106 ng/mL (12800 U/mL)
	rIL-12	eBioscience (14-8121)	4 ng/mL	400 ng/mL
	anti-IL-4 (11B11)	eBioscience (16-7041)	1 μg/mL	100 μg/mL
Th17 cells	rTGF-β human TGF-β_1	R&D Systems (240-B-010)	10 ng/mL	1000 ng/mL
	IL-6	eBioscience (14-8061)	50 ng/mL	5000 ng/mL
	IL-23	eBioscience (14-8231)	4 ng/mL	400 ng/mL
	anti-IL-4 (11B11)	eBioscience (16-7041)	1 μg/mL	100 μg/mL
	anti-IFN-γ	eBioscience (16-7311)	1 μg/mL	100 μg/mL
	anti-IL-2	eBioscience (16-7021)	1 μg/mL	100 μg/mL

Isolation of $CD4^+CD62L^+$ naïve T cells

Mouse splenocytes were first removed isolated from DO11.10 mice, which express an MHC class II restricted T cell receptor specific for $OVA_{323-339}$. Mouse $CD4^+$ T cells were purified using a Miltenyi $CD4^+$ T cell negative isolation kit (Cat#130-090-860). Naïve $CD4^+$ T cells were further purified using the Miltenyi anti-CD62L positive selection magnetic beads (cat#130-049-701).

In vitro Th1 polarization

$CD4^+CD62L^+$ naïve T cells with or without $CD25^+$ T cells were cultured in serum free HL-1 media in the presence of rIL-2 (1 ng/mL), rIL-12 (4 ng/mL) and anti-IL-4 (1 μg/mL). Activity of murine B7-H4-Ig was tested in a number of formats (soluble and insoluble presentation of B7-H4-

Ig). Murine B7-H4-Ig was also assessed for activity on T cells activated non-specifically or with antigen-specific stimulation. To provide murine B7-H4-Ig in an immobilized format, 96-well flat bottom plates were first coated with murine B7-H4-Ig, 100 µl per well at 1 µg/mL, and incubated at 37°C for 2 hours. To provide murine B7-H4-Ig in soluble form, murine B7-H4-Ig was added to the tissue culture at 1 µg/mL or as indicated in the brief describes of the drawings and on the figures for dose dependent studies. For non-specific antigen activation, Mouse CD3/CD28 T Cell Expander beads (Dynabeads®; Invitrogen, Cat#11452D) were added into each well, at a 1:1 cell to bead ratio. For OVA specific activation, splenocytes were first isolated from Balb/C mice as antigen presenting cells (APC) followed by irradiation at 3000 rads for 45 minutes and then added into each well, at a 1:1, APC to responder cell, ratio. $OVA_{323-339}$ peptide was added into the culture at 20 µg/mL.

In vitro Th17 polarization

$CD4^+$ $CD62L^+$ naïve T cells with or without $CD25^+$ T cells were cultured in serum free HL-1 media in the presence of rTGF-β (10 ng/mL), IL-6 (50 ng/mL), IL-23 (4 ng/mL), anti-IL-4 (1 µg/mL), anti-IFN-γ (1 µg/mL) and anti-IL-2 (1 µg/mL). To provide murine B7-H4-Ig in an immobilized format, 96-well flat bottom plates were first coated with murine B7-H4-Ig, 100 µl per well at 1 µg/mL, and incubated at 37°C for 2 hours. To provide murine B7-H4-Ig in soluble form, murine B7-H4-Ig was added to the tissue culture at 1 µg/mL or as indicated in the brief description of the drawings and on the figures for dose dependent studies. For non-specific antigen activation, Mouse CD3/CD28 T Cell Expander beads (Dynabeads®; Invitrogen, Cat#11452D) was added into each well, at a 1:1 cell to bead ratio. For OVA specific activation, splenocytes were first isolated from Balb/C mice as antigen presenting cells (APC) followed by irradiation at 3000 rads for 45 minutes and then added into each well, at a 1:1, APC to responder cell, ratio. $OVA_{323-339}$ peptide was added into the culture at 20 µg/mL.

Proliferation Analysis

$[^3H]$-Thymidine (1 µCi/well) was added into each well 24 hr post co-incubation. Proliferation was determined by uptake of $[^3H]$-thymidine

detected at 48 hr post [^{3}H]-thymidine addition using a Topcount Microplate Scintillation Counter (Packard Instruments, Meridan, CT). Results are expressed as the mean of triplicate cultures ± SEM.

Cytokine Analysis

Supernatants were collected from plates without [^{3}H]-Thymidine at 72 hr for cytokine analysis. Cytokine measurements were performed using the Mouse Cytokine 10-Plex system (Millipore) and Luminex Liquidchip analyzer (Qiagen, Valencia, CA) or ELISA.

Results

To demonstrate murine B7-H4-Ig bioactivity *in vitro*, B7-H4-Ig was added in the T cell polarization culture. The ability of B7-H4-Ig to inhibit T cell proliferation was determined by [^{3}H]-thymidine incorporation and cytokine production via MILLIPLEX™MAP. In addition, the interaction between B7-H4-Ig and Treg cells was evaluated by comparing the T cell polarization culture outcome in the presence or absence of $CD4^+CD25^+$ Treg cells.

B7-H4-Ig treatment alters $CD4^+$ T cell activation and differentiation under both Th1 cell- and Th17 cell *in vitro* polarization culture conditions. Mouse $CD4^+CD62L^+$ T cells were first isolated from DO11.10 mice using Miltenyi kits. As shown in Figure 21, naïve $CD4^+CD62L^+$ T cells activated in the presence of Th1 cell-promoting conditions *in vitro* (with IL2, IL-12 and anti-IL4) in the presence of murine B7-H4-Ig significantly inhibited mouse $CD4^+CD62L^+$ naïve T cell proliferation after stimulation with either Dynabeads® Mouse CD3/CD28 T Cell Expander beads (anti-CD3+anti-CD28) for non-specific stimulation, and also when naïve $CD4^+CD62L^+$ T cells were activated in the presence of $OVA_{323-339}$ pulsed APC (APC/OVA). Similar results were obtained when the $CD4^+$ T cells were activated under both bead and APC activating conditions. Additionally, Figure 22 shows that murine B7-H4-Ig significantly decreased the level of IFN-γ produced from mouse $CD4^+CD62L^+$ naïve T cells under these same conditions. In all cases similar results were seen whether B7-H4-Ig was added in solution (Sol) or bound (Plate) to plates.

It is believed that both MS and EAE are Th1 cell/Th17 cell-mediated, therefore, it was next determined if B7-H4-Ig was able to inhibit naïve

$CD4^+CD62L^+$ T cell differentiation when activated in the presence of Th17 cell-promoting *in vitro* culture conditions. As shown in Figure 23, naïve $CD4^+CD62L^+$ T cells were activated in the presence of Th17 cell-promoting conditions *in vitro* (with rTGF-β, IL-6, IL-23, anti-IL-4, anti-IFN-γ and anti-IL-2). Murine B7-H4-Ig significantly inhibited mouse $CD4^+CD62L^+$ naïve T cell proliferation after stimulation with either Dynabeads® Mouse CD3/CD28 T Cell Expander beads (anti-CD3+anti-CD28) for non-specific stimulation, or when naïve $CD4^+CD62L^+$ T cells were activated in the presence of $OVA_{323-339}$ pulsed APC (APC/OVA). Similar results were obtained when the $CD4^+$ T cells were activated under both bead and APC activating conditions. Additionally, Figure 24 and 25, respectively, show that murine B7-H4-Ig significantly decreased the level of IL-17 and TNF-α produced from mouse $CD4^+CD62L^+$ naïve T cells induced under these same conditions Similar results were seen when B7-H4-Ig was added in solution (Sol) or bound (Plate) to plates.

The above *in vitro* Th1/Th17 assay was repeated 3 times using B7-H4-Ig from the same batch, which consistently demonstrated that B7-H4-Ig inhibited Th1/Th17 cell proliferation and IFN-γ and IL17 production. B7-H4-Ig has no impact on Th2 cells using the same target, naïve $CD4^+CD62L^+$ T cells, under Th2 *in vitro* polarization conditions: IL-2, IL-4, anti-IL12 and anti-IFN-γ (Figure 26).

Identical *in vitro* bioactivity of murine B7-H4-Ig from different batches has been demonstrated. As shown in Figures 27 and 28, B7-H4-Ig from Lot#22 and Lot#23 resulted in similar inhibition of IFN-γ and IL-17 production in a dose dependent manner under the Th1 and Th17 *in vitro* polarization conditions, respectively. This data not only proves consistency of the Th1/Th17 assay but also consistency of the B7-H4-Ig production process.

Example 6: Human B7-H4-Ig inhibits mouse Th17 Cells

Materials and Methods

The human and mouse B7-H4 proteins are 95% homologous. Human B7-H4-Ig was tested for cross-reactivity with murine T cells. Naïve $CD4^+$ T cells were isolated as described above. Upon purification, murine naïve

$CD4^+$ T cells were polarized in the presence of IL-2, IL-12 and anti-IL4 for Th1 cell-promoting conditions, or TGF-β, IL-6, IL-23, anti-IL4, anti-IFNγ and anti-IL-2 for Th17 cell-promoting conditions, as described in Example 5 for murine B7-H4-Ig *in vitro* bioactivity. Human B7-H4-Ig was directly added into the culture at 0, 0.1, 1 or 10 μg/mL. Human Control IgG1 (Synagis®) was added into the culture to bring the final protein concentration to 10 μg/mL.

Results

Data presented in Figures 29, 30, and 31 show human B7-H4-Ig cross reacted with mouse naïve $CD4^+$ T cells and blocked murine Th1/Th17 proliferation (Figure 29) in a dose dependent manner, which correlated with reduced IFN-γ production in Th1 cells (Figure 30) and IL-17 production in Th17 cells (Figure 31). The same results were obtained when using human B7-H4-Ig from a different batch.

Example 7: B7-H4-Ig affects Tregs

Methods and Materials

Depletion of $CD4^+CD25^+$ T cells

$CD4^+CD25^+$ Treg cells were depleted using anti-mCD25-PE (Miltenyi Cat#120-000-900) and anti-PE magnetic beads (Miltenyi Cat#120-000-294) prior to the CD62L positive selection. After depletion of $CD4^+CD25^+$ T cells, $CD25^+/P3^+$ cells were decreased from approximately 5% to 1%.

Results

$CD4^+CD25^+$ Treg cells were optionally depleted (-$CD25^+$ T cells) from DO11.10 mouse $CD4^+CD62L^+$ naïve T cell population prior to *in vitro* Th_1 polarization in the presence of $OVA_{323-339}$ peptide pulsed APC and B7-H4-Ig.

It was found that the extent to which B7-H4-Ig decreased the level of naïve $CD4^+CD62L^+$ T cell proliferation and cytokine production when activated in the presence of either Th1 cell- or Th17 cell-promoting conditions was correlated to the age of the mouse from which the naïve $CD4^+CD62L^+$ T cells were isolated. Initial selection for naïve $CD4^+CD62L^+$ T cells was based upon negative selection for $CD4^+$ T cells followed by positive selection for $CD62L^+$ cells via use of the AutoMax. Since the

number of Treg cells present within a mouse increases with age and Treg cells are $CD4^+CD62L^+CD25^+$, it was next determined if depletion of $CD25^+$ cells, *i.e.*, Treg and activated $CD4^+$ T cells, during the $CD4^+$ T cell negative selection would alter the ability of B7-H4-Ig to inhibit naïve $CD4^+CD62L^+$ T cell production of IFN-γ when activated in the presence of Th1 cell-promoting conditions.

$CD4^+CD62L^+$ naïve T cells were first isolated from DO11.10 mice and polarized to Th1 cells in the presence of rIL-2 (1 ng/mL), rIL-12 (4 ng/mL) and anti-IL-4 (1 μg/mL), and stimulated with $OVA_{323-339}$ pulsed APC (APC/OVA). Murine B7-H4-Ig or Control IgG at various doses was added directly to the culture. As shown in Figure 32A, depletion of Treg (open circle) resulted in higher IFN-γ production as compared to IFN-γ levels when Treg cells were present (solid circle). The B7-H4-Ig mediated decrease in IFN-γ production was dose dependent (open triangles), and the inhibition was more profound in the presence of $CD4^+CD25^+$ Treg cells (solid triangle). Conversely, B7-H4-Ig increased IL-10 production in a dose dependent manner (solid triangles – Figure 32B).

The effect of depletion of $CD4^+CD25^+$ Treg cells (-$CD25^+$ T cells) from DO11.10 mouse $CD4^+CD62L^+$ naïve T cells prior to *in vitro* Th17 polarization in the presence of $OVA_{323-339}$ peptide pulsed APC and various amounts of murine B7-H4-Ig was also tested. $CD4^+CD62L^+$ naïve T cells were first isolated from DO11.10 mice. $CD4^+CD25^+$ included or depleted cells (-CD25+ T cells) were polarized to Th17 cells in the presence of rTGF-β (10 ng/mL), IL-6 (50 ng/mL), IL-23 (4 ng/mL), anti-IL-4 (1 μg/mL), anti-IFN-γ (1 μg/mL) and anti-IL-2 (1 μg/mL), and stimulated with $OVA_{323-339}$ pulsed APC (APC/OVA). Murine B7-H4-Ig or Control IgG at various doses was added directly to the culture. As shown in Figure 33A, the depletion of Treg (open circle) resulted in higher IL-17 production as compared to IL-17 levels when Treg cells were present (solid circle). The decrease in IL-17 production was found to be dose dependent (open triangles). The inhibition was consistently higher when $CD4^+CD25^+$ Treg cells were present (solid triangle). In contrast, Figure 33B shows that murine B7-H4-Ig increased IL-10 production in a dose dependent manner (open triangles) and the increase

in IL-10 was greater when $CD4^+CD25^+$ Treg cells were present (solid triangle).

In vitro B7-H4-Ig inhibits proliferation and differentiation of naïve $OVA_{323-339}$-specific transgenic $CD4^+$ T cells into either Th1 or Th17 cells when stimulated with either $OVA_{323-339}$-pulsed syngeneic APC or antigen non-specific anti CD3/CD28 coated beads. Furthermore, B7-H4-Ig reduces the level of IFN-γ and IL-17/TNFα produced by Th1 cells and Th17 cells, respectively. The reduction of IFN-γ and IL-17 production was less pronounced when $CD4^+CD25^+$ T regulatory cells are depleted from the purified $CD4^+CD62L^+$ naïve $CD4^+$ T cells. Most importantly, B7-H4-Ig dose dependently increases IL-10 production during Th1/Th17 polarization when $CD4^+CD25^+$ T regulatory cells are present. These data show that B7-H4-Ig may act in part upon Treg cells to upregulate IL-10 expression and inhibit Th1 and Th17 effector differentiation and function.

Example 8: B7-H4-Ig promotes iTreg induction

Materials and Methods

In vitro induction of iTreg cells

$CD4^+CD62L^+$ naïve T cells were first labeled with CFSE (5 μM) for 10 min at room temperature. The dye was quenched by the addition of 0.5 volumes of ice-cold fetal calf serum. The cells were incubated on ice for 5 min followed by centrifugation to collect the cell pellet. The cells were washed 2 more times in HL-1 culture media. The cells were then cultured in the presence of TGF-β (10 ng/mL) and IL-2 (10, 50, 100 U/mL) for 3 days before intracellular staining with anti-FoxP3 *APC.* FACS analysis was conducted to detect the $FoxP3^+$, *in vitro* expanded iTreg cells.

Results

The above findings suggest that B7-H4-Ig induces naïve $CD4^+CD62L^+$ T cells to differentiate toward a Treg cell phenotype and/or directly enhances Treg cell function. Therefore, it was next examined if B7-H4-Ig treatment of naïve $CD4^+CD62L^+$ T cells in the presence of inducible Treg (iTreg) cell-promoting conditions would results in an increase in the numbers of $CD4^+CD25^+FoxP3^+$ iTreg cells. First the *in vitro* iTreg induction culture conditions were optimized to allow for experimental conditions to assess what additional effect, if any, B7-H4-Ig has on iTreg induction. $CD4^+$

T cells were isolated from female SJL mice. Purified mouse $CD4^+CD62L^+$ T cells were first labeled with CSFE and induced to iTreg cells in the presence of TGF-β (10 ng/mL) and IL-2 at concentrations of 0, 50 or 100 U/mL. FACS analysis was performed 3 days later to detect FoxP3 expression and CFSE content. A FoxP3 positive and CFSE diluted cell population was detected when using 100 U/mL of IL-2 for iTreg differentiation. The size of this cell population decreased when using less IL-2.

To assess the role of B7-H4-Ig in iTreg differentiation, the suboptimal iTreg conditions (10 ng/mL of TGF-β and 50 U/mL of IL-2), were used. This was done so that if B7-H4-Ig did in fact induce an increase in the percentage of $CD4^+CD25^+FoxP3^+$ cells, it would be clear that the B7-H4-Ig-induced increase was not masked by the TGF-β/IL-2 effect. FACS analysis of staned cells revealed naïve $CD4^+CD62L^+$ T cells were induced to express FoxP3 and CD25 *in vitro*, *i.e.*, iTreg, when the naïve $CD4^+CD62L^+$ T cells were activated in the presence of suboptimal iTreg cell-promoting condition, 10 ng/mL of TGF-β and 50 U/mL of IL-2. Different amounts of murine B7-H4-Ig (0, 1, 5 or 10 μg/mL) were added. B7-H4-Ig increased the percentage of $FoxP3^+CD25^+$ iTregs in a dose-dependent manner with the highest percentage of $FoxP3^+CD25^+$ T cells induced when the naïve $CD4^+CD62L^+$ T cells were activated in the presence of 10 μg/ml of B7-H4-Ig. In contrast, when naïve $CD4^+CD62L^+$ T cells were activated in the presence of 10 μg/ml control IgG, no increase in $FoxP3^+CD25^+$ iTregs was seen.

Example 9: B7-H4-Ig modulates $CD4^+$ T cell activation and nTreg suppression function

Materials and Methods

In vitro suppression assay

Spleens and lymph nodes were harvested from 10 FoxP3-GFP mice on a B6 background. Cells were made into a single cell suspension, and a total of 1.76×10^9 cells were collected. 1×10^8 cells were set aside to be irradiated for APCs in the assay. Naïve $CD4^+$ T cells were purified from the rest of the cells as described above. A total of 6.2×10^8 naïve $CD4^+$ T cells were collected. 5×10^7 cells were set aside to be effector T cells in the experiment. The remainder of the cells (5.7×10^8) were stained with anti-

CD4 PE-Cy7, and the PE-Cy7$^+$/GFP$^+$ cells were sorted via MoFlo. A total of 4 x 10^6 nTreg (CD4$^+$/FoxP3$^+$) cells were collected from the MoFlo at 99% purity. The suppression assay cultures were set up with 1 x 10^5 effector T cells + 1 x 10^5 irradiated APCs + αCD3 (1 μg/mL) at a final volume of 200 μl in a round bottom plate. The culture wells also received various ratios of nTreg cells: B7-H4-Ig.

Results

To further determine the effect of B7-H4-Ig on Treg cell function, an *in vitro* Treg suppression assay using natural Treg (nTreg) cells purified from B6/FoxP3-GFP mouse spleens and lymph nodes was conducted. In this transgenic mouse, the GFP transgene is under the regulation of the FoxP3 specific promoter, allowing the detection of nTreg cells expressing the endogenous FoxP3 by green fluorescence.

nTreg cells (CD4$^+$/CD62L$^+$/FoxP3-GFP$^+$) were isolated from B6/FoxP3-GFP mice by MoFLo sorting. Naïve GFP$^-$ T cells CD4$^+$/CD62L$^+$/FoxP3-GFP$^-$) were used as responder cells. Increasing numbers of the nTreg cells were added to constant numbers of naïve CD4$^+$CD62L$^+$ T cells, irradiated splenocytic APCs, and anti-CD3 (Figure 34). As shown in Figure 35, various responder:nTreg ratios were employed using a fixed responder cell number (1 x 10^5) with increasing Treg cells (0, 1.25 x 10^4, 2.5 x 10^4, 5 x 10^4, 1 x 10^5, 2 x 10^5) resulting in ratios of 1:0, 1:0.12, 1:0.25, 1:0.5, 1:1 and 1:2 fixed responder/nTreg. Various amounts of B7-H4-Ig (0, 1, 5, or 10 mg/mL) were added to the suppression assays and responder cell proliferation was assessed by [^{3}H]-thymidine 3 days later.

As shown in Figure 35, in the absence of nTreg cells (ratio at 1:0) B7-H4-Ig suppressed CD4$^+$ T cell activation and proliferation in a dose dependent manner. In the absence of B7-H4-Ig (closed circles), nTreg prevented CD4$^+$ T cell activation, also in a dose dependent fashion. At the ratio 1:2 (1 responder : 2 nTreg), nTreg cells almost abolished CD4$^+$ T cell activation. Significant suppression was observed when both nTreg and murine B7-H4-Ig were present. At the 1:0.12 and 1:0.25 ratios of T cell responder : nTreg no suppression was detected in the absence of murine B7-H4-Ig. However, 5 or 10 μg/mL of B7-H4-Ig completely blocked anti-CD3

induced T cell activation. In the absence of B7-H4-Ig, suppression increased only in the presence of more nTreg, ratios of 1:0.5, 1:0, 1:1 and 1:2 responder/nTreg.

Example 10: B7-H4-Ig modulates the induction and progression of disease in the $PLP_{139-151}$ peptide induced relapsing-EAE (R-EAE)

Methods and Materials

PLP induced R-EAE model

For $PLP_{139-151}$-induced R-EAE, 6- to 7-wk-old female SJL mice were immunized s.c. with 100 μL of an emulsion containing 200 μg of *M. tuberculosis* H37Ra and 50 μg of $PLP_{130-151}$ (HSLGKWLGHPDKF) (SEQ ID NO:139) distributed over three spots on the flank. Individual animals were observed daily and clinical scores assessed in a blinded fashion on a 0–5 scale as shown in Table 8 (Miller, et al., Curr. Protocols Immulo., Chapter 12, Unit 15.1). Unless otherwise mentioned, all mice were age and sex-matched for all experiments.

Figure 36 shows a typical B7-H4-Ig treatment regimen. Mice were randomly divided into four groups. For the disease prevention model, B7-H4-Ig or control IgG at 3mg/kg was injected intraperitoneally (i.p.) on the same day as $PLP_{130-151}$ priming. For the therapeutic model, B7-H4-Ig or control IgG at 3mg/kg was given i.p. beginning on approximately Day 21 post $PLP_{130-151}$ priming. In both cases, B7-H4-Ig was administrated, 3 times a week, for 2-4 weeks.

Delayed-Type Hypersensitivity (DTH) Responses

DTH responses were quantitated using a 24 hr ear swelling assay as previously described. Pre-challenge ear thickness was determined using a Mitutoyo model 7326 engineer's micrometer (Schlesinger's Tools, Brooklyn, New York). Immediately thereafter, DTH responses were elicited by injecting 10 μg of peptide in 10 μL of PBS into the dorsal surface of the ear using a 100 μL Hamilton syringe fitted with a 30 gauge needle. The increase in ear thickness over pre-challenge measurements was determined 24 hr after ear challenge. Results are expressed in units of 10^{-4} inches ± SEM. Results are expressed as the change in ear thickness in units of 10^{-4} inches ± SEM. The measurements were carried out independently by 2

investigators who did not know the identity of the experimental groups. Significance of ear swelling in murine B7-H4-Ig treated mice over Control IgG injected mice was assessed by the Student's *t* test.

In Vitro Antigen-Specific Recall Responses

Draining lymph nodes (axillary, brachial, and inguinal) were harvested, and single cell suspensions were obtained by mashing through sterile 60-mesh wire screens. In 96-well microtiter plates, 5×10^5 erythrocyte-free (Tris-NH_4Cl-treated) lymph node cells per well were incubated in supplemented culture medium with or without antigen at 37°C in 7% CO_2 for 24 hr and then pulsed with 1 μCi/well of [^{3}H]-Thymidine for the final 48 h of culture. Proliferation was determined by uptake of [^{3}H]-Thymidine detected using a Topcount Microplate Scintillation Counter (Packard Instruments, Meridan, CT). Results are expressed as the mean of triplicate cultures from individual animal ± SEM. Supernatants were collected at 72 hr for cytokine analysis. Cytokine measurements were performed using the Mouse Cytokine 10-Plex system and Luminex Liquidchip analyzer (Qiagen, Valencia, CA) or ELISA.

Results

T cells specific for the inducing epitope ($PLP_{139-151}$ peptide) in the PLP R-EAE animal model, cause acute CNS damage resulting in induction of T cell responses to endogenous encephalitogenic myelin epitopes, which are exposed to the immune system as a result of the initial acute damage. This progression of the relapsing-remitting disease course in R-EAE has been shown to be mediated by *de novo* activation of naïve T cells specific for $PLP_{178-191}$ peptides, a process known as *epitope spreading*. During the disease course of R-EAE mice develop an ascending paralytic demyelinating disease characterized by a relapsing-remitting clinical course, which is a validated model for MS (Miller, et al., *Curr Protoc Immunol.*, Chapter 15:Unit 15.1 (2010)).

To determine the therapeutic benefit of B7-H4-Ig, murine B7-H4-Ig was tested in the $PLP_{139-151}$-induced R-EAE mouse model both for prevention of disease (treatment begun on the same day of disease induction) and therapeutic intervention (treatment begun during the disease remission) settings (Figure 36). In both cases the disease course was followed for about

2 months to assess clinical disease following the grading system shown in Table 9.

Table 9: Grading System for Clinical Assessment of EAE

Score	Clinical Signs
0	Normal mouse; no overt signs of disease
1	Limp tail[a] or hind limb weakness[b] but not both
2	Limp tail[a] or hind limb weakness[b]
3	Partial hind limb paralysis[c]
4	Complete hind limb paralysis[d]
5	Moribund state; death by EAE: sacrifice for humane reasons

[a]Limp tail: complete flaccidity of the tail, and absence of curling at the tip of the tail when mouse is picked up.

[b]Hind limb weakness: observed as a waddling gait, the objective sign being that, in walking, mouse's hind limbs fall through the wire cage tops.

[c]Partial hind limb paralysis: mouse can no longer use hind limbs to maintain rump posture or walk but can still move one or both limbs to some extent.

[d]Complete hind limb paralysis: total loss of movement in hind limbs; mouse drags itself only on its forelimbs. Mice at this stage are given food on the cage floor, long sipper tubes, and daily subcutaneous saline injections to prevent death by dehydration.

This was done along with monitoring DTH responses and *in vitro* recall responses to spread epitopes by assaying for cytokine secretion following *ex vivo* stimulation of T cells with peptides.

B7-H4-Ig Prevents Relapsing Disease in the R-EAE Model

Female SJL mice were first immunized with PLP139-151 peptide emulsified in CFA and then randomized into 2 groups for either prevention or therapeutic treatment. For each treatment regimen there were 2 subgroups, with one subgroup receiving Control IgG and the other subgroup receiving murine B7-H4-Ig. Both Control IgG and murine B7-H4-Ig were given at 3 mg/kg, 3 times a week for 4 weeks. Prevention treatment (Figure 37) means that murine B7-H4-Ig or Control IgG was administered starting on the day at

PLP immunization (*t=0*). Therapeutic treatment (Figure 38) means that murine B7-H4-Ig or Control IgG was administered beginning on Day 21 (*t=21*). The data is presented as the mean clinical score over the 55 day time course with 5 mice per group, and the clinical scores and injection were conducted double blinded.

The data presented in Figures 37 and 38 show the clinical scores for murine B7-H4-Ig versus control IgG in both the preventative and therapeutic treatment regimens. The data shows that murine B7-H4-Ig decreased and/or prevented R-EAE disease relapse with both treatment regimens. The clinical scores on the control IgG injected R-EAE mice averaged 1.5 to 2.5, versus 0 – 0.5 for B7-H4-Ig treated animals. The difference between the murine B7-H4-Ig treated mice and Control IgG injected mice was significant.

B7-H4-Ig prevents epitope spreading

To determine the effect of B7-H4-Ig on blocking $CD4^+$ T cell mediated activity specific for the primary myelin-derived epitope $PLP_{139-151}$ and epitope spreading, peptide-specific responses *in vivo* via DTH on all 4 treatment groups were analyzed. Mice were ear challenged with 10 μg of the indicated peptides on Day 50, and swelling was measured 24 h later. Ear swelling was evaluated and plotted in Figure 39 (*DTH response significantly less than Control IgG injected mice, *p less than 0.01*). The present data show that murine B7-H4-Ig treatment significantly reduced the response to the dominant epitope $PLP_{139-151}$ with treatment starting on Day 0, and no inhibition of the $PLP_{139-151}$ response if murine B7-H4-Ig treatment was begun on Day 21. In contrast, the response to the spread epitope $PLP_{178-191}$-specific response was significantly reduced when murine B7-H4-Ig treatment was started on both Day 0 and Day 21.

B7-H4-Ig reduced $PLP_{178-191}$ specific T cell proliferation

Lymph nodes were isolated from the murine B7-H4-Ig and Control IgG injected EAE mice as described above. T cells were harvested and were elicited with $PLP_{139-151}$, the disease inducing dominant epitope, and $PLP_{178-191}$, the spread epitope-specific peptide, *in vitro*. [^{3}H]-thymidine was added to the *in vitro* stimulation assay to analyze T cell proliferation. *In vitro* peptide recall stimulation assays show that both murine B7-H4-Ig treatment regimens reduced $PLP_{178-191}$-specific (the spreading epitope) T cell

proliferation *in vitro* (Figure 40, *[^{3}H]-thymidine incorporation significantly less than Control IgG injected mice, *p less than 0.01*). There was no significant difference in $PLP_{139-151}$-specific (the primary peptide) T cell proliferation.

B7-H4-Ig down regulated $PLP_{139-151}$ and $PLP_{178-191}$ specific T cell response

Lymph nodes were isolated from Control IgG and murine B7-H4-Ig treated mice as described above. T cells were harvested and immune responses were elicited with $PLP_{139-151}$, the disease inducing dominant peptide, and $PLP_{178-191}$, the spread epitope peptide, *in vitro*. Supernatant was collected and analyzed for IFN-γ via commercially available ELISA kit.

The above experiments clearly show that B7-H4-Ig impacted the relapsing disease. DTH analysis (Figure 39) and IFN-γ production as measured in *ex vivo* T cell recall studies using the $PLP_{139-151}$ peptide (Figure 41, *IFN-γ production significantly less than Control IgG injected mice, *p less than 0.01*) demonstrate B7-H4-Ig specific inhibition of immune response to $PLP_{139-151}$ peptide when B7-H4-Ig was administered on Day 0, suggesting B7-H4-Ig may affect the acute disease phase.

B7-H4-Ig prevents epitope spreading

To clarify the effect of B7-H4-Ig on the EAE acute phase, a 2nd *in vivo* experiment was conducted in the $PLP_{139-151}$ induced R-EAE model. DTH responses in murine B7-H4-Ig and Control IgG injected EAE mice were assayed as described above. Mice were ear challenged with 10 μg of $PLP_{139-151}$ on Day 10, and swelling was measured 24 h later. In this study, two different doses of B7-H4-Ig were given to SJL mice (n=10 per treatment group) on Day 0, at 60μg (3 mg/kg) or 300 μg (15 mg/kg). After 5 injections of B7-H4-Ig, on Day 10 post disease induction, five mice were used for DTH analysis followed by *ex vivo* T cell antigen recall analysis. As shown in Figure 42 (*DTH response significantly less than Control IgG injected mice), when B7-H4-Ig was given on Day 0, both 3 mg/kg (60 μg) and 15 mg/kg (300 μg) doses significantly inhibited the immune response to the inducing $PLP_{139-151}$ peptide as measured by the DTH response to $PLP_{139-151}$ peptide versus $OVA_{323-339}$ peptide (negative control).

Draining lymph nodes were harvested from the animals, and single cell suspensions prepared as described above and reactivated *in vitro* in the presence of anti-CD3 (0.1-10 μg/mL), $PLP_{139-151}$ (1-20 μg/mL), or $OVA_{323-339}$ (1-20 μg/mL). T cell proliferation *in vitro* was analyzed by [^{3}H] thymidine incorporation. Figure 43 (*[^{3}H]-thymidine incorporation significantly less than Control IgG injected mice, *p less than 0.01*) shows robust T cell proliferation with cells from both Control IgG and B7-H4-Ig injected mouse groups when stimulated with anti-CD3, while the negative control peptide, $OVA_{323-339}$, did not elicit any response. In contrast, T cells from B7-H4-Ig injected groups, at both 3 and 15 mg/kg doses, showed little to no response to $PLP_{139-151}$ peptide stimulation. Both the DTH response and the *ex vivo* T cell proliferation upon primary $PLP_{139-151}$ peptide recall demonstrate that B7-H4-Ig protects against the EAE acute phase in addition to its clear effect on relapsing disease.

Example 11: B7-H4-Ig blocks pathogenic CD4$^+$ T cell infiltration and promotes accumulation of Tregs

Materials and Methods

Immunochemical Staining

Mice were anesthetized with nembutal and perfused with phosphate-buffered saline (PBS). Brains and spinal cords from each mouse were frozen in OCT (Miles Laboratories; Elkhart, IN) in liquid nitrogen. Tissue from the lower lumbar region of the spinal cord was sectioned at 6 μm on a Reichert-Jung 1800 cryotome and mounted on Superfrost Plus electrostatically charged slides. Cross-sections (10 μm thick for brains and 6 μm for spinal cords) from longitudinal sections of brain and spinal cord were performed. Tissues were stained with biotin-conjugated antibody to mouse CD4, PLP and FoxP3. Positive staining of biotinylated antibodies was visualized by a Tyramide Signal Amplification (TSA) Direct kit (NEN, Boston, MA) according to manufacturer's instructions and fluoroscein anti-mouse IgG (Vector Laboratories). Sections were counterstained with 4,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich) and then coverslipped with Vectashieldmounting medium (Vector Laboratories). Slides were examined and images were acquired via epifluorescence using the SPOT RT camera

(Diagnostic Instruments, Sterling Heights, MI). Sections from each group were analyzed at 40 or 100x magnification.

Results

The *in vitro* iTreg induction study with B7-H4-Ig provides evidence that B7-H4-Ig promotes iTreg differentiation (Example 8). Using purified nTreg cells from FoxP3-GFP transgenic mice in the *in vitro* suppression assay, a decrease in activation and proliferation of $CD4^+$ T effector cells by B7-H4-Ig was demonstrated (Example 9, Figure 35). The addition of B7-H4-Ig to the suppression assay in the presence of low numbers of nTreg cells has a significant effect on blocking effector T cell activation and proliferation *in vitro*.

The effect of B7-H4-Ig treatment on the number of Treg cells *in vivo* was analyzed in this study. The effect of B7-H4-Ig on the number and phenotype of $CD4^+$ T cells infiltrating into the CNS following B7-H4-Ig treatment, the relevant site for activity *in vivo,* was determined. As shown in Figure 44, SLJ mice (10 mice per groups) were immunized with $PLP_{139-151}$ following the standard protocol to induce R-EAE disease. SJL mice were first immunized with $PLP_{139-151}$ peptide. B7-H4-Ig treatment started during remission (Day 23). Mice received mouse Control IgG, 100 μg, or B7-H4-Ig at either 60 or 300 μg, 3 times per week for 2 weeks or 4 weeks. Half of the animals (5 mice from each group) were euthanized on Day 35, after 6 doses of B7-H4-Ig. The rest of the animals (5 mice from each group) were euthanized on Day 50, after 12 doses of B7-H4-Ig. Mouse spleens, draining lymph nodes, spinal cords and brains were collected, tissues were made into a single cell suspension and counted via the use of a hemocytometer, and analyzed for the presence of effector/memory $CD4^+$ T cells ($CD4^+CD44^+$) and Treg cells ($CD4^+CD25^+FoxP3^+$). Whole cerebellar and lumbar spinal cord tissue samples were snap frozen and processed as described above to analyze the number of $CD4^+$ and $FoxP3^+$ cells present within the CNS by histology.

As demonstrated in Figures 45A, 45B, and 45C, treatment with murine B7-H4-Ig showed a trend toward increasing numbers of $CD4^+$ T cells in the spleens (45A) and the draining lymph nodes (45B), and in contrast a

decrease in the total numbers of infiltrating $CD4^+$ T cells within the CNS (45C) after 6 treatments.

Cells isolated from spleen, draining lymph node and also lumbar spinal cord were stained for CD4, CD44 and FoxP3 followed by FACS analysis to obtain the number of total $CD4^+$ T cells, Treg ($CD4^+$/$Foxp3^+$) and effector/memory $CD4^+$ T cells ($CD4^+$/$CD44^+$). The data is presented in Figures 46A, 46B, and 46C as the mean number of cells for each phenotype from individual mouse. When the number of Treg ($CD4^+$/$FoxP3^+$) cells was calculated, B7-H4-Ig treatment remarkably increased the number of Treg cells within the spleen (46A) and lymph node (46B), suggesting the increase in the $CD4^+$ T cell population was due in part to the increase of Treg cells. B7-H4-Ig treatment also decreased effector/memory T cells ($CD4^+$/$CD44^+$) within the CNS when compared to Control IgG treated mice (46C). Similar data was obtained after the full course of 12 treatments. Figures 46A, 46B, and 46C also reveals that while there were fewer $CD4^+$ T cells infiltrating into the CNS, the amount of Treg cells in the CNS appeared constant (46C), indicating that B7-H4-Ig altered the ratio of Treg cells to total $CD4^+$ T cells within the CNS. Indeed, as shown in Figure 47, the percentage of Treg cells among $CD4^+$ T cells was significantly higher in the CNS from B7-H4-Ig treated mice compared with CNS from Control IgG injected mice.

The level of demyelination via anti-PLP staining in control IgG and B7-H4-Ig treated mice was also analyzed. The results indicate that there is not a significant, detectable difference in the level of PLP staining between groups, i.e., no significant difference in the level of demyelination. However, the T cell infiltrates into the CNS were also examined histologically, by staining and counting the total number of $CD4^+$ T cells, and $FoxP3^+$ cells in cross section samples taken from the lumbar spinal cord. Histological data correlates with the flow cytometric analysis with regard to the total number of $CD4^+$ T cells and the number of $FoxP3^+$ Treg cells present. The histology data is in line with the FACS data in demonstrating that B7-H4-Ig treatment increases the number of $FoxP3^+$ cells within the CNS. It also shows that the $FoxP3^+$ cells are co-localized with effector $CD4^+$ T cells within the CNS, allowing them to exert their suppressive effect on pathogenic T cells.

Overall the data clearly demonstrate that B7-H4-Ig treatment favorably alters the ratio of Treg cells to total $CD4^+$ T cells within the CNS, and is consistent with the proposed mechanism of action which suggests that B7-H4-Ig treatment both inhibits CD4+ T cell activation and increases Treg cell function and/or numbers. Similar findings were achieved after the full 12 doses (Day 50 post disease induction,).

The impact of B7-H4-Ig treatment on epitope spreading was also examined. To do so, spleens and draining lymph nodes were collected from the same mice that were analyzed for the number and phenotype of $CD4^+$ T cells. SJL mouse were immunized with 50 μg of $PLP_{139-151}$ peptide emulsified in CFA. Mice were treated B7-H4-Ig during remission: 60 or 100 ug per dose, 3 doses/wk, for 2 weeks (6 doses). On Day 35 total splenocytes and lymph node cells ($5x10^5$ cells per 200ul culture) were activated in separate wells per mouse in the presence of anti-CD3 (1ug/ml), $PLP_{139-151}$ or $PLP_{178-191}$ (20 μg/mL). At 24 hours following the initiation of culture, 1 μCi of ^{3}[H] tritiate thymidine was added to each well and wells were analyzed at 72 hours post the initiation of culture. This presented as the mean CPM. As shown in Figures 48A and 48B, treatment of mice with B7-H4-Ig decreased the proliferative response to both $PLP_{139-151}$ and $PLP_{178-191}$. Therefore, B7-H4-Ig treatment during remission of ongoing R-EAE in SJL mice appears to decrease epitope spreading via an increase in the number of Treg cells. The mean clinical score of this study presented in Figure 49 show that B7-H4-Ig at both 3 mg/kg (60 μg/dose) and 15 mg/kg (300 μg/dose) doses significantly prevented the primary relapsing. The higher dose appears more efficacious to lower the disease score at later time points (beyond Day 42 post disease initiation).

Example 12: *In vivo* bioactivity of human B7-H4-Ig in PLP induced R-EAE model

Human B7-H4-Ig was also tested for its therapeutic efficacy in the $PLP_{139-151}$-induced R-EAE mouse model. SJL mice were first immunized with $PLP_{139-151}$ peptide. Female SJL mice were first immunized with $PLP_{139-151}$ peptide emulsified in CFA and on Day 23 randomized into 3 groups. One group received control human IgG1 (Synagis), at 5 mg/kg (100 μg), 3 times a week for 4 weeks. The other 2 groups were given B7-H4-Ig at 5 or 25

mg/kg, 3 times a week for 4 weeks (Figure 50). The disease course was followed for approximately 2 months to assess clinical disease following the grading system shown in Table 9 and long-term relapse rate as described above.

As shown in the Figures 51A and 51B, at two different dosing levels, 5 or 25 mg/kg, human B7-H4-Ig inhibited the primary disease relapse as determined by a reduction of mean clinical score (51A) and reduction in long-term relapse rate (51B). The higher dose (25 mg/kg) appears to result in a lower mean clinical score (51A); however, there was no difference in the relapse frequency between the low dose and high dose groups (51B); both doses were equally as effective in preventing relapsing episodes.

The data presented in Examples 5-12 show the highly effective and reproducible, therapeutic efficacy of B7-H4-Ig treatment in $PLP_{139-151}$-induced relapsing-remitting EAE (R-EAE). In addition, the bioactivity of B7-H4-Ig was determined both *in vivo* and *in vitro*. The data show that murine B7-H4-Ig inhibited proliferation and differentiation of naïve $OVA_{323-339}$-specific transgenic $CD4^+$ T cells stimulated in Th1 and Th17 lineage driving conditions *in vitro*. B7-H4-Ig also decreased the level of IFN-γ and IL-17 produced by Th1 cells and Th17 cells, respectively. The reduction of IL-17 skewing was less pronounced when $CD4^+CD25^+$ T regulatory cells were depleted from the AutoMax-purified $CD4^+CD62L^+$ naïve $CD4^+$ T cells, which in turn suggested that B7-H4-Ig also acts directly upon Tregs to inhibit Th17 effector differentiation and function. The effect of murine B7-H4-Ig on induction of inducible regulatory T cells (iTreg) *in vitro* using naïve $CD4^+$ T cells expanded in the presence of suboptimal concentrations of TGF-β and IL-2 for the induction of iTreg differentiation was also tested. The data showed that B7-H4-Ig enhances the differentiation of naïve $CD4^+$ T cells toward an iTreg phenotype in a dose-dependent manner as determined by FACS analysis of the $FoxP3^+/CD25^+$ cell population. Thus, B7-H4-Ig simultaneously targets multiple key pathogenic, inflammatory pathways involved in MS and other autoimmune diseases and further suppresses inflammation by inducing Tregs. This is the first direct evidence that B7-H4-Ig promotes iTreg induction. Coupled with its effects on Th1 and Th17

cell differentiation, this distinguishes B7-H4-Ig from all other drugs being developed for MS.

B7-H4-Ig was further tested for its ability to modulate the suppressive function of Tregs in an *in vitro* suppression assay. Natural Treg (nTreg) cells were purified from FoxP3-GFP transgenic mice using a MoFlo cell sorter to obtain $CD4^+$/FoxP3-GFP^+ nTreg cells. $CD4^+$/GFP^- T responder cells were stimulated with an anti-CD3 antibody in the presence or absence of $CD4^+$/GFP^+ nTreg cells and murine B7-H4-Ig. In the absence of nTreg cells, B7-H4-Ig inhibited $CD4^+$ T cell activation and proliferation in a dose-dependent fashion, confirming previous findings. In the absence of murine B7-H4-Ig, nTreg cells prevented $CD4^+$ T cell activation and proliferation in a cell number dependent manner. However, a significant increase in the level of immune suppression of responder $CD4^+$/GFP^- T cells was observed when both nTreg and B7-H4-Ig were present. The above *in vitro* analysis of B7-H4-Ig supports the model that B7-H4-Ig not only blocks naïve $CD4^+$ T cell activation and inhibits the differentiation of naïve helper T cells into pro-inflammatory Th1 and Th17 subsets, but also enhances naïve $CD4^+$ T cell differentiation into iTreg.

The ability of B7-H4-Ig to modulate the induction and progression of R-EAE *in vivo* was also tested. In the 1st *in vivo* study, the initiation of B7-H4-Ig treatment on the day of $PLP_{139-151}$/CFA peptide priming did not affect disease symptoms during the acute phase of R-EAE, but inhibited the primary disease relapse as determined by a significant reduction in mean clinical score and DTH responses to the spreading $PLP_{178-191}$ epitope. Likewise, initiation of B7-H4-Ig treatment during disease remission (Day 21 post disease induction) significantly reduced the severity of disease relapse concomitant with inhibition of T cell responses to the spreading $PLP_{178-191}$ epitope. In a repeat R-EAE study, in contrast to the first *in vivo* study, B7-H4-Ig treatment starting on the day of $PLP_{139-151}$/CFA peptide priming was shown to affect the disease symptoms during the acute phase of R-EAE. Subsequently, it was demonstrated that murine B7-H4-Ig increased Treg cell number in the periphery of treated mice. B7-H4-Ig blocked pathogenic $CD4^+$ T cell infiltration into the CNS and increased the percentage of protective

Tregs in the CNS of R-EAE mice. This may explain the effect of B7-H4-Ig on both the primary and relapsing state of disease.

Human B7-H4-Ig was tested both *in vitro* and *in vivo*. Results from *in vitro* bioanalysis show that human B7-H4-Ig cross-reacted with murine naïve $CD4^+$ T cells and that it blocked murine Th1/Th17 proliferation and differentiation. Results from an *in vivo* R-EAE experiment revealed that human B7-H4-Ig inhibited disease as determined by a reduction in both the mean clinical score and the long-term relapse rate. Furthermore, identical *in vitro* and *in vivo* bioactivity was shown for B7-H4-Ig from different batches (lots) demonstrating consistency of the B7-H4-Ig production process. Taken together the present findings suggest that B7-H4-Ig induces an increase in the number and/or function of Tregs, and decreases Th1/Th17 responses.

Example 13: Diabetes mouse models

CTLA4KD/NOD mice

Methods and Materials

Both female and male CTLA4KD/NOD mice (Chen *et al., PNAS,* 103(44):16400-16405 (2006)) at age of 2 weeks were randomly assigned into experimental groups: vehicle (11 mice), and B7-H4-Ig (12 mice). Mice were treated with B7-H4-Ig 3 times per week for 4 weeks (15 mg/kg, per i.p. injection), and monitored 3 times per week for glucose content in the urine first with Diastix. If Diastix showed positive, plasma was collected for blood glucose level. Diabetes was determined when blood glucose reached $\geq$ 600 mg/dL.

Results

Figure 52 shows B7-H4-Ig prevents autoimmune diabetes development in $CTLA_4KD$/NOD mice. $CTLA_4KD$/NOD mice at age of 2 weeks were injected with either vehicle or B7-H4-Ig 3 times per week for 4 weeks (15 mg/kg, per i.p. injection). In line with published data (*Chen et al., PNAS, Modeling CTLA4-linked autoimmunity with RNA interference in mice, 2006*), 30-40% of the vehicle injected mice developed diabetes when the mice were 5 weeks old (3 weeks post vehicle injection). The B7-H4-Ig treated $CTLA_4KD$/NOD mice did not develop diabetes when the mice were 24 weeks old (22 weeks post B7-H4-Ig treatment).

$CTLA_4$KD/BDC2.5/NOD mice

Methods and Materials

CTLA4KD/NOD mice (Chen, et al., *PNAS* (2006)) were bred with BDC2.5 TCR transgenic mice (Katz, et al., *Cell*, 74(6):1089-100 (1993)). Both female and male mice at age of 11 weeks were randomly assigned into experimental groups: vehicle (3 mice), and B7-H4-Ig (5 mice). Mice were treated with B7-H4-Ig 3 times per week for 4 weeks (15 mg/kg, per i.p. injection), and monitored 3 times per week for glucose content in the urine first with Diastix. If Diastix showed positive, plasma was collected for blood glucose level. Diabetes was determined when blood glucose reached ≥ 600 mg/dL.

Results

Figure 53 shows B7-H4-Ig delayed autoimmune diabetes in CTLA4KD/BDC2.5/NOD mice. CTLA4KD/BDC2.5/NOD mice at age of 11 weeks were injected with either vehicle or B7-H4-Ig 3 times per week for 4 weeks (15 mg/kg, per i.p. injection). In line with historical data (in communication with Dr. Chen), 100% of the vehicle injected mice developed diabetes when the mice were 16 weeks old (5 weeks post vehicle injection). The B7-H4-Ig treated CTLA4KD/BDC2.5/NOD mice did not develop diabetes until the mice reached 21 weeks old (10 weeks post B7-H4-Ig treatment).

Example 14: B7-H4-Ig enhances Treg activity

Methods and Materials

Preparation of enteroantigen from fecal extracts

Extracts were prepared by removing the colon and cecum from mice and placing the content in PBS. This was sonicated 3 times for 30 seconds on ice, followed by centrifugation at 10,000g for 10 min to remove insoluble material. The supernatant was collected, sterile filtered, and stored at -60°C. The protein concentration in the supernatants was typically 1 to 1.5 mg/mL as determined by the bicinchoninic acid (BCA) method.

B7-H4-Ig treatment

Female wild-type Balb/c, 7 to 9 weeks of age, were injected with either 60 μg or 300 μg of B7-H4-Ig, intraperitoneally (IP), 3 times a week for 2 weeks. There were 5 mice in each group. B7-H4-Ig treated mice and also

naïve control mice were euthanized for T cell isolation after 2 week treatment.

Preparation of $CD4^+CD25^-$ T cells and $CD4^+$ $CD25^+$ T cells

The $CD4^+CD25^-$ T cells were obtained as follows: $CD4^+$ T cells were positively selected from spleen single-cell suspensions using a mouse anti-CD4 monoclonal antibody-coated DYNABEAD® and the DETACHABEAD® system (Dynal AS, Oslo, Norway) according to the manufacturer's instructions. Then the $CD4^+$ T cells (<98% pure assessed by flow cytometry) were separated into $CD25^+$ and $CD25^-$ T cell populations by Miltenyi's magnetic bead technology (MACS®, Miltenyi Biotech, Belgisch Gladbach, Germany) using PE-labeled anti-CD25 monoclonal antibody, followed by the addition of anti-PE microbeads and depletion according to the manufacturer's instructions.

Preparation and Pulse of Antigen-Presenting Cells

Normal spleen cells from BALB/c mice were used as antigen-presenting cells (APC). The spleen cells were adjusted to 8×10^6 cells/mL, and 400 μg extract enteroantigen in a final volume of 2 mL was mixed in 24-well plates for antigen presentation. After incubation for 18 hours, the cells were washed 3 times in medium and irradiated (3000 rad) to eliminate APC proliferation.

Proliferation Assay

APCs were adjusted to 1.0×10^6 cells/mL, and 100 μL was added to each well of a 96-well round-bottom culture plate. CD4+$CD25^-$ T cells isolated from Balb/C mice were adjusted to 1×10^6 cells/mL, and 100 μL was added to the APCs. After 4 days of culture, proliferation was measured by adding 0.5 μCi of [^{3}H]-thymidine to each well, incubating for 18 hours, and harvesting the cells to count the incorporated thymidine.

Results

In a standard *in vitro,* enteroantigen priming and proliferation assay, normal mouse $CD4^+CD25^-$ T cells were first added into 96-well plate and mixed with enteroantigen pulsed APC. $CD4^+CD25^+$ Treg cells were isolated from control or B7-H4-Ig treated mice and added in the above culture at 0, 6250, 12500 and 25000 Treg/well. Figure 54 shows that Tregs from B7-H4-Ig treated mice were more potent in blocking enteroantigen priming and T

cell proliferation when compared to Tregs from control mice. In addition, mice treated with a higher dose B7-H4-Ig (300 μg) gave rise to more effective Treg when compared to lower dose B7-H4-Ig (60 μg) treated ones, indicating a dose-dependent effect.

$CD4^+CD25^-$ T cells from B7-H4-Ig treated mice were also assessed for their responsiveness to enteroantigen priming and proliferation. Figure 55 shows $CD4^+CD25^-$ T effector cells from B7-H4-Ig treated mice responded well to enteroantigen pulsed APC and that B7-H4-Ig treatment barely affected enteroantigen specific $CD4^+CD25^-$ T effector cell response.

The data suggest that Treg cells recovered from lymph nodes of B7-H4-Ig treated animals exhibit an increased activity compared with Treg cells obtained from control mice in blocking antigen priming and T cell proliferation. B7-H4-Ig treatment marginally affects enteroantigen specific $CD4^+CD25^-$ T effector cells.

Example 15: B7-H4-Ig functions early during the activation of naïve cells and differentiation into Th17 cells to inhibit IL-17A production

Methods and Materials

$CD4^+CD62L^+$ naïve T cells were isolated from BALB/c mice as in the above examples, and activated by anti-CD3 and anti-CD28 bound beads in the presence of Th17 differntiation cocktail (TGF-β1 (10 ng/mL), IL-6 (50 ng/mL), IL-23 (4 ng/mL), anti-IL-4 (10 μg/mL), anti-IFN-γ (5 μg/mL) and anti-IL-2 (5 μg/mL)). Murine B7-H4-Ig (10 μg/mL) or retinoic acid (RA) (10 μM) was added to the cultures on day 0, day 1, or day 2 of the 4 day culture. IL-17A levels were measured by ELISA at the end of the 4 day culture.

Results

The Th17 assay is 4 days in duration. In order to determine when B7-H4-Ig is acting, the Th17 assay was performed with the addition of 10 μg/mL of murine B7-H4-Ig, 10 μg/mL of mouse IgG control, or 10 mM retinoic acid on Day 0, Day 1, or Day 2.

Both murine B7-H4-Ig and RA inhibited IL-17A most potently when added at the beginning of the assay (day 0), indicating that B7-H4-Ig

functions early during the differentiation of naïve T cells to Th17 T cells to inhibit IL-17A production and/or secretion (Figure 56).

Example 16: B7-H4-Ig downregulates expression of genes associated with differentiation of Th17 cells

Methods and Materials

$CD4^+CD62L^+$ naïve T cells were isolated from BALB/c mice as in the above examples, and activated by anti-CD3 and anti-CD28 bound beads in the presence of Th17 differntiation cocktail (TGF-β1 (10 ng/mL), IL-6 (50 ng/mL), IL-23 (4 ng/mL), anti-IL-4 (10 μg/mL), anti-IFN-γ (5 μg/mL) and anti-IL-2 (5 μg/mL)). Cells were incubated with either B7-H4-Ig (two different lots - #22 and #23 – at 1 μg/mL) or isotype control. RNA was isolated from the cells and used for quantitative RT-PCR to test the expression levels of a large number of mRNAs associated with Th17 cells, Tregs, autoimmune disiorders and inflammation (SA Biosciences mouse Th17 panel).

Human T cells were activated by anti-CD3 and anti-CD28 bound beads in the presence of Th17 differntiation cocktail (TGF-β1 (10 ng/mL), IL-6 (50 ng/mL), IL-23 (4 ng/mL), anti-IL-4 (10 μg/mL), anti-IFN-γ (5 μg/mL) and anti-IL-2 (5 μg/mL)). Cells were incubated with two different variants of B7-H4-Ig (1 μg/mL) identified as Q or L, or a humanized monoclonal IgG antibody directed against an epitope in the A antigenic site of the F protein of the Respiratory Syncytial Virus (Synagis), as an isotype control.

Results

The quantitative RT-PCR results show that B7-H4-Ig downregulates the expression of mRNA involved in Th17 cell differentiation, such as the master Th17 transcription factor, RORc, and the Th17 effector molecules, IL-17A, IL-17F, IL-21, and IL-22 (Table 10 and Figures 57 and 58).

Table 10: mRNA upregulation or downregulation by B7-H4-Ig in activated Th17 cells

Description	Symbol	Fold Up-or Down Regulation			
		Test Sample / Control Sample			
		Lot 22	Lot 23	L Version	Q Version
Calcyclin binding protein	Caybp	-1.02	-1.00	-1.20	-1.05
Chemokine (C-C motif) ligand 1	Ccl1	3.75	-1.33		
Chemokine (C-C motif) ligand 2	Ccl2	1.18	-1.11	2.07	1.03
Chemokine (C-C motif) ligand 20	Ccl20	-2.22	-2.00	1.38	-3.04
Chemokine (C-C motif) ligand 22	Ccl22	1.36	1.15	1.17	1.10
CD2 antigen	Cd2	-1.19	-1.06	-1.06	-1.14
CD247 antigen	Cd247	1.23	1.26	-1.00	1.23
CD28 antigen	Cd28	1.10	1.10	1.20	1.01
CD3 antigen, delta polypeptide	Cdl3d	1.23	1.26	-1.04	1.03
CD3 antigen, epsilon polypeptide	Cd3e	1.27	1.37	1.08	1.04
CD3 antigen, gamma polypeptide	Cd3g	1.27	1.21	-1.02	1.00
CD4 antigen	Cd4	1.37	1.30	1.05	1.07
CD40 ligand	Cd40lg	1.26	1.15	1.05	-1.10
CD8 antigen, alpha chain	Cd8a	-1.19	-1.08	-1.35	-1.13
CCAAT/enhancer binding protein (C/EBP), beta	Cetpb	-1.27	-1.11	1.01	-1.09
Colony stimulating factor 2 (granulocyte-macrophage)	Csf2	-1.97	-2.06	-1.30	-1.95
Colony stimulating factor 3 (granulocyte)	Csf3	2.50	2.55	-1.95	-1.44
Sphingosine-1-phosphate receptor 1	S1pr1	1.57	1.72	-1.05	-1.18
Forkhead box P3	Foxp3	1.38	1.64	-1.03	1.24
GATA binding protein 3	Gata3	1.47	1.41	1.06	-1.17
Intercellular adhesion molecule 1	Icam1	1.14	1.32	1.42	-1.04
Inducible T-cell co-stimulator	Icos5	-1.04	1.05	-1.04	-1.09
Interferon gamma	Ifng	1.36	1.54	-1.08	-1.02
Interleukin 10	Il10	-1.63	-1.26	-1.48	-1.87
Interleukin 12 receptor, beta 1	Il12rb1	1.24	1.06	-1.12	-1.05
Interleukin 12 receptor, beta 2	Il12rb2	-1.09	-1.05	-1.47	-1.02
Interleukin 13	Il13	-1.24	-1.07	-1.27	-1.22
Interleukin 17A	Il17A	-5.29	-6.88	-1.29	-3.31
Interleukin 17F	Il17f	-5.15	-3.79	-2.26	-3.31
Interleukin 17 receptor B	Il17rb	1.92	2.61	1.09	1.13
Interleukin 17 receptor E	IL17re	1.43	1.25	1.17	1.16

Interleukin 18	Il18	-1.36	-1.28	1.59	1.39
Interleukin 1 beta	Il1b	-2.98	1.01	1.22	-2.95
Interleukin 2	Il2	-1.09	-1.39	1.13	-1.11
Interleukin 21	Il21	-1.45	-1.51	-1.25	-1.04
Interleukin 22	Il22	-4.16	-3.71	-2.11	-2.57
Interleukin 23, alpha subunit p19	Il23a	-1.49	-1.36	-1.23	-1.76
Interleukin 23 receptor	Il23r	-2.26	-1.58	-1.42	-1.66
Interleukin 4	Il4	1.15	1.34	-1.70	-1.11
Interleukin 6 receptor, alpha	Il6ra	1.35	1.26	1.60	1.69
Interleukin 7 receptor	Il7r	1.33	1.26	1.78	1.69
Interferon-stimulated protein	Isg20	2.16	1.88	1.30	1.25
Janus kinase 1	Jak1	1.40	1.39	1.08	1.02
Janus kinase 2	Jak2	1.09	-1.00	-1.18	-1.18
Matrix metallopeptidase 13	Mmp13	-1.02	-1.89		
Myeloid differentiation primary response gene 88	Myd88	1.19	1.17	1.72	1.14
Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	Nfatc2	1.15	1.23	1.09	1.06
Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2	Ffkb1	1.33	1.29	1.54	-1.21
RAR-related orphan receptor gamma	Rorc	-3.93	-2.40	-1.10	-1.37
Suppressor of cytokine signaling 1	Socs1	1.01	1.08	-1.33	-1.13
Suppressor of cytokine signaling 3	Socs3	1.12	1.16	1.34	-1.47
Signal transducer and activator of transcription 3	Stat3	1.31	1.31	1.11	1.04
Signal transducer and activator of transcription 4	Stat4	2.89	4.29		
Signal transducer and activator of transcription 5A	Stat5a	-1.06	-1.01	-1.05	-1.05
Signal transducer and activator of transcription 6	Stat6	-1.00	1.02	1.20	1.12
Spleen tyrosine kinase	Syk	-1.92	-1.75	1.22	-1.42
Transforming growth factor, beta 1	Tgfb1	1.03	1.05	1.10	-1.02
Toll-interleukin 1 receptor (TIR) domain-containing adaptor protein	Tlrap	-1.02	1.10	1.15	1.02
Toll-like receptor 4	Tlr4	1.49	1.17	-1.06	-1.32
Tumor necrosis factor	Tnf	-1.19	-1.13	1.05	-1.09
Tnf receptor-associated factor 6	Traf6	1.05	1.15	1.33	-1.01
YY1 transcription factor	Yy1	-1.09	1.21	-1.19	-1.03
Glucuronidase, beta	Gusb	1.06	1.02	1.02	1.04

Hypoxanthine guanine phosphoribosyl transferase 1	Hprt1	1.03	-1.02	-1.01	1.03
Heat shock protein 90 alpha (cytosolic), class B member 1	Hsp90ab1	-1.16	-1.11	-1.11	-1.16
Glyceraldehyde-3-phosphate dehydrogenase	Gapdh	-1.17	-1.12	-1.03	-1.02
Actin, beta	Actb	1.24	1.26	1.13	1.10
Low mRNA: less certain					
Chemokine (C-C motif) ligand 1	Ccl1	3.75	-1.33	-2.47	-2.33
Chemokine (C-C motif) ligand 7	Ccl7	1.24	1.31	-3.32	-3.17
CD34 antigen	Cd34	1.08	-1.65	1.27	1.66
C-type lectin domain family 7, member a	Clec7a	-4.21	3.21	-4.75	-3.42
Chemokine (C-X3-C motif) ligand 1	Cx3cl1	7.42	8.71	1.09	2.66
Chemokine (C-X-C motif) ligand 1	Cxcl1	1.24	1.31	1.09	1.15
Chemokine (C-X-C motif) ligand 12	Cxcl12	2.13	1.31	1.09	1.15
Chemokine (C-X-C motif) ligand 2	Cxcl2	-1.04	1.65	1.35	-3.17
Chemokine (C-X-C motif) ligand 5	Cxcl5	1.67	-1.80	2.88	1.15
Interleukin 25	Il25	3.67	1.31	1.09	1.15
Interleukin 12B	Il12b	1.24	1.31	1.09	1.15
Interleukin 15	Il15	-2.31	-2.56	-1.16	1.12
Interkeukin 17C	Il17c	2.58	2.14	-1.03	1.43
Interleukin 17D	Il17d	1.24	1.31	1.09	1.15
Interleukin 17 receptor C	Il17rc	2.32	2.12	-1.70	1.07
Interleukin 17 receptor D	Il17d	-1.16	1.59	-1.79	-1.67
Interleukin 27	Il27	1.65	1.59	1.99	2.87
Interleukin 3	Il3	-1.41	2.27	-1.74	1.38
Interleukin 5	Il5	2.59	-1.21	1.09	1.68
Interleukin 6	Il6	1.14	1.21	1.13	-1.15
Matrix metallopeptidase 13	Mmp13	-1.02	-1.89	5.00	1.75
Matrix metallopeptidase 3	Mmp3	1.24	1.31	1.09	1.15
Matrix metallopeptidase 9	Mmp9	1.78	-1.24	-2.87	-1.53
Signal transducer and activator of transcription 4	Stat4	2.89	4.29	-1.07	-2.12
T-box 21	Tbx21	-1.14	1.15	-1.07	-2.78

Example 17: Activity of Murine B7-H4-Ig in the Mouse TH17 Assay

Methods and Materials

Animals

BALB/c mice, female, 5 – 7 week old (Harlan) were used.

Isolation of Naïve CD4 Cells

Cell strainer

ACK lysis buffer

10× Hank's Balanced Salt Solution (Sigma H1641)

CD4 Negative selection kit (Miltenyi Biotec 130-090-860)

Anti-mouse CD25-Biotin (Miltenyi Biotec 130-092-569)

CD62L beads (Miltenyi Biotec 130-049-701)

96 Well Cell Culture Cluster (Costar 3595)

Culture Medium

Dynabeads® Mouse CD3/CD28 T cell Expander (Invitrogen 11452D)

HL-1 media (Lonza 344017)

1000× β-mercaptoethanol (2-Me, Invitrogen 21985-023)

Penicillin/streptomycin (P/S, Invitrogen 15070-063)

Non-essential Amino Acids (Invitrogen 11140-050)

L-Glutamine (Invitrogen 25030-081)

TH17 Differentiation Cocktail

Recombinant human TGF-β1 (R&D Systems 240-B-010)

Recombinant mouse IL-6 (eBioscience 4-8061)

Recombinant mouse IL-23 (eBioscience 14-8231)

Anti-mouse IL-2 (eBioscience 16-7021)

Anti-mouse IL-4 (eBioscience 16-7041)

Anti-mouse IFN-γ (eBioscience 16-7311)

B7-H4-Ig and Murine B7-H4-Ig

Murine B7-H4-Ig Lot 22

Murine B7-H4-Ig Lot 23

Human B7-H4-Ig (Q)

Human B7-H4-Ig (L)

Positive and Negative Controls

All trans-Retinoic Acid (ATRA, Sigma R2625)

ChromoPure Mouse IgG (Jackson ImmunoResearch 015-000-003)

Synagis® (MedImmune)

Analysis

Mouse IL-17A ELISA kit

Peripheral T cells were obtained from spleens and inguinal lymph nodes of 6-9 week old BALB/c mice by mechanical disruption through a cell strainer followed by red blood cell (RBC) lysis.

Naïve helper T cells ($CD4^+CD62L^+$) were obtained using the Miltenyi Biotec microbead system. $CD4^+$ cells are enriched by negative selection, and followed by a positive selection of $CD62L^+$. In some experiments CD25+ cells were also depleted.

After selection, cells are activated *in vitro* with anti-CD3/CD28 beads at a 1:1 cell:bead ratio in the presence of the T_H17 differentiation cocktail (TGF-β1 (10 ng/ml), IL-6 (50 ng/ml), IL-23 (4 ng/ml), anti-IL-2 (5 μg/ml), anti-IL- 4 (10 μg/ml), and anti-IFN-γ (5 μg/ml)). Murine B7-H4-Ig is added at a final concentration of 1.25 -20 μg/ml. Retinoic acid (RA) is used as positive control and mouse IgG is used as a negative control.

After 4 days of culture, cultures are spun down and supernatants are harvested and stored at <-65 °C until analyzed for IL-17A levels by ELISA.

Flow cytometry and intracellular staining (ICS) were used to assess the purity of the starting cell popoulations and the success of the differentiation protocol.

Results

Initial experiments were performed to demonstrate that murine B7-H4-Ig acts to reduce IL-17A expression by murine Th17 cells. $CD4^+CD62L^+$ T cells are stimulated and cultured for four days in Th17 promoting conditions in the presence of murine B7-H4-Ig, control mouse IgG, or retinoic acid (RA).

Supernatants were harvested on day 4 and analyzed by IL-17A ELISA. As shown in Figure 59, both murine B7-H4-Ig and RA treatment lead to reduced IL-17A expression, relative to the control mouse IgG.

An experiment was preformed to see whether human B7-H4-Ig is active in the assay. Species cross-reactivity should be possible because the B7-H4 extracellular domain is 95% identical in human B7-H4 and its murine analog. Protein concentrations of 20, 10, 5, 2.5, and 1.25 μg/mL were tested. Two variants of human B7-H4-Ig were compared with Synagis®, an irrelevant human IgG1 antibody therapeutic, and two lots of murine B7-H4-Ig were compared with mouse IgG control.

As shown in Figures 60 and 61, both human B7-H4-Ig and its murine analog are active on mouse cells in the Th17 assay.

Example 18: MRL/lpr lupus mouse model

Materials and Methods

Treatment regimen

MRL/*lpr* mice at 4 weeks of age were used in this experiment. Animals were tagged with metal tags on their right ears for identification. Cyclophosphamide (CTX) is the primary drug used for diffuse proliferative glomerulonephritis in patients with renal lupus, Daikh and Wofsy reported that combination treatment with CTX and CTLA4-Ig was more effective than either agent alone in reducing renal disease and prolonging survival of NZB/NZW F1 lupus mice with advanced nephritis (Daikh and Wofsy, *J Immunol.,* 166(5):2913-6 (2001)). In the proof-of-concept study, combination treatments with recombinant murine B7-H4-Ig and CTX were tested. Mice received single or combination treatment of murine B7-H4-Ig (Lot#DEV-110-5-006) at 5 mg/kg with or without CTX at 50 mg/kg via IP injection, once every 2 weeks (Figure 62). Murine B7-H4-Ig dosing regimen at 5 mg/kg (100 μg/mouse/injection), twice a week, was chosen based on *in vivo* studies of monoclonal antibody and fusion proteins. Both single and combination treatments were diluted with PBS to generate a similar 500 μL injection sample which was administered using a 3 ml syringe with a gauge 27 needle. Blood samples (~200 μL) were taken from the submandibular vein (ARC SOP 8050.02.08) 3 days before the protein treatment and the every other week during and after treatments. Blood samples were collected in Microtainer® Blood Collection Tubes, and plasma was harvested from the supernatant of blood sample after centrifugation and stored at -80°C freezer until use.

Anti-dsDNA autoantibody analysis

A positive anti-dsDNA autoantibody control was generated by pooling plasma from 5 aged MRL/*lpr* mice, approximately 8 months of age. The positive control plasma was aliquoted and stored at -80°C freezer before use.

The dsDNA solution (10 mg/mL) was first generated by dissolving salmon testes DNA in PBS at 37°C in a water bath followed by filtration through a 0.45 μm membrane filter. The dsDNA solution was aliquoted and stored at -80°C before use.

On the day prior to the autoantibody ELISA, 100 μL of the dsDNA stock solution was first added to 10 mL of PBS resulting in a final concentration of 100 μg/ml. 100 μl of the diluted dsDNA was then added to each well of Immulon 2HB 96-well flat bottom microtiter plates. Plates were placed in a humidified incubator at 37°C without lids for overnight coating. Plates were washed 4 times with 300 μL of PBS/T [0.1% (v/v) Tween-20 (polysorbate 20) in PBS] using a microtiter plate washer (hydroFLEX, TECAN. Software: HydroControl) and then blocked with 200 μL of blocking buffer (10% fetal bovine serum in PBS) at room temperature for at least 1 h followed by 4 washes with 300 μL of PBS/T. The negative control (normal Balb/C mouse plasma, 1:2000 dilution), positive controls (1:5000, 1:10000, 1:20000 dilutions), and samples (1:2000 dilution) were diluted with blocking buffer. Next, 100 μL of each diluted control and sample was added to corresponding wells (duplicate wells) and incubated at room temperature for 2 h or at 4°C overnight followed by 4 washes with 300 μL of PBS/T. For detection, 100 μL of anti-mouse IgG-HRP (Sigma, A9309, diluted to 1:10000 in blocking buffer) was added to each well and the plate was incubated at room temperature for 1 h followed by 4 washes with 400 μL of PBS/T.

Later, 100 μL of room temperature, pre-warmed TMB Substrate Reagent was added to each well. When the color developed optimally (about 10 min), 100 μL of stop solution (1% sulfuric acid) was added. The plate was then read for absorbance at 450 nm (OD_{450}) using a microtiter plate reader (SPECTRAmax, Molecular Devices. Software: SoftMax Pro5.2).

All the blood samples were analyzed at the end of the study. The OD_{450} value of test samples was normalized relatively to the internal positive control at the 1:10000 dilution. The relative value rather than the absolute Ab concentration was used for comparison.

Histology

To evaluate glomerulonephritis, mouse kidneys were harvested and fixed in 10% formalin (SAFEFIX II) and shipped to AML Laboratories (Rosedale, MD). Sections of 7 nm were obtained and stained via standard H&E staining at AML Labs. Images were taken under the light microscope at low and high magnifications.

Proteinuria analysis

Proteinuria was measured by testing fresh urine samples using urinalysis dipsticks (Germaine Laboratories, catalog # 52100). A fresh urine drop was collected on the dip reagent pad. Approximately 1 – 2 minutes later, the color of the pad was compared with the color chart to determine the proteinuria level on a scale of 0 to 4+, where 0/trace = negative, 1+ = 30, 2+ = 100, 3+ = 300, and 4+ = >2,000 mg/dl.

Results

Female MRL/*lpr* mice at age of 4 weeks were divided into 4 groups (Figure 62): vehicle, CTX alone, murine B7-H4-Ig alone and CTX plus murine B7-H4-Ig combination treatment (Combo). CTX was given IP, 50 mg/kg, once every 2 weeks. Murine B7-H4-Ig was administrated IP, 100 µg (5 mg/kg), and twice every week. The Combo group received CTX injection every 2 weeks and murine B7-H4-Ig 2 times every week at 5 mg/kg. Mice were treated for 7 weeks.

Table 11: MRL/lpr mouse dsDNA autoantibody data table

	Mouse #	Mouse Age (weeks)							
		4	7	8	9	10	11	15	19
Control	246	0.01	0.16	0.48	2.24	1.44	3.09	2.25	7.00
	247	0.20	0.20	0.19	1.23	1.16	3.05	3.79	5.97
	248	0.01	3.33	11.41	12.27	9.18	7.63	10.16	9.50
	249	0.11	2.53	4.07	2.68	1.66	2.75	3.98	4.54
	250	0.21	0.12	0.40	1.16	0.86	0.96	6.05	6.10
CTX+ murine B7-H4-Ig	251	0.04	0.16	0.27	0.08	0.13	0.20	0.33	1.39
	252	0.09	0.11	0.62	0.94	0.71	0.60	2.37	0.85
	253	0.45	0.29	1.25	0.48	0.44	0.28	0.49	0.97
	254	0.17	0.36	0.19	0.58	0.74	0.55	0.59	0.91

	255	0.49	1.07	1.20	1.95	1.27	0.93	1.44	*
Murine B7-H4-Ig	256	0.12	0.14	1.63	1.33	1.36	2.19	3.35	4.52
	257	0.15	0.53	4.64	6.25	4.66	6.85	6.02	5.98
	258	0.22	0.73	0.76	0.92	0.54	0.71	1.24	1.57
	259	0.48	0.48	1.89	1.77	1.30	1.16	0.87	1.55
	260	0.13	0.33	1.09	0.82	0.60	0.56	2.27	3.16
CTX	261	0.15	0.55	2.50	3.46	1.50	2.97	2.93	2.61
	262	0.46	0.21	0.33	2.09	4.05	4.67	4.87	11.76
	263	0.05	1.07	2.03	2.26	2.95	3.75	2.62	3.05
	264	0.09	0.10	0.17	0.79	1.97	2.21	1.76	2.58
	265	0.01	0.10	0.06	0.26	0.39	0.55	1.11	1.26
		0.01 (BW)	- 0.02	0.00 (BW)	0.02	-0.02 (BW)	- 0.01	0.00 (BW)	0.01
PC-1 (1:5k)		0.00 (BW)	2.03 (PC)	0.00 (BW)	2.04 (PC)	0.00 (BW)	1.06 (PC)	0.00 (BW)	1.94 (PC)
PC-1 (1:10k)		0.00 (BW)	1.00 (PC)	0.00 (BW)	1.00 (PC)	0.00 (BW)	1.00 (PC)	0.00 (BW)	1.00 (PC)
PC-1 (1:20k)		0.00 (BW)	0.49 (PC)	0.00 (BW)	0.43 (PC)	0.00 (BW)	0.49 (PC)	0.00 (BW)	0.53 (PC)

PC: autoantibody positive controls; diluted at 1:5000, 1:10000, 1:20000

BW: Blank wells

Control Group: Negative plasma (1:2000) from Balb/C mice

- All MRL/lpr mouse plasma was diluted at 1:2000.
- OD450 readings were normalized against the readings of positive control diluted at 1:10000 on the same ELISA plate.

*mouse accidently died at the previous blood collection time point.

Plasma was collected from MRL/*lpr* mice pre-treatment (4 wk) and periodically up to 21 weeks of age. Anti-ds-DNA auto antibody was assessed and normalized against an internal control, which was a pool of plasma collected from older MRL/*lpr* mice (Table 11).

Combination of murine B7-H4-Ig and CTX significantly reduced anti-dsDNA auto antibody development in the MRL/*lpr* lupus mouse model. Figure 63 shows the relative anti-dsDNA auto antibody measurements, demonstrating that combination treatment with murine B7-H4-Ig and CTX prevented auto antibody development in the treated MRL/*lpr* mice and that the combination treatment was more efficacious than CTX or murine B7-H4-Ig treatment alone. At later time points, weeks 19 and 21, murine B7-H4-Ig alone treatment also demonstrated significantly lower autoantibody level,

suggesting murine B7-H4-Ig alone may be effective with an optimized dosing regimen.

This experiment was terminated when mice were 21 week old. Lymphoid organs were collected from control vehicle injected and treated mice. Combination of murine B7-H4-Ig and CTX significantly reduced lymphoproliferation in the MRL/*lpr* lupus mouse model. The combination treatment with murine B7-H4-Ig and CTX prevented enlargement of the spleen and lymph nodes. Proteinuria was analyzed using an AimStrip protein paper strip prior to euthanizing the animals. Figure 64 reveals that proteinuria was significantly reduced in the mice with combination treatment of murine B7-H4-Ig and CTX compared with mice in the remaining groups.

Kidneys were harvested for histology. H&E staining of the kidney sections showed that the murine B7-H4-Ig and CTX combination treatment decreased vasculitis and prevented glomerular damage. In the vehicle injected mouse kidney, massive lymphocytic infiltration was observed around the blood vessels and red blood cells were seen inside the glomerulus.

In the MRL/*lpr* mice, combination treatment with recombinant murine B7-H4-Ig protein and CTX remarkably prevented lupus disease progression, demonstrated by lower anti-dsDNA autoantibody, no enlargement of lymphoid organs, lower proteinuria, less lymphocyte infiltration in the kidney and evidence of an intact glomerulus.

Example 19: Generation of Immature Dendritic Cells

Small scale DC generation from about 50 mL of total blood (n = 3-5 donors)

- Fresh GM-CSF and IL-4 every 2 days + 10% human AB serum
- 7 day culture

Compare to serum free method

- Adding GM-CSF and IL-4 at the beginning of the differentiation
- 5 – 6 day culture

Harvest imDC

- Marker analysis: CD3, 14, 80, 83, 86, 11C, HLA-DR
- APC-B7-H4-Ig binding, competition with anti-B7-H4

B7-H4-Ig in vitro assay

- Use LPS + 10, 50, 250 μg/mL of B7-H4-Ig or control IgG1
- Collect supernatant for cytokine analysis – first TNFa and IL-6; save supernatant for other cytokine analysis in the future
- Harvest cells for DC maturation marker analysis: e.g. CD80, CD83, CCR7

Determining the role of B7-H4-Ig on DC maturation

- Use DC maturation cocktail and study role of B7-H4 on DC maturation
    - LPS alone; TNFa + PGE2; IL-1b + IL6 + TNFa + PGE2 …
    - 2 day maturation versus 1 day maturation; regular dish or ultra-low adherent dish; minimize cell numbers for each well/assay
        - Collect supernatant for cytokine analysis
        - Harvest cells for DC maturation marker analysis: e.g. CD80, CD83, CCR7
- Cells from 3 – 5 donors will be tested
- Determine release criteria on imDC, e.g. cell markers and B7-H4-Ig binding
- Determine maturation cocktail, timing and criteria on inhibition of cytokine and/or maturation markers

Reproducibility at a small scale

- Process cells from 3 donors using the defined imDC parameters
- Apply defined DC maturation cocktail and B7-H4-Ig
- Analyze cytokine and mDC markers

Large scale generation of imDC

- Start processing 2 -3 leukopaks using the defined imDC conditions from small scale
- 1 leukopak generally result in $5x10^8$ to $1x10^9$ imDC

- Cryopreserve imDC
- Thaw cells for DC maturation & B7-H4-Ig function analysis
- Use maturation condition defined by small scale
- Try to minimize cell numbers/well
- Test robustness and reproducibility of the assay: cytokine and mDC markers

- Provide imDC for receptor discovery - If small amount of cells are needed for the receptor discovery, imDC will be available from the small scale cell process after the confirmative studies

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We claim:

1. A fusion protein comprising a first fusion partner comprising a B7-H4 polypeptide or fragment thereof fused to a second polypeptide directly or indirectly via a linker peptide or polypeptide sequence that is fused to the second polypeptide, wherein the B7-H4 polypeptide or fragment thereof comprises the IgV of SEQ ID NO:63 or the IgV of SEQ ID NO:64 or the B7-H4 fusion protein lacks the KRRS sequence of SEQ ID NO:57.

2. The fusion protein of claim 1, wherein the B7-H4 polypeptide is a human or murine B7-H4 polypeptide.

3. The fusion protein of claim1, wherein the B7-H4 polypeptide comprises the extracellular domain of human B7-H4, or a fragment thereof having B7-H4 binding activity.

4. The fusion protein of claim 3 wherein the B7-H4 polypeptide is a fragment of at least 50 amino acids of the extracellular domain of human B7-H4 selected from the group consisting of

32-249, 32-248, 32-247, 32-246, 32-245, 32-244, 32-243, 32-242, 32-241, 31-249, 31-248, 31-247, 31-246, 31-245, 31-244, 31-243, 31-242, 31-241, 30-249, 30-248, 30-247, 30-246, 30-245, 30-244, 30-243, 30-242, 30-241, 29-249, 29-248, 29-247, 29-246, 29-245, 29-244, 29-243, 29-242, 29-241, 28-249, 28-248, 28-247, 28-246, 28-245, 28-244, 28-243, 28-242, 28-241, 27-249, 27-248, 27-247, 27-246, 27-245, 27-244, 27-243, 27-242, 27-241, 26-249, 26-248, 26-247, 26-246, 26-245, 26-244, 26-243, 26-242, 26-241, 25-249, 25-248, 25-247, 25-246, 25-245, 25-244, 25-243, 25-242, 25-241, 24-249, 24-248, 24-247, 24-246, 24-245, 24-244, 24-243, 24-242, 24-241, of SEQ ID NO:49, or SEQ ID NO:52, or

32-245, 32-244, 32-243, 32-242, 32-241, 32-240, 32-239, 32-238, 32-237, 31-245, 31-244, 31-243, 31-242, 31-241, 31-240, 31-239, 31-238, 31-237, 30-245, 30-244, 30-243, 30-242, 30-241, 30-240, 30-239, 30-238, 30-237, 29-245, 29-244, 29-243, 29-242, 29-241, 29-240, 29-239, 29-238, 29-237, 28-245, 28-244, 28-243, 28-242, 28-241, 28-240, 28-239, 28-238, 28-237, 27-245, 27-244, 27-243, 27-242, 27-241, 27-240, 27-239, 27-238, 27-237, 26-245, 26-244, 26-243, 26-242, 26-241, 26-240, 26-239, 26-238, 26-237, 25-245, 25-244, 25-243, 25-242, 25-241, 25-240, 25-239, 25-238,

25-237, 24-245, 24-244, 24-243, 24-242, 24-241, 24-240, 24-239, 24-238, 24-237, of SEQ ID NO:50 or SEQ ID NO:53, or

32-259, 32-258, 32-257, 32-256, 32-255, 32-254, 32-253, 32-252, 32-251, 31-259, 31-258, 31-257, 31-256, 31-255, 31-254, 31-253, 31-252, 31-251, 30-259, 30-258, 30-257, 30-256, 30-255, 30-254, 30-253, 30-252, 30-251, 29-259, 29-258, 29-257, 29-256, 29-255, 29-254, 29-253, 29-252, 29-251, 28-259, 28-258, 28-257, 28-256, 28-255, 28-254, 28-253, 28-252, 28-251, 27-259, 27-258, 27-257, 27-256, 27-255, 27-254, 27-253, 27-252, 27-251, 26-259, 26-258, 26-257, 26-256, 26-255, 26-254, 26-253, 26-252, 26-251, 25-259, 25-258, 25-257, 25-256, 25-255, 25-254, 25-253, 25-252, 25-251, 24-259, 24-258, 24-257, 24-256, 24-255, 24-254, 24-253, 24-252, 24-251, of SEQ ID NO:51 or SEQ ID NO:54, or

24-241, 24-240, 24-239, 24-238, 24-237, 24-236, 24-235, 24-234, 24-233, 23-241, 23-240, 23-239, 23-238, 23-237, 23-236, 23-235, 23-234, 23-233, 22-241, 22-240, 22-239, 22-238, 22-237, 22-236, 22-235, 22-234, 22-233, 21-241, 21-240, 21-239, 21-238, 21-237, 21-236, 21-235, 21-234, 21-233, 20-241, 20-240, 20-239, 20-238, 20-237, 20-236, 20-235, 20-234, 20-233, 19-241, 19-240, 19-239, 19-238, 19-237, 19-236, 19-235, 19-234, 19-233, 18-241, 18-240, 18-239, 18-238, 18-237, 18-236, 18-235, 18-234, 18-233, 17-241, 17-240, 17-239, 17-238, 17-237, 17-236, 17-235, 17-234, 17-233, 16-241, 16-240, 16-239, 16-238, 16-237, 16-236, 16-235, 16-234, 16-233, of SEQ ID NO:43 or SEQ ID NO:46, or

24-237, 24-236, 24-235, 24-234, 24-233, 24-232, 24-231, 24-230, 24-229, 23-237, 23-236, 23-235, 23-234, 23-233, 23-232, 23-231, 23-230, 23-229, 22-237, 22-236, 22-235, 22-234, 22-233, 22-232, 22-231, 22-230, 22-229, 21-237, 21-236, 21-235, 21-234, 21-233, 21-232, 21-231, 21-230, 21-229, 20-237, 20-236, 20-235, 20-234, 20-233, 20-232, 20-231, 20-230, 20-229, 19-237, 19-236, 19-235, 19-234, 19-233, 19-232, 19-231, 19-230, 19-229, 18-237, 18-236, 18-235, 18-234, 18-233, 18-232, 18-231, 18-230, 18-229, 17-237, 17-236, 17-235, 17-234, 17-233, 17-232, 17-231, 17-230, 17-229, 16-237, 16-236, 16-235, 16-234, 16-233, 16-232, 16-231, 16-230, 16-229, of SEQ ID NO:44 or SEQ ID NO:47, or

24-251, 24-250, 24-249, 24-248, 24-247, 24-246, 24-245, 24-244,

24-243, 23-251, 23-250, 23-249, 23-248, 23-247, 23-246, 23-245, 23-244, 23-243, 22-251, 22-250, 22-249, 22-248, 22-247, 22-246, 22-245, 22-244, 22-243, 21-251, 21-250, 21-249, 21-248, 21-247, 21-246, 21-245, 21-244, 21-243, 20-251, 20-250, 20-249, 20-248, 20-247, 20-246, 20-245, 20-244, 20-243, 19-251, 19-250, 19-249, 19-248, 19-247, 19-246, 19-245, 19-244, 19-243, 18-251, 18-250, 18-249, 18-248, 18-247, 18-246, 18-245, 18-244, 18-243, 17-251, 17-250, 17-249, 17-248, 17-247, 17-246, 17-245, 17-244, 17-243, 16-251, 16-250, 16-249, 16-248, 16-247, 16-246, 16-245, 16-244, 16-243, of SEQ ID NO:45 or SEQ ID NO:48.

5. The fusion protein of claim 1, wherein the B7-H4 polypeptide comprises at least 25 amino acids of the IgV domain as set forth in SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:83, SEQ ID NO:84 or SEQ ID NO:85.

6. The fusion protein of claim 1, wherein the B7-H4 polypeptide is a fragment of at least 50 amino acids of the IgV domain of human B7-H4 selected from the group consisting of

16-144, 16-145, 16-146, 16-147, 16-148, 16-149, 16-150, 16-151, 16-152, 17-144, 17-145, 17-146, 17-147, 17-148, 17-149, 17-150, 17-151, 17-152, 18-144, 18-145, 18-146, 18-147, 18-148, 18-149, 18-150, 18-151, 18-152, 19-144, 19-145, 19-146, 19-147, 19-148, 19-149, 19-150, 19-151, 19-152, 20-144, 20-145, 20-146, 20-147, 20-148, 20-149, 20-150, 20-151, 20-152, 21-144, 21-145, 21-146, 21-147, 21-148, 21-149, 21-150, 21-151, 21-152, 22-144, 22-145, 22-146, 22-147, 22-148, 22-149, 22-150, 22-151, 22-152, 23-144, 23-145, 23-146, 23-147, 23-148, 23-149, 23-150, 23-151, 23-152, 24-144, 24-145, 24-146, 24-147, 24-148, 24-149, 24-150, 24-151, 24-152, of any of SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, or SEQ ID NO:48, or

24-152, 24-153, 24-154, 24-155, 24-156, 24-157, 24-158, 24-159, 24-160, 25-152, 25-153, 25-154, 25-155, 25-156, 25-157, 25-158, 25-159, 25-160, 26-152, 26-153, 26-154, 26-155, 26-156, 26-157, 26-158, 26-159, 26-160, 27-152, 27-153, 27-154, 27-155, 27-156, 27-157, 27-158, 27-159, 27-160, 28-152, 28-153, 28-154, 28-155, 28-156, 28-157, 28-158, 28-159, 28-160, 29-152, 29-153, 29-154, 29-155, 29-156, 29-157, 29-158, 29-159, 29-160, 30-152, 30-153, 30-154, 30-155, 30-156, 30-157, 30-158, 30-159, 30-160, 31-152, 31-153, 31-154, 31-155, 31-156, 31-157, 31-158, 31-159,

31-160, 32-152, 32-153, 32-154, 32-155, 32-156, 32-157, 32-158, 32-159, 32-160, of any of SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, or SEQ ID NO:54,

20-148, 20-149, 20-150, 20-151, 20-152, 20-153, 20-154, 20-155, 20-156, 21-148, 21-149, 21-150, 21-151, 21-152, 21-153, 21-154, 21-155, 21-156, 22-148, 22-149, 22-150, 22-151, 22-152, 22-153, 22-154, 22-155, 22-156, 23-148, 23-149, 23-150, 23-151, 23-152, 23-153, 23-154, 23-155, 23-156, 24-148, 24-149, 24-150, 24-151, 24-152, 24-153, 24-154, 24-155, 20-156, 25-148, 25-149, 25-150, 25-151, 25-152, 25-153, 25-154, 25-155, 25-156, 26-148, 26-149, 26-150, 26-151, 26-152, 26-153, 26-154, 26-155, 26-156, 27-148, 27-149, 27-150, 27-151, 27-152, 27-153, 27-154, 27-155, 27-156, 28-148, 28-149, 28-150, 28-151, 28-152, 28-153, 28-154, 28-155, 28-156, of any of SEQ ID NO:65, SEQ ID NO:66, or SEQ ID NO:67, or

24-152, 24-153, 24-154, 24-155, 24-156, 24-157, 24-158, 24-159, 24-160, 25-152, 25-153, 25-154, 25-155, 25-156, 25-157, 25-158, 25-159, 25-160, 26-152, 26-153, 26-154, 26-155, 26-156, 26-157, 26-158, 26-159, 26-160, 27-152, 27-153, 27-154, 27-155, 27-156, 27-157, 27-158, 27-159, 27-160, 28-152, 28-153, 28-154, 28-155, 28-156, 28-157, 28-158, 28-159, 28-160, 29-152, 29-153, 29-154, 29-155, 29-156, 29-157, 29-158, 29-159, 29-160, 30-152, 30-153, 30-154, 30-155, 30-156, 30-157, 30-158, 30-159, 30-160, 31-152, 31-153, 31-154, 31-155, 31-156, 31-157, 31-158, 31-159, 31-160, 32-152, 32-153, 32-154, 32-155, 32-156, 32-157, 32-158, 32-159, 32-160, of any of SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:77, SEQ ID NO:78, or SEQ ID NO:79,

optionally with one to five amino acids of a signal peptide attached to the N-terminal end.

7. The fusion protein of claim 1, wherein the second polypeptide comprises an immunoglobulin constant domain.

8. The fusion protein of claim 7 wherein the immunoglobulin constant domain comprises the hinge, C_H2 and C_H3 regions of a murine immunoglobulin Cγ2a chain.

9. The fusion protein of claim 8, wherein the immunoglobulin constant domain is encoded by a nucleotide sequence having at least 80%,

85%, 90%, 95%, 99% or 100% identity to SEQ ID NO:86, SEQ ID NO:87, or SEQ ID NO:90.

10. The fusion protein of claim 1, wherein the immunoglobulin constant domain comprises the hinge, C_H2 and C_H3 regions of a human immunoglobulin Cγ1 chain.

11. The fusion protein of any of claims 1-10, further comprising a domain that mediates dimerization or multimerization of the fusion protein to form homodimers, heterodimers, homomultimers, or heteromultimers.

12. The fusion protein of claim 11, wherein the domain that mediates dimerization or multimerization is selected from the group consisting of one or more cysteines that are capable of forming an intermolecular disulfide bond with a cysteine on the partner fusion protein, a coiled-coil domain, an acid patch, a zinc finger domain, a calcium hand domain , a C_H1 region, a C_L region, a leucine zipper domain, an SH2 (src homology 2) domain, an SH3 (src Homology 3) domain, a PTB (phosphotyrosine binding) domain, a WW domain, a PDZ domain, a 14-3-3 domain, a WD40 domain, an EH domain, a Lim domain, an isoleucine zipper domain, and a dimerization domain of a receptor dimer pair.

13. The fusion protein of claim 1 comprising the polypeptide SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, or SEQ ID NO:133.

14. The fusion protein of claim 1 comprising the polypeptide SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, or SEQ ID NO:137.

15. A dimeric protein comprising a first and a second fusion protein, wherein the first and the second fusion proteins comprise the fusion protein of any of claims 1-14, wherein the first and the second fusion proteins are bound to one another by covalent or noncovalent bonds to form a dimer.

16. The dimeric or multimeric protein of any of claims 1-15, wherein the fusion proteins are bound together by disulfide bonds.

17. An isolated nucleic acid molecule comprising a nucleic acid sequence that encodes the fusion protein of any of claims 1-16.

18. A vector comprising the nucleic acid of claim 17.

19. A host cell comprising the vector of claim 18.

20. A method for treating or inhibiting one or more symptoms of an inflammatory response in an individual in need thereof comprising

administering to the individual a B7-H4 fusion protein of any of claims 1-16 in an amount effective to reduce or inhibit the one or more symptoms of the inflammatory response in the individual.

21. The method of claim 20 wherein the inflammatory response is associated with an autoimmune disease or disorder.

22. The method of claim 21 wherein the individual has an autoimmune disease selected from the group consisting of rheumatoid arthritis, systemic lupus erythematosus, alopecia areata, anklosing spondylitis, antiphospholipid syndrome, autoimmune addison's disease, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, autoimmune lymphoproliferative syndrome (alps), autoimmune thrombocytopenic purpura (ATP), Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac sprue-dermatitis, chronic fatigue syndrome immune deficiency, syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, cicatricial pemphigoid, cold agglutinin disease, Crest syndrome, Crohn's disease, Dego's disease, dermatomyositis, dermatomyositis - juvenile, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia – fibromyositis, grave's disease, guillain-barre, hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), Iga nephropathy, insulin dependent diabetes (Type I), juvenile arthritis, Meniere's disease, mixed connective tissue disease, multiple sclerosis, relapsing-remitting multiple sclerosis, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychondritis, polyglancular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, Raynaud's phenomenon, Reiter's syndrome, rheumatic fever, sarcoidosis, scleroderma, Sjogren's syndrome, stiff-man syndrome, Takayasu arteritis, temporal arteritis/giant cell arteritis, ulcerative colitis, uveitis, vasculitis, vitiligo, and Wegener's granulomatosis.

23. A method for treating inflammation in a subject comprising administering an effective amount of the B7-H4 fusion protein of any of claims 1-16 to the subject to inhibit or reduce differentiation of, proliferation

of, activity of, and/or cytokine production and/or secretion by an immune cell selected from the group consisting of Th1, Th17, Th22, other cells that secrete, or cells that cause other cells to secrete, inflammatory molecules.

24. The method of claim 23 wherein the B7-H4 fusion protein of any of claims 1-18 is administered in an effective amount to inhibit or reduce differentiation of, proliferation of, activity of, and/or cytokine production and/or secretion by Th17, Th1 or Th22 cells.

25. A method for treating inflammation in a subject comprising administering to the subject an effective amount of the B7-H4 fusion protein of any of claims 1-16 to enhance their suppressive effect on Th1 or Th17 cells.

26. A method for treating inflammation in a subject comprising administering to the subject an effective amount of the B7-H4 fusion protein of any of claims 1-16 to promote or enhance IL-10 production by Tregs.

27. A method for treating inflammation in a subject comprising administering to the subject an effective amount of the B7-H4 fusion protein of any of claims 1-16 to increase cell numbers or increase populations of Tregs.

28. A method for treating inflammation in a subject comprising administering to the subject an effective amount of the B7-H4 fusion protein of any of claims 1-16 to inhibit the Th1 and Th17 pathways and to enhance the suppressive activity of Tregs on the Th17 pathway or to promote or enhance IL-10 secretion by Tregs.

29. A method for reducing proinflammatory molecule production in a subject comprising administering to the subject an effective amount of the B7-H4 fusion protein of any of claims 1-18, or fragments thereof to inhibit the production of one or more cytokines in the subject.

30. Any one of the methods of claims 20-29 further comprising administering a second therapeutic agent.

31. A method for selecting a subject for treatment with a B7-H4 fusion protein comprising

screening subjects for levels of one or more cytokines selected from the group consisting of IL-1β, TNF-α, TGF-beta, IFN-γ, IL-10, IL-17, IL-6, IL-23, IL-22, and IL-21, or MMPs,

comparing the levels of the cytokines to levels of the levels of cytokines or MMPs in a control subject that does not have an inflammatory disorder, and

administering to the subject an effective amount of a B7-H4 fusion protein of any of claims 1-16 to inhibit or reduce one or more symptoms of an inflammatory disorder if the levels of one or more cytokines are elevated in the subject compared to levels in the control subject that does not have an inflammatory disorder.

32. A method for selecting a subject for treatment with a B7-H4 fusion protein comprising screening subjects for levels of mRNA encoding one or more cytokines selected from the group consisting of IL-1β, TNF-α, TGF-beta, IFN-γ, IL-10, IL-17, IL-6, IL-23, IL-22, and IL-21, or MMPs,

comparing the levels of the mRNAs encoding cytokines or MMPs to levels of the mRNAs encoding cytokines or MMPs in a control subject that does not have an inflammatory disorder, and

administering to the subject an effective amount of a B7-H4 fusion protein of any of claims 1-16 to inhibit or reduce one or more symptoms of an inflammatory disorder if the levels of one or more mRNAs encoding cytokines are elevated in the subject compared to levels in the control subject that does not have an inflammatory disorder.

33. A method for for selecting a subject for treatment with a B7-H4 fusion protein comprising

screening subjects for polymorphisms in genes encoding one or more cytokines selected from the group consisting of IL-1β, TNF-α, TGF-beta, IFN-γ, IL-10, IL-17, IL-6, IL-23, IL-22, and IL-21, or MMPs, and

administering to the subject an effective amount of a B7-H4 fusion protein of any of claims 1-16 to inhibit or reduce one or more symptoms of an inflammatory disorder if one or more of the genes encoding cytokines or MMPs has a polymorphism.

34. The method of any of claims 20-31wherein the subject did not previously respond to treatment with TNF blockers.

35. The method of any of claims 20-34 wherein the subject has chronic and persistent inflammation.

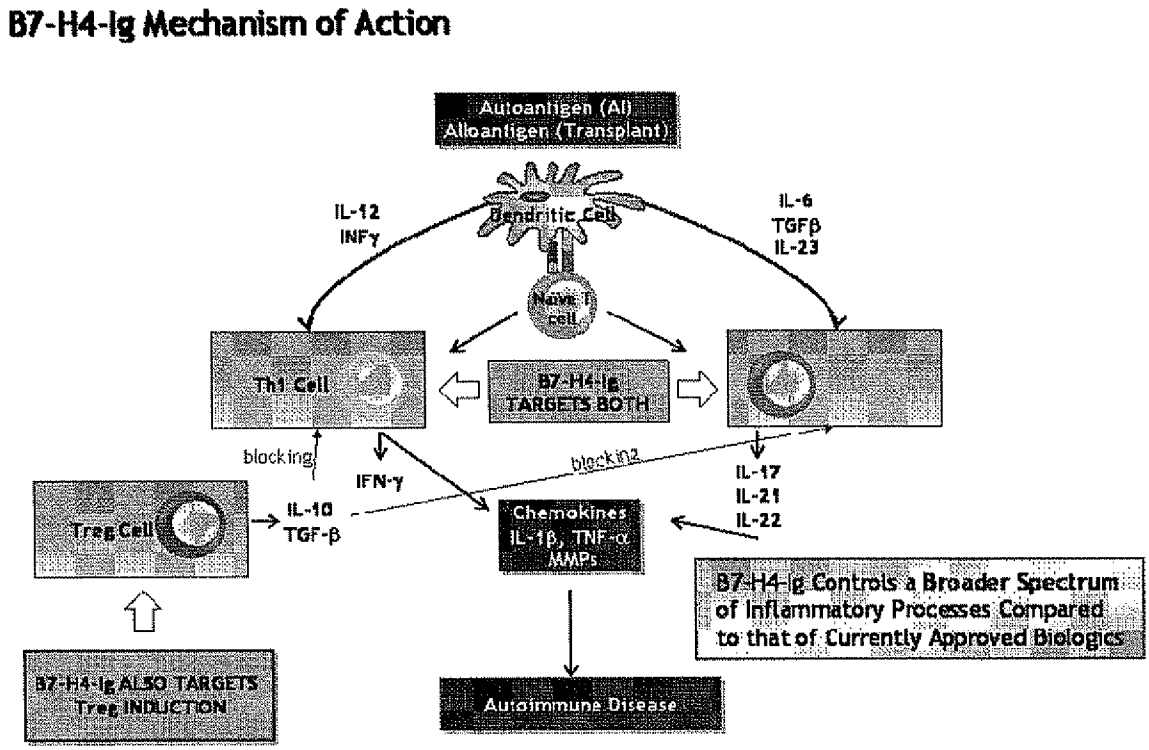

B7-H4-Ig Mechanism of Action
Autoantigen (Al)
Alloantigen (Transplant)
IL-12
INFγ
Dendritic Cell
IL-6
TGFβ
IL-23
Naive T cell
Th1 Cell
B7-H4-Ig
TARGETS BOTH
blocking
IFN-γ
blocking2
IL-17
IL-21
IL-22
Treg Cell
IL-10
TGF-β
Chemokines
IL-1β, TNF-α
MMPs
B7-H4-Ig Controls a Broader Spectrum
of Inflammatory Processes Compared
to that of Currently Approved Biologics
B7-H4-Ig ALSO TARGETS
Treg INDUCTION
Autoimmune Disease

Figure 1A

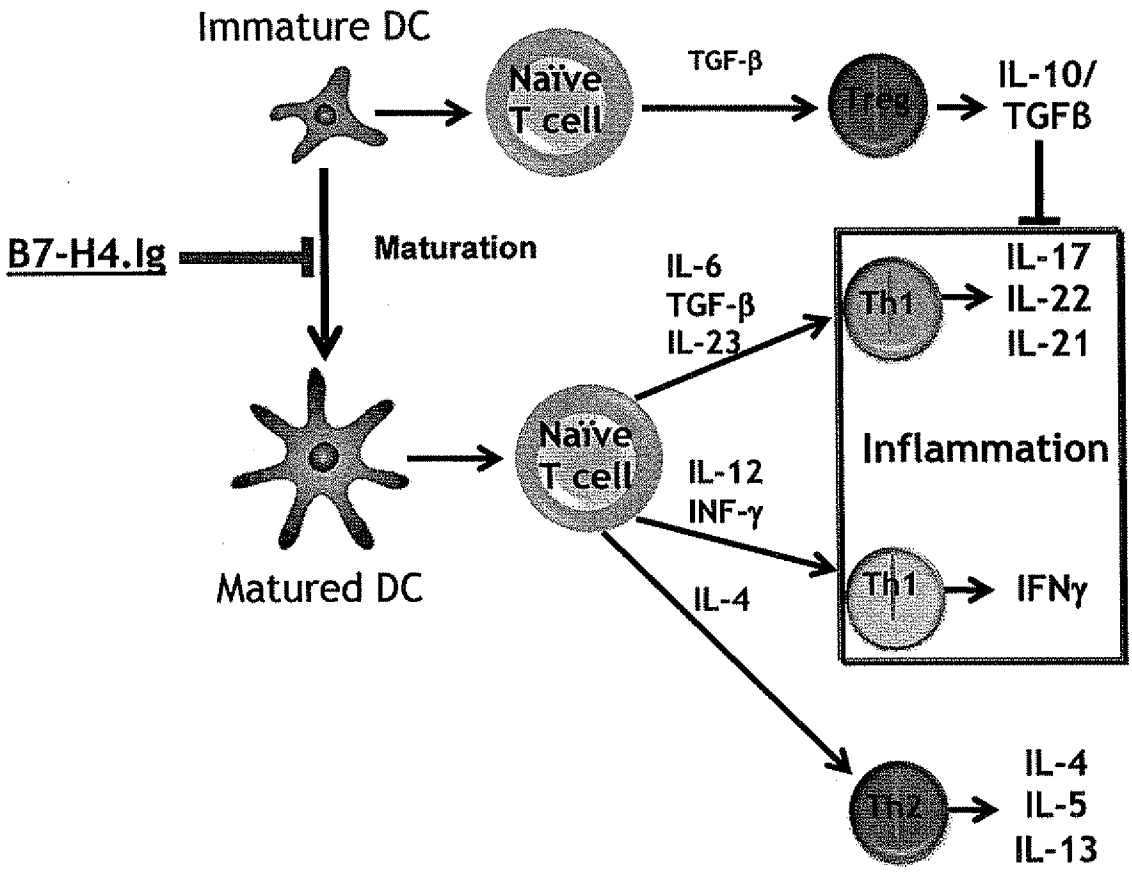


Figure 1B

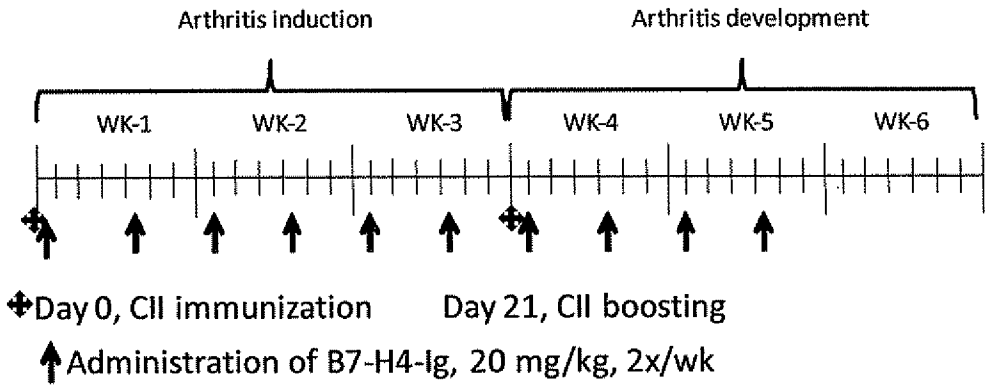


Figure 2

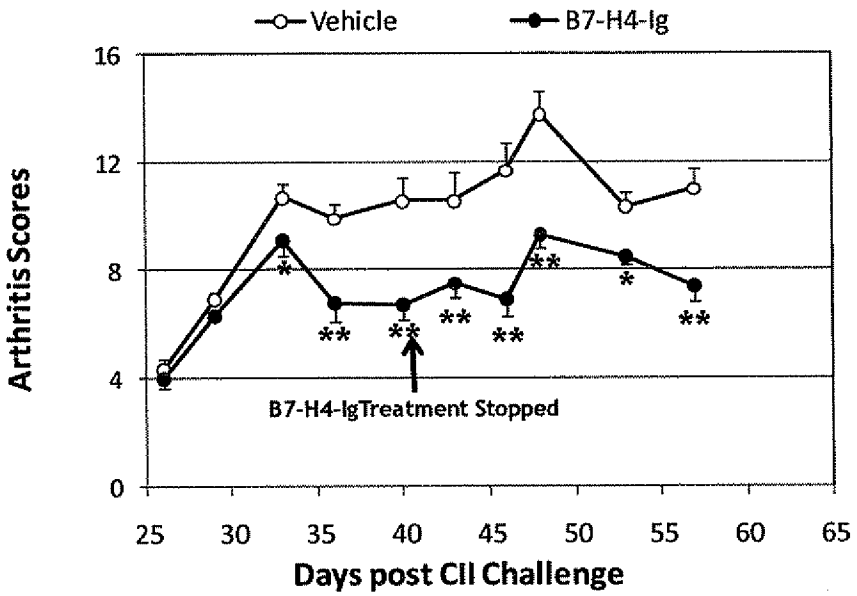


Figure 3

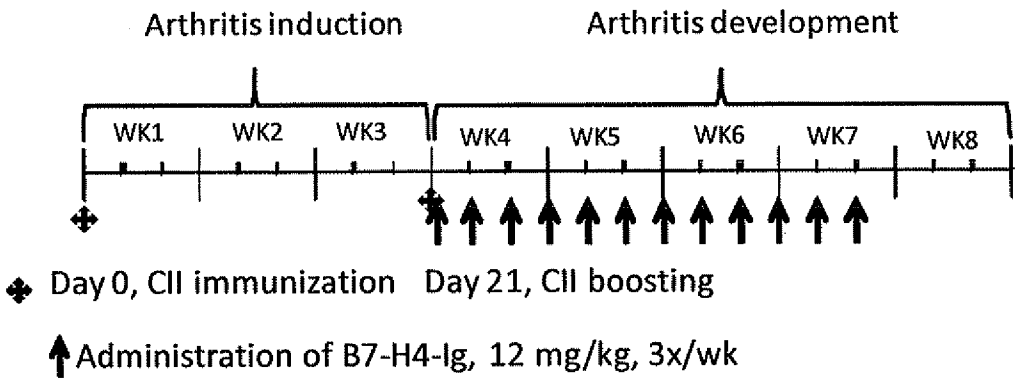


Figure 4

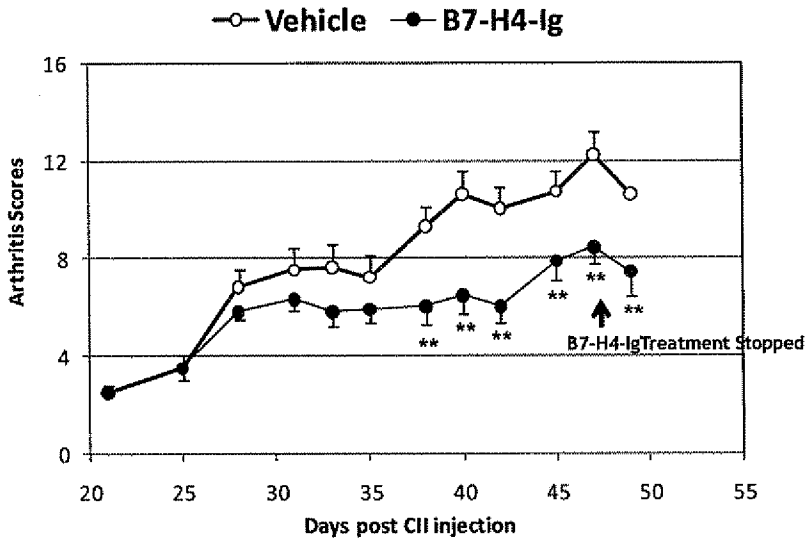


Figure 5

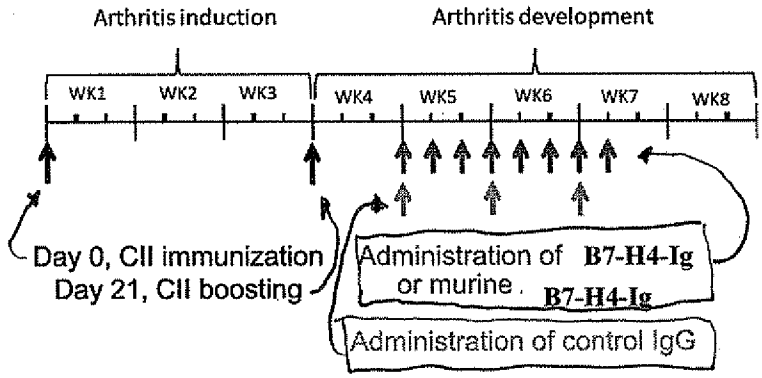


Figure 6

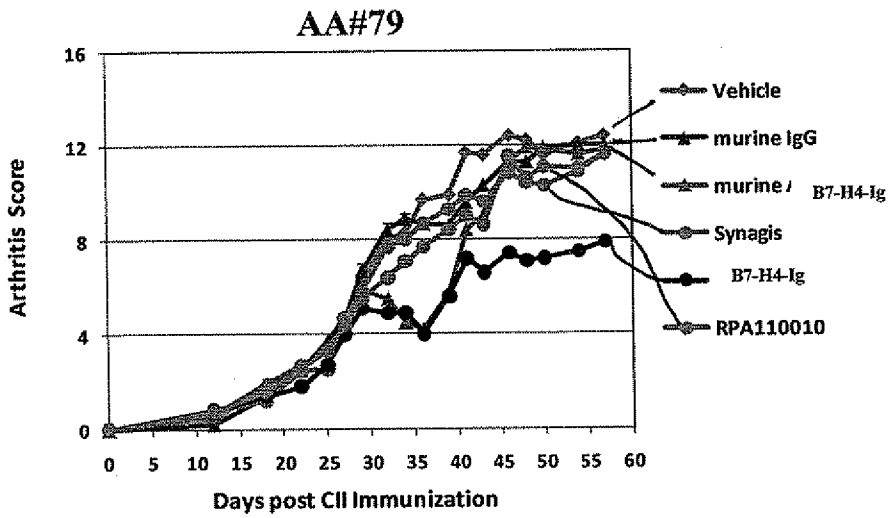


Figure 7

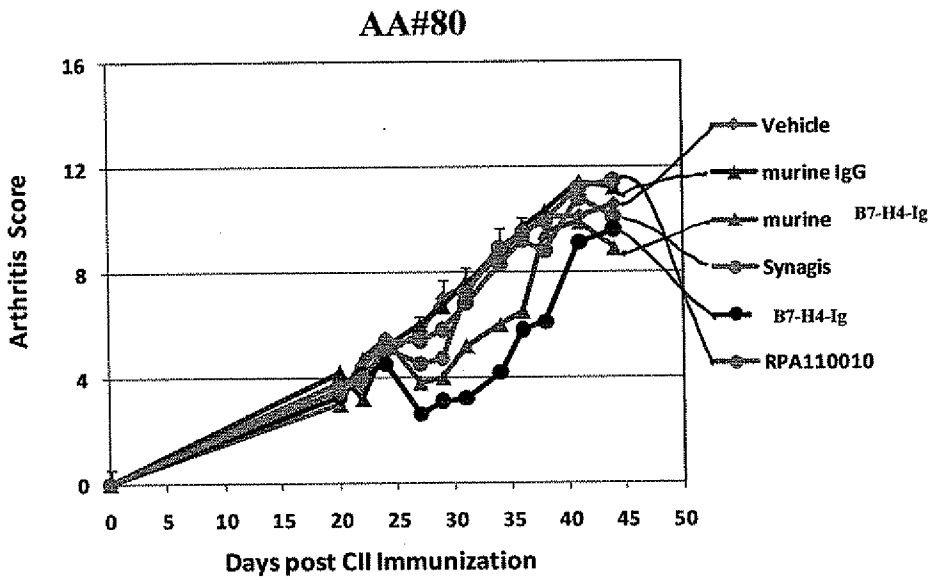


Figure 8

			Sample #	1	2	3	7	8	9	13	14	15	4	5	6	10	11	12	16	17	18
			Group	Human IgG1 Control									Human B7-H4-Ig								
			Mouse #	#3324	#3329	#3330	#3324	#3329	#3330	#3324	#3329	#3330	#3332	#3334	#3338	#3332	#3334	#3338	#3332	#3334	#3335
			Day	27	27	27	34	34	34	41	41	41	27	27	27	34	34	34	41	41	41
Analytes	Units	LLOQ		2	4	5	10	8	8	12	9	10	2	3	2	5	4	4	9	6	9
clinical score	ug/mL	10.4		37.6	53	44.4	48	41.3	50.3	47.7	39	43.2	57.3	53.7	42.7	37.8	48.5	56.1	46.8	49.5	46
Apo A1 (Apolipoprotein A1)	pg/mL	12		39.9	119	151	57.1	59.2	135	39.9	86.7	95.4	135	135	151	96.7	142	136	108	105	140
CD40	pg/mL	92		1800	1120	1930	1730	1120	1470	1850	2260	1270	1980	1550	1320	1370	2030	2380	1680	1630	1830
CD40 Ligand	ug/mL	0.83		10.8	10.9	9.26	13.1	16.6	12.7	17.2	13.9	21.3	13.4	8.16	7.58	17.2	8.79	12.4	17.1	10.6	12.1
CRP (C Reactive Protein)	pg/mL	39.1		18.4	26.8	22.4	21.4	20.4	18.4	22.4	20.4	28.9	18.4	23.4	28.4	22.4	14.3	20.4	28.4	26.3	18.4
EGF (Epidermal Growth Factor)	pg/mL	66.95		20.2	22.2	42.6	32.2	25.9	92.5	25.9	31.2	78.5	20.2	20.2	17.1	22.2	22.2	20.2	21.2	20.2	16
Endothelin-1	pg/mL	12.25		558	662	778	599	580	712	619	582	587	626	613	623	630	602	700	628	493	694
Eotaxin	pg/mL	0.953		25.6	32.4	48.6	25	34.3	37.5	27.7	29.7	36.9	33	29.1	26.4	31.7	33	29.1	36.9	30.4	27.7
Factor VII	ng/mL	0.9926		<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	0.196	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>
FGF-9 (Fibroblast Growth Factor-9)	ng/mL	0.577		15.7	17.8	17.8	17.8	16.8	17.8	18.8	17.8	17.8	18.8	17.8	16.8	17.8	17.8	17.8	19.9	17.8	18.8
FGF-basic (Fibroblast Growth Factor-basic)	ug/mL	170		<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	207	<LOW>	<LOW>	<LOW>	135	<LOW>	<LOW>	<LOW>	118	<LOW>
Fibrinogen	ug/mL	15.3		15.3	17.2	19	14.1	17.4	15.5	17.2	18.8	15.7	18	19.5	16.6	17.1	18.1	16.2	26.9	16.3	20.9
GCP-2 (Granulocyte Chemotactic Protein-2)	pg/mL	0.0245		<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>
GM-CSF (Granulocyte Macrophage-Colony Stimulating Factor)	pg/mL	8.746		<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	0.308	<LOW>	<LOW>	<LOW>	<LOW>	4.16	2.96	1.01	0.477	1.54
GSF-alpha (Glutathione S-Transferase alpha)	ng/mL	0.42		135	119	133	115	200	97.4	251	215	235	161	204	123	281	362	160	267	270	193
Haptoglobin	ug/mL	0.84		7.14	8.58	7.14	8.58	8.58	5.96	7.14	7.14	7.14	<LOW>	<LOW>	7.14	7.14	7.14	8.58	8.58	8.58	7.14
IFN-gamma (Interferon-gamma)	pg/mL	57.82		26.6	58.1	8.66	15.6	43.1	9.7	59.3	13.4	15.2	18.7	69.7	36.1	91	12.7	45.4	12.3	59.9	14.1
IgA (Immunoglobulin A)	ug/mL	1.89		<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	65	<LOW>	<LOW>	<LOW>	94.2	94.2	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>
IL-10 (Interleukin-10)	pg/mL	109.26		<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	40.9	32.4	21.3	<LOW>	<LOW>	27	17.1	17.1	17.1	17.1	<LOW>	<LOW>
IL-11 (Interleukin-11)	pg/mL	87.38		<LOW>	25.2	21.8	<LOW>	<LOW>	<LOW>	<LOW>	0.0914	0.0532	0.0532	<LOW>	0.0532	0.0725	<LOW>	<LOW>	<LOW>	<LOW>	0.0914
IL-12p70 (Interleukin-12p70)	ng/mL	0.5712		<LOW>	<LOW>	<LOW>	<LOW>	0.0532	0.0832	<LOW>	0.0914	0.0532	0.0532	<LOW>	<LOW>	0.00451	0.0152	0.00808	0.00572	0.00572	0.00572
IL-13 (Interleukin-13)	ng/mL	0.152		<LOW>	0.00572	<LOW>	0.00451	<LOW>	<LOW>	0.00572	0.00808	<LOW>	<LOW>	<LOW>	<LOW>	0.00451	0.0152	0.00808	0.00572	0.00572	<LOW>
IL-17 (Interleukin-17)	pg/mL	0.674		10.9	13.3	12.3	11.9	11.1	11.1	14.1	13.1	12.3	12.3	14.1	11.7	11.7	10.9	13.1	13.3	11.7	11.5
IL-18 (Interleukin-18)	pg/mL	44.92		386	310	575	398	297	470	447	529	2200	483	284	361	361	447	587	488	284	1950
IL-1alpha (Interleukin-1alpha)	pg/mL	0.447		3.58	4.36	5.87	4.36	5.12	4.36	5.12	4.36	3.58	4.36	4.16	1.98	5.12	3.58	5.12	4.36	5.12	5.12
IL-1beta (Interleukin-1beta)	pg/mL	67		<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	8.57	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>
IL-2 (Interleukin-2)	pg/mL	21.274		<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>
IL-3 (Interleukin-3)	pg/mL	79.54		<LOW>	17.7	<LOW>	17.7	17.7	17.7	35.7	35.7	<LOW>	<LOW>	27.9	17.7	17.7	<LOW>	17.7	35.7	17.7	17.7
IL-4 (Interleukin-4)																					

Figure 9

			Sample #	1	2	3	7	8	9	13	14	15	4	5	6	10	11	12	16	17	18
			Group	Human IgG1 Control									Human B7-H4-Ig								
			Mouse #	#3324	#3329	#3330	#3324	#3329	#3330	#3324	#3329	#3330	#3332	#3334	#3339	#3332	#3336	#3338	#3332	#3334	#3338
			Day	27	27	27	34	34	34	41	41	41	27	27	27	34	34	34	41	41	41
Analytes	Units	LDD																			
IL-5 (Interleukin-5)	pg/mL	0.194		0.488	0.403	0.264	0.333	0.474	0.333	0.616	0.399	0.333	0.474	0.333	0.333	0.474	0.264	0.403	0.474	0.264	0.264
IL-6 (Interleukin-6)	pg/mL	19.852		6.27	6.27	<LOW>	13.2	98	34.1	74.2	67.5	93.7	9.38	9.23	<LOW>	106	5.57	14.5	49.5	66.2	14.5
IL-7 (Interleukin-7)	pg/mL	0.309		0.0693	<LOW>	<LOW>	0.0583	<LOW>	<LOW>	0.0593	0.0593	<LOW>	<LOW>	<LOW>	0.102	0.0823	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>
IP-10 (Inducible Protein-10)	pg/mL	40.28		65.3	52	55.5	68.1	52	84.3	54.7	68.1	66.8	57.3	55.5	38.6	81	59	54.2	69.3	47.6	59
KC/GROalpha (Melanoma Growth Stimulatory Activity Protein)	pg/mL	0.1739		0.0804	0.0795	0.0683	0.165	0.405	0.273	0.293	0.298	0.626	0.156	0.0826	0.049	0.376	0.0813	0.148	0.164	0.221	0.0683
LIF (Leukemia Inhibitory Factor)	pg/mL	43.6		1650	1350	1470	1620	1620	1480	1850	1380	1470	1540	1500	1500	1430	1500	1480	1540	1540	1580
Lymphotactin	pg/mL	85.48		198	178	189	219	161	205	178	183	194	164	169	194	196	162	197	187	148	187
MCP-1 (Monocyte Chemoattractant Protein-1)	pg/mL	16.929		74.4	64.7	70.5	99.8	78.5	117	90.2	129	106	72.6	56.4	66.9	84.6	56	101	73.9	53.1	76.8
MCP-3 (Monocyte Chemoattractant Protein-3)	pg/mL	91.96		168	169	138	188	160	193	235	236	242	169	139	129	208	101	139	197	115	132
MCP-5 (Monocyte Chemoattractant Protein-5)	pg/mL	48.38		35.7	37.1	35.5	37.5	46.2	41	46.5	47.4	52.8	41.4	33.8	28.6	57.8	29.2	46.7	48.8	35.7	37.9
M-CSF (Macrophage-Colony Stimulating Factor)	pg/mL	0.01795		6.19	6.6	6.59	6.48	6.3	6.09	6.38	6.22	6.17	6.88	6.51	6.21	5.79	6.35	6.51	6.02	5.67	6.07
MDC (Macrophage-Derived Chemokine)	pg/mL	21.85		1250	1310	1070	1270	1320	1130	1910	1430	1150	1250	1110	1040	1390	1120	1360	1350	1110	1290
MIP-1alpha (Macrophage Inflammatory Protein-1alpha)	ng/mL	0.2255		3.03	3.82	3.03	3.13	2.88	2.94	3.03	3.13	3.32	2.83	3.13	3.03	3.03	2.88	3.08	3.13	2.93	3.03
MIP-1beta (Macrophage Inflammatory Protein-1beta)	pg/mL	77.66		178	173	198	259	157	358	144	115	152	110	115	173	181	127	277	148	70.7	115
MIP-1gamma (Macrophage Inflammatory Protein-1gamma)	ng/mL	0.0736		36.9	37.1	37.9	42.2	41	40.9	45.4	44.8	44.6	43.2	38.6	32.1	50.4	30.1	41	42.8	32.7	42.5
MIP-2 (Macrophage Inflammatory Protein-2)	pg/mL	7.274		16.1	22.3	23.4	27.2	32.3	33.6	33.6	33.6	50.3	20.9	16.1	21.6	36.8	26.6	22.3	25.9	29.4	19.7
MIP-3beta (Macrophage Inflammatory Protein-3beta)	ng/mL	0.488		2.89	3.05	2.6	2.63	2.52	2.63	3.27	2.73	2.35	2.6	2.79	2.52	2.79	2.69	3.11	3.05	2.87	2.87
MMP-9 (Matrix Metalloproteinase-9)	ng/mL	10		166	177	431	207	259	288	272	250	1120	266	140	266	583	334	365	253	218	561
MPO (Myeloperoxidase)	ng/mL	0.95		216	191	265	263	253	254	259	263	240	248	227	191	247	254	242	208	228	285
Myeloperin	ng/mL	34		8.95	11.4	23.8	10	9.69	12.7	49.8	90.2	385	17.3	12.1	9.69	14.6	9.33	30.5	10	8.58	59.6
OSM (Oncostatin M)	ng/mL	0.13		<LOW>	0.0103	<LOW>	<LOW>	<LOW>	0.00578	<LOW>	0.0103	0.0103	<LOW>	<LOW>	0.00578	0.0103	0.0103	<LOW>	<LOW>	<LOW>	<LOW>
RANTES (Regulation Upon Activation, Normal T-Cell Expressed and Secreted)	pg/mL	0.5		0.0826	<LOW>	<LOW>	0.0826	<LOW>	<LOW>	<LOW>	0.165	<LOW>	0.0826	<LOW>	0.0826	0.105	0.105	<LOW>	0.0826	<LOW>	0.105
SAP (Serum Amyloid P)	ug/mL	5.4		43.5	34.2	42.9	38.4	48.6	38.3	49.2	46.2	48.8	41	39.5	39.7	51.8	31	42.6	47.9	38.6	46
SCF (Stem Cell Factor)	pg/mL	75.12		226	207	220	214	182	214	214	249	201	204	194	275	182	220	217	175	198	210
SGOT (Serum Glutamic-Oxaloacetic Transaminase)	ug/mL	1.86		84.6	79.7	75.4	75	140	81.9	84	93.9	78.4	74.3	84.6	94.9	114	73.1	55.8	89.8	93.9	71.1
TIMP-1 (Tissue Inhibitor of Metalloproteinase Type 1)	ng/mL	0.18		1.38	1.13	1.23	1.7	1.88	1.31	3.04	3.21	4.33	1.38	1.31	1.14	4.85	1.22	1.49	4.76	1.74	2.16
Tissue Factor	ng/mL	0.5175		9.17	10	9.17	10.8	10	12.5	10.8	9.17	11.7	8.32	11.7	10	10.8	10.4	13.3	10.8	9.17	9.17
TNF-alpha (Tumor Necrosis Factor-alpha)	pg/mL	0.137		0.0965	<LOW>	<LOW>	0.0778	<LOW>	0.0326	0.122	0.027	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>	<LOW>
TPO (Thrombopoietin)	pg/mL	2.66		38.9	34.2	38.9	36.1	34.2	39.8	38	39.8	32.4	38	39.6	34.2	63.4	30.4	26.1	38	32.4	36.1
VCAM-1 (Vascular Cell Adhesion Molecule-1)	ng/mL	19		2260	2080	3160	2290	2120	2460	2150	1680	1930	2250	2120	2130	1860	2190	2470	1840	1940	2390
VEGF (Vascular Endothelial Cell Growth Factor)	pg/mL	38.2		144	136	139	93.6	128	114	159	171	212	159	128	160	160	141	155	125	120	155
vWF (von Willebrand Factor)	ng/mL	98.6		125	131	168	107	92.4	139	131	103	211	143	123	107	101	137	135	113	111	133

Figure 10

Analytes	Units	Human IgG1 control vs. human B7-H4 Ig				R2 vs. clin. score
		D27	D34	D41	All	
Clinical score		0.12	0.00	0.08	0.04	
Apo A1 (Apolipoprotein A1)	ug/mL	0.19	0.48	0.10	0.10	0.04
CD40	pg/mL	0.17	0.17	0.07	0.01	0.19
CD40 Ligand	pg/mL	0.42	0.12	0.40	0.21	0.00
CRP (C-Reactive Protein)	ug/mL	0.41	0.33	0.11	0.13	0.14
EGF (Epidermal Growth Factor)	pg/mL	0.20	0.36	0.25	0.11	0.04
Eotaxin-1	pg/mL	0.14	0.08	0.04	0.01	0.05
Eotaxin	pg/mL	0.26	0.40	0.45	0.40	0.00
Factor VII	ng/mL	0.05	0.41	0.48	0.12	0.01
FGF-9 (Fibroblast Growth Factor-9)	ng/mL					
FGF-basic (Fibroblast Growth Factor-basic)	ng/mL	0.24	0.19	0.18	0.09	0.19
Fibrinogen	ug/mL					
GCP-2 (Granulocyte Chemotactic Protein-2)	ng/mL	0.34	0.13	0.30	0.10	0.00
GM-CSF (Granulocyte Macrophage-Colony Stimulating Factor)	pg/mL					
GST-alpha (Glutathione S-Transferase-alpha)	ng/mL					
Haptoglobin	ug/mL	0.31	0.19	0.37	0.17	0.11
IFN-gamma (Interferon-gamma)	pg/mL		0.13	0.06	0.44	0.08
IgA (Immunoglobulin A)	ug/mL	0.32	0.35	0.49	0.29	
IL-10 (Interleukin-10)	pg/mL					
IL-11 (Interleukin-11)	pg/mL					
IL-12p70 (Interleukin-12p70)	ng/mL					
IL-17 (Interleukin-17)	ng/mL					
IL-18 (Interleukin-18)	ng/mL	0.32	0.27	0.13	0.50	0.01
IL-1alpha (Interleukin-1alpha)	pg/mL	0.28	0.20	0.43	0.42	0.15
IL-1beta (Interleukin-1beta)	ng/mL	0.19	0.50	0.19	0.34	0.10
IL-2 (Interleukin-2)	pg/mL					
IL-3 (Interleukin-3)	pg/mL					
IL-4 (Interleukin-4)	pg/mL					
IL-5 (Interleukin-5)	ng/mL	0.44	0.50	0.20	0.26	0.03
IL-6 (Interleukin-6)	pg/mL	0.33	0.44	0.06	0.22	0.52
IL-7 (Interleukin-7)	ng/mL					
IP-10 (Inducible Protein-10)	pg/mL	0.06	0.40	0.29	0.46	0.13
KC/GRO-alpha (Melanoma Growth Stimulatory Activity Protein)	ng/mL	0.36	0.31	0.07	0.11	0.34
LIF (Leukemia Inhibitory Factor)	pg/mL	0.40	0.13	0.41	0.26	0.12
Lymphotactin	pg/mL	0.16	0.33	0.24	0.10	0.04
MCP-1 (Monocyte Chemoattractant Protein-1)	pg/mL	0.23	0.18	0.02	0.02	0.29
MCP-2 (Monocyte Chemoattractant Protein-2)	pg/mL	0.18	0.15	0.01	0.01	0.44
MCP-3 (Monocyte Chemoattractant Protein-3)	pg/mL	0.37	0.37	0.07	0.29	0.23
MCP-5 (Monocyte Chemoattractant Protein-5)	ng/mL	0.39	0.40	0.50	0.50	0.12
M-CSF (Macrophage-Colony Stimulating Factor)	pg/mL	0.23	0.33	0.26	0.28	0.16
MDC (Macrophage-Derived Chemokine)	ng/mL	0.19	0.44	0.14	0.11	0.05
MIP-1alpha (Macrophage Inflammatory Protein-1alpha)	pg/mL	0.10	0.22	0.18	0.11	0.01
MIP-1beta (Macrophage Inflammatory Protein-1beta)	ng/mL	0.42	0.44	0.08	0.22	0.27
MIP-1gamma (Macrophage Inflammatory Protein-1gamma)	pg/mL	0.37	0.31	0.03	0.05	0.39
MIP-2 (Macrophage Inflammatory Protein-2)	ng/mL	0.12	0.16	0.25	0.30	0.01
MIP-3beta (Macrophage Inflammatory Protein-3beta)	ng/mL	0.37	0.05	0.27	0.39	0.10
MMP-9 (Matrix Metalloproteinase-9)	ng/mL	0.46	0.06	0.30	0.25	0.26
MPO (Myeloperoxidase)	ng/mL	0.38	0.16	0.12	0.13	0.16
Myoglobin	ng/mL					
OSM (Oncostatin M)	pg/mL					
RANTES (Regulation Upon Activation, Normal T-Cell Expressed and Secreted)	ug/mL	0.32	0.50	0.13	0.26	0.32
SAP (Serum Amyloid P)	pg/mL	0.41	0.43	0.07	0.19	0.03
SCF (Stem Cell Factor)	ug/mL	0.26	0.27	0.43	0.29	0.01
SGOT (Serum Glutamic-Oxaloacetic Transaminase)	ng/mL	0.39	0.25	0.24	0.48	0.32
TIMP-1 (Tissue Inhibitor of Metalloproteinase Type-1)	ng/mL	0.31	0.38	0.21	0.37	0.08
Tissue Factor	ng/mL					
TNF-alpha (Tumor Necrosis Factor-alpha)	ng/mL	0.50	0.49	0.34	0.39	0.00
TPO (Thrombopoietin)	ng/mL	0.19	0.33	0.33	0.23	0.04
VCAM-1 (Vascular Cell Adhesion Molecule-1)	pg/mL	0.28	0.01	0.05	0.47	0.00
VEGF (Vascular Endothelial Cell Growth Factor)	ng/mL	0.19	0.28	0.37	0.49	0.07
vWF (von Willebrand Factor)						

Figure 11

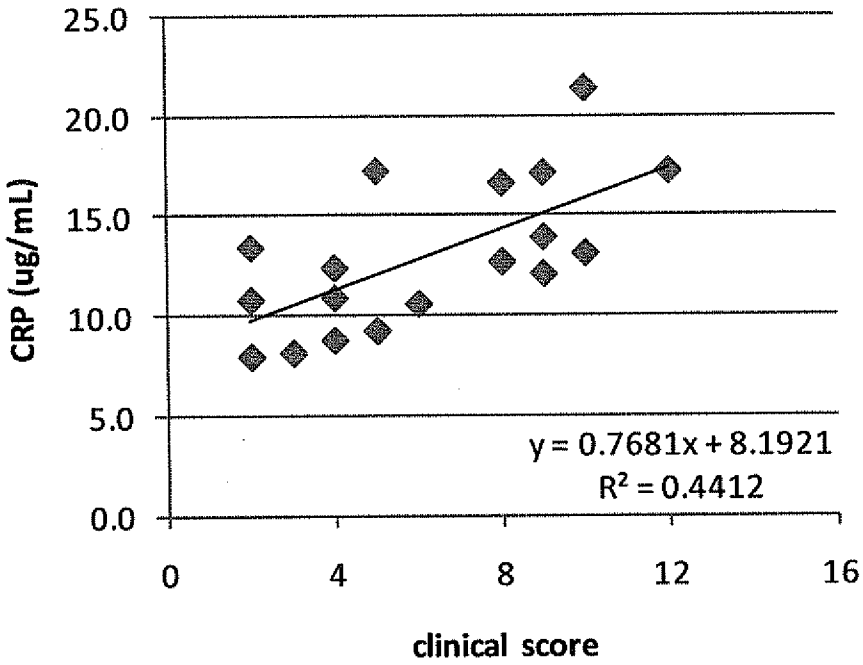


Figure 12

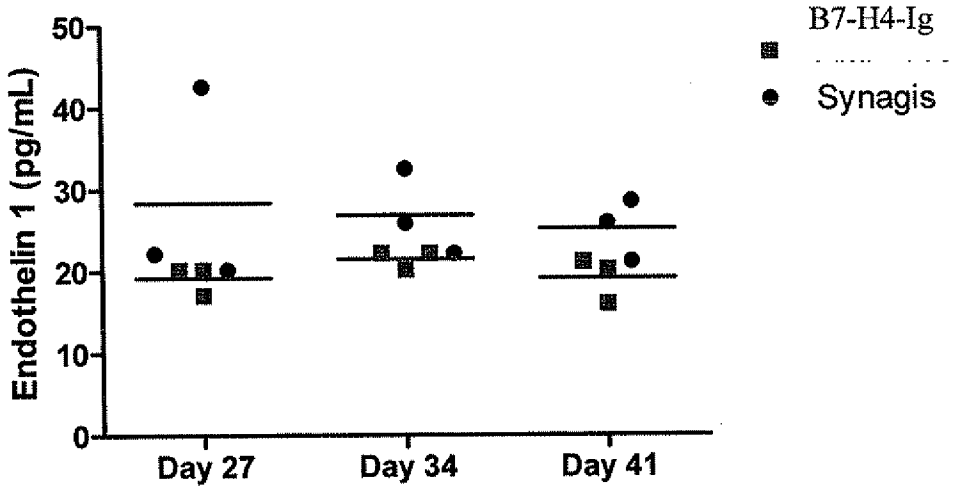


Figure 13

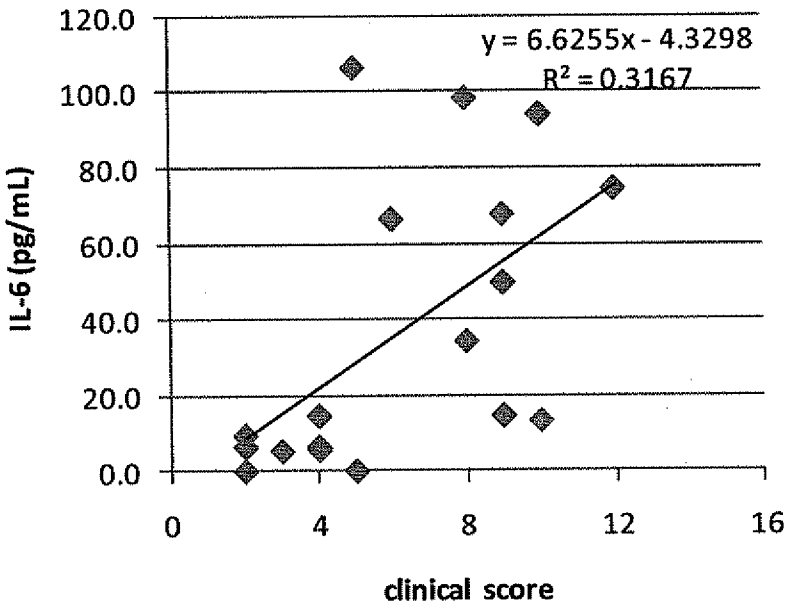


Figure 14

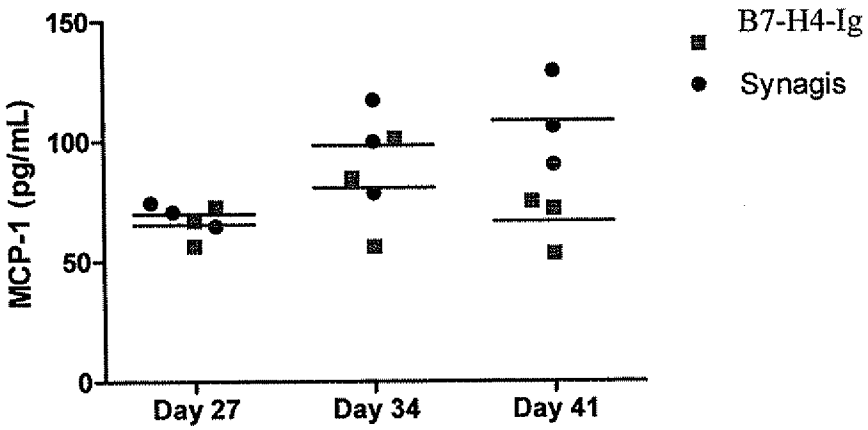


Figure 15

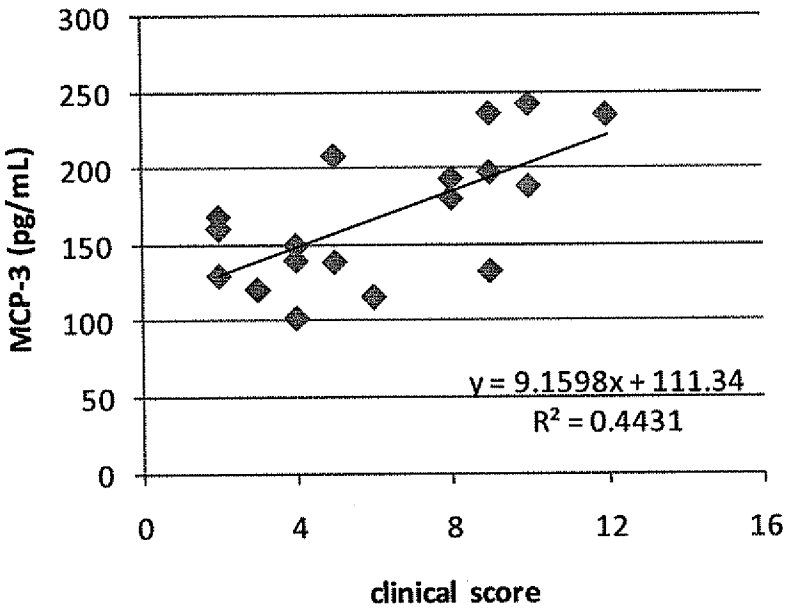


Figure 16

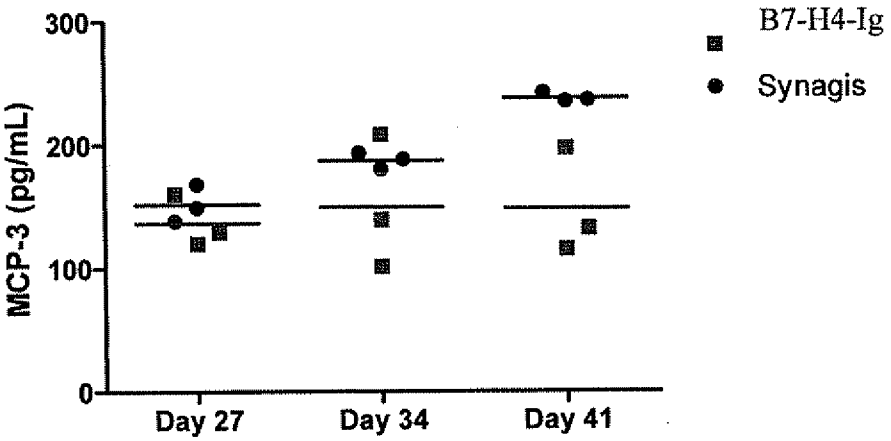


Figure 17

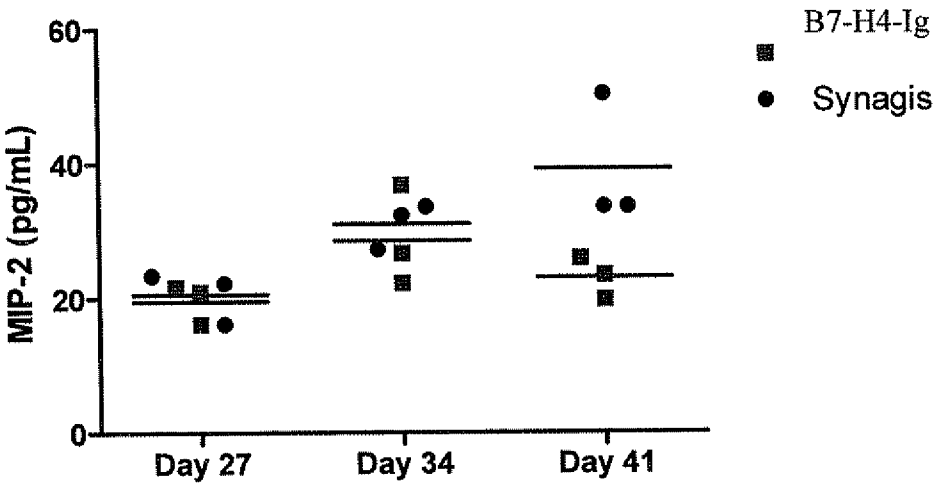


Figure 18

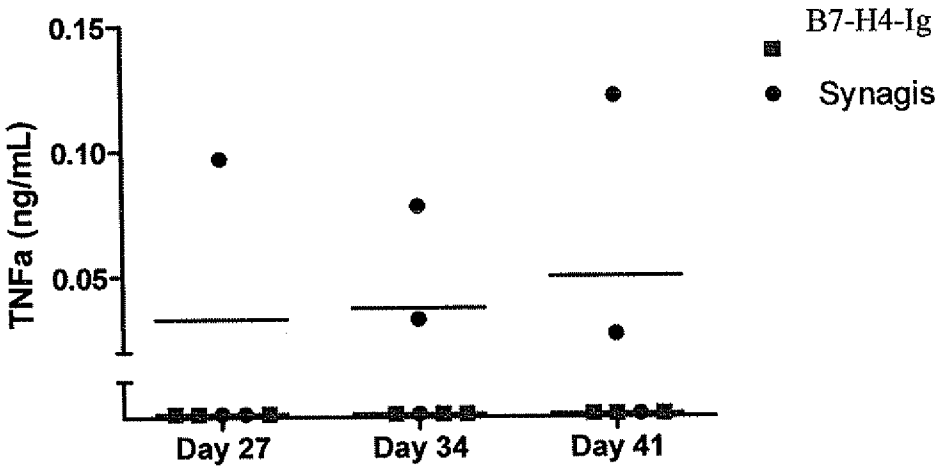


Figure 19

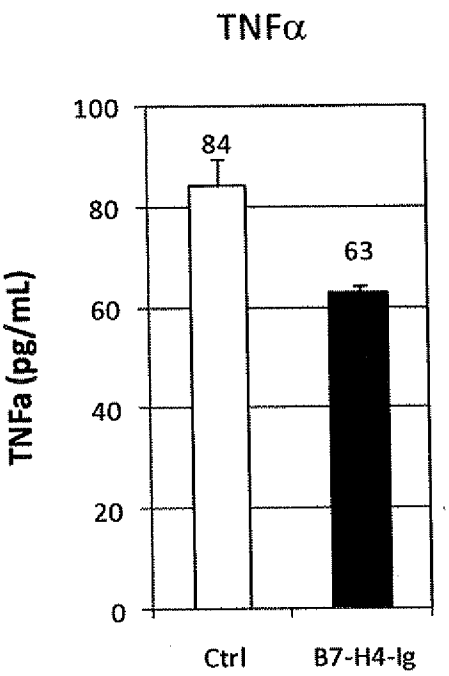


Figure 20A

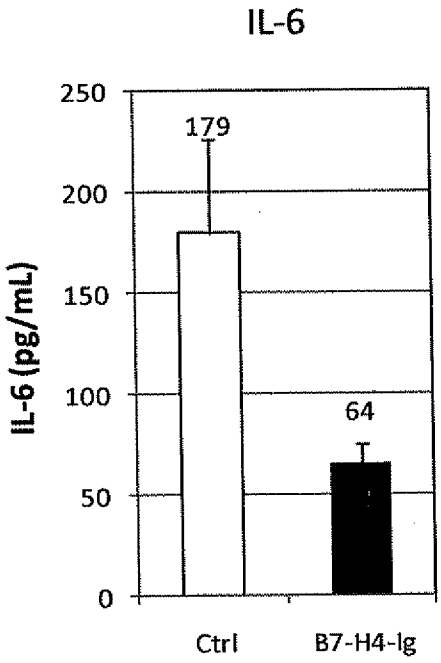


Figure 20B

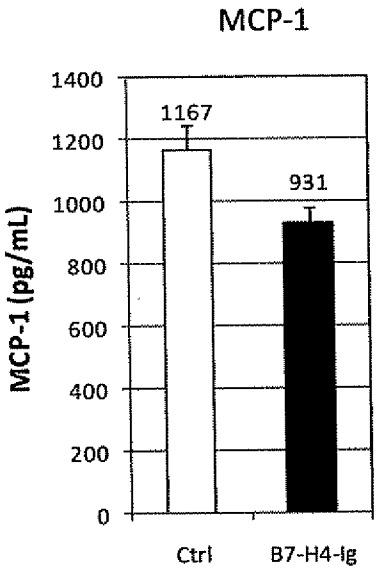


Figure 20C

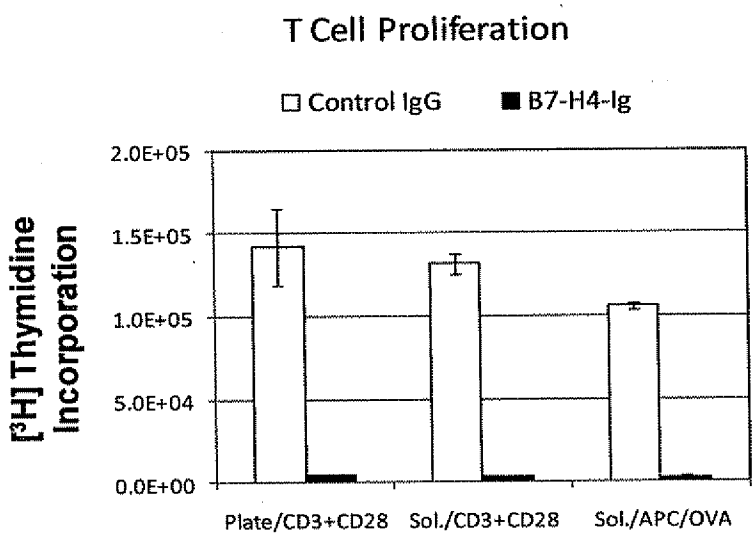


Figure 21

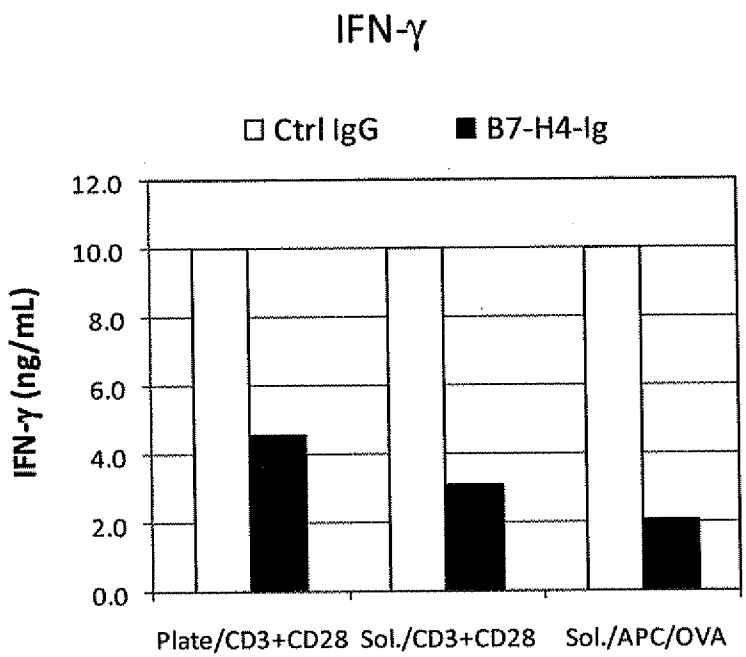


Figure 22

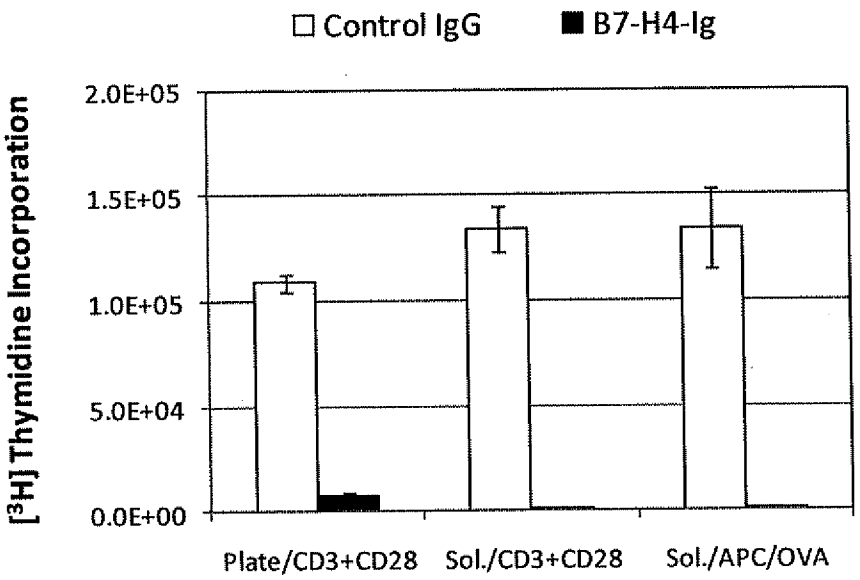


Figure 23

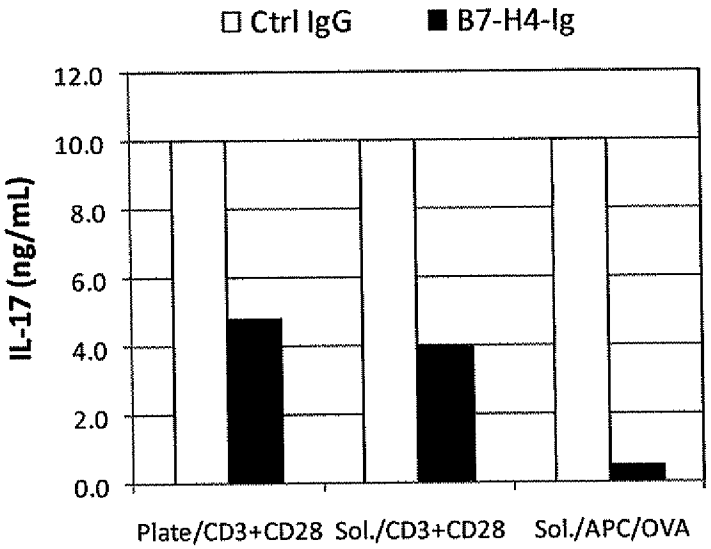


Figure 24

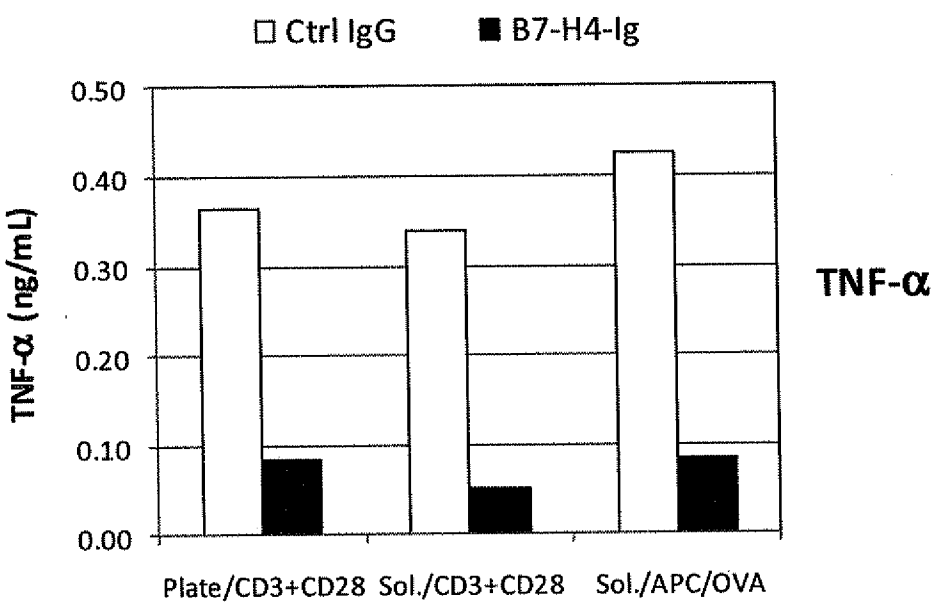


Figure 25

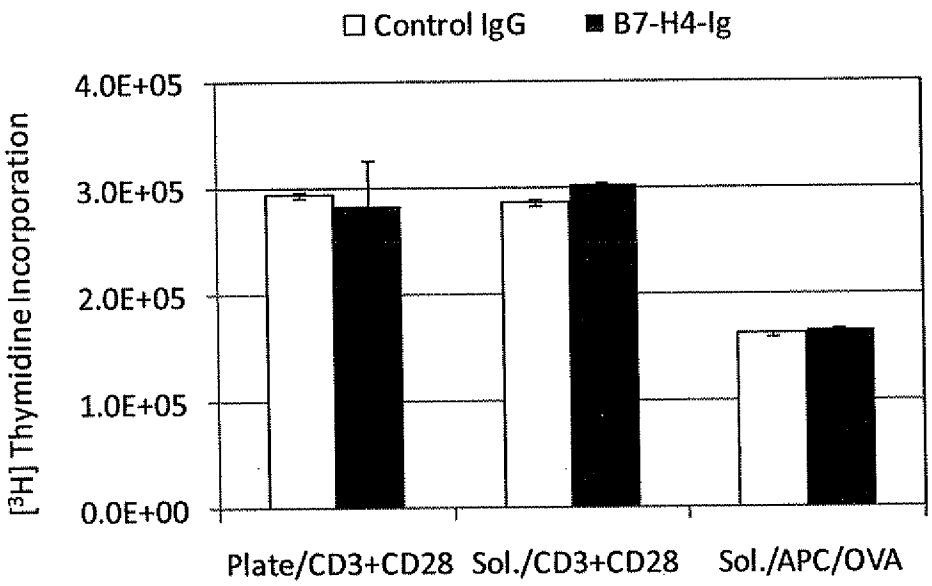


Figure 26

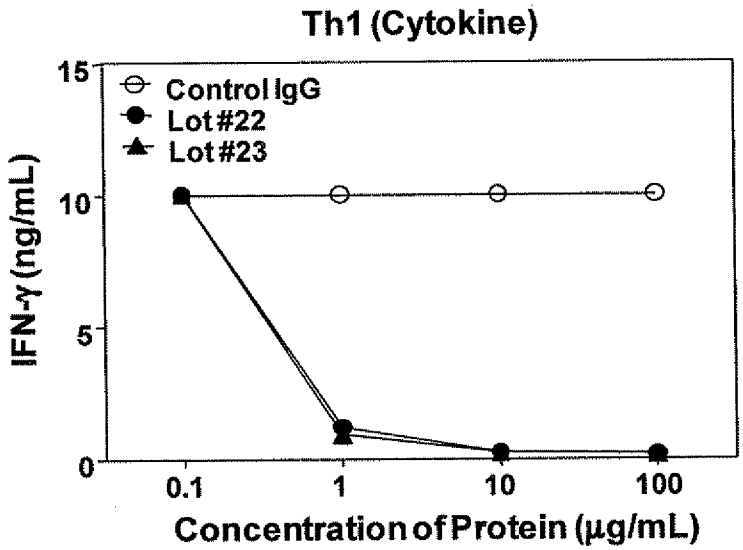


Figure 27

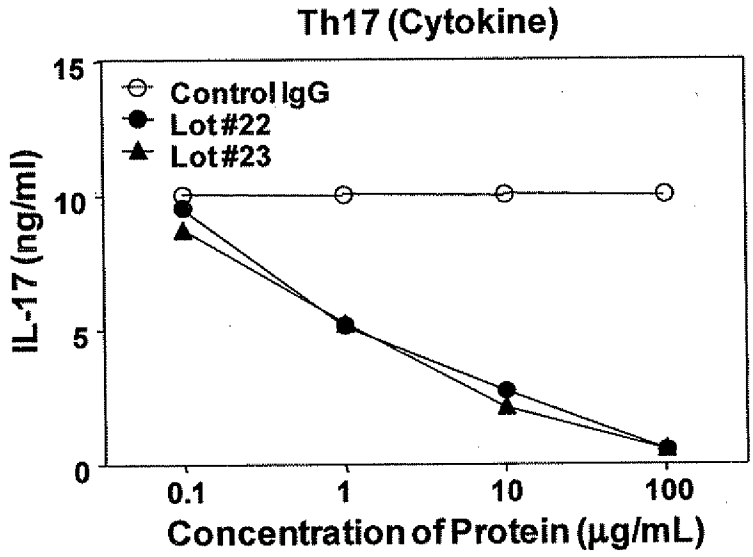


Figure 28

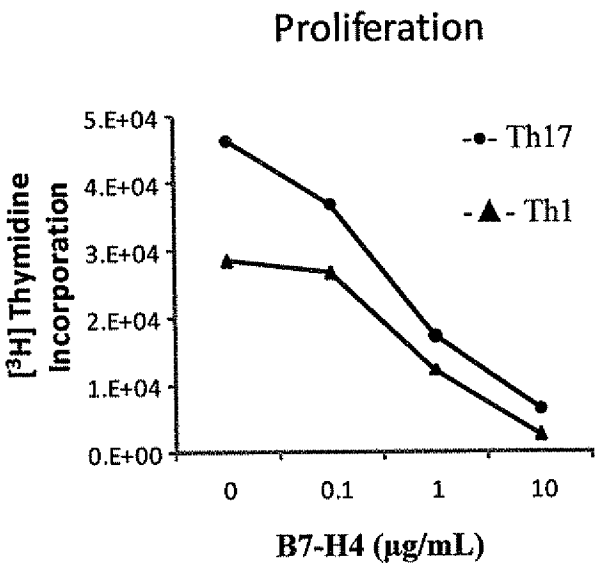


Figure 29

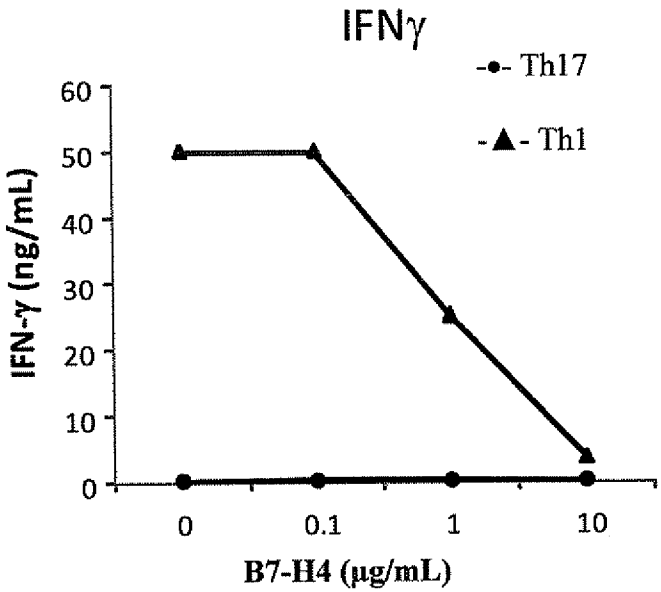


Figure 30

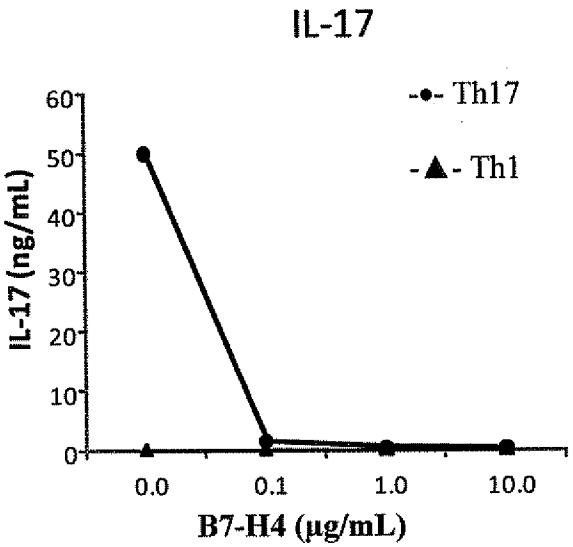


Figure 31

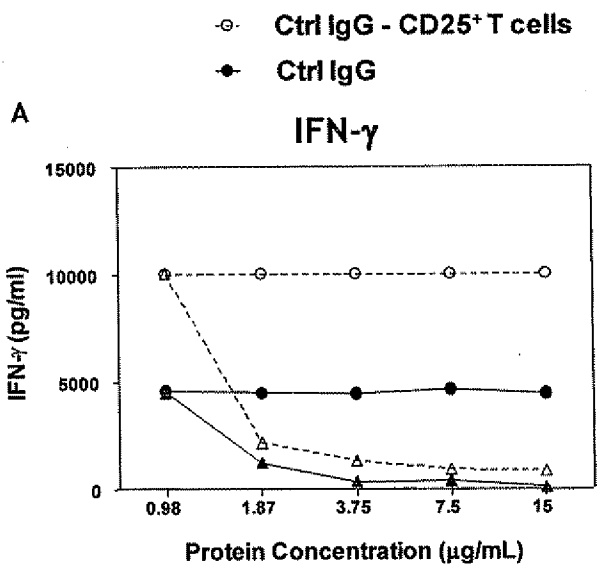


Figure 32 A

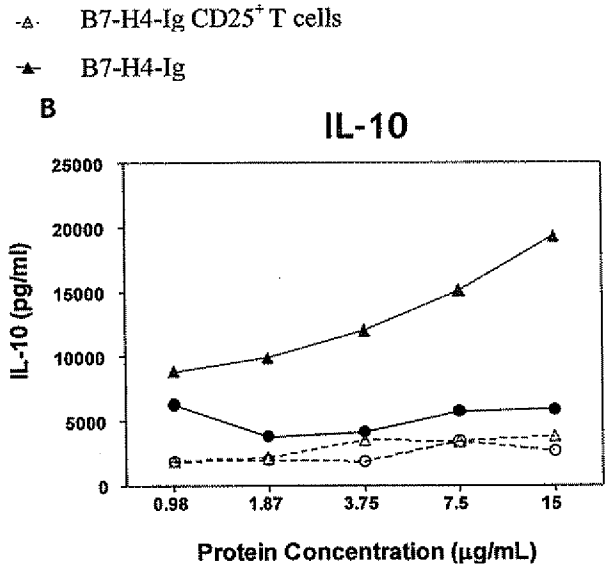


Figure 32B

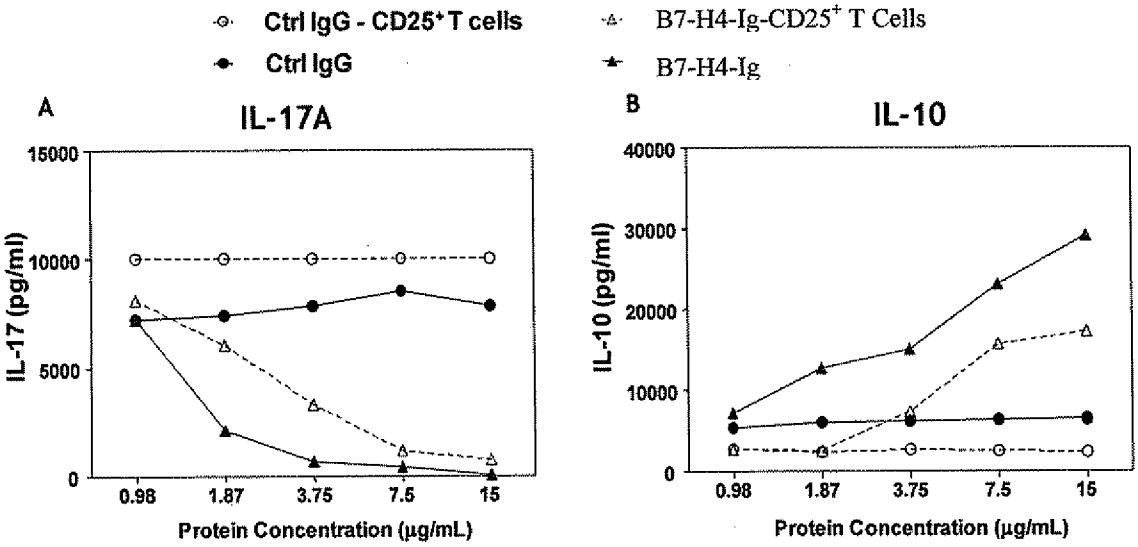


Figure 33 A

Figure 33 B

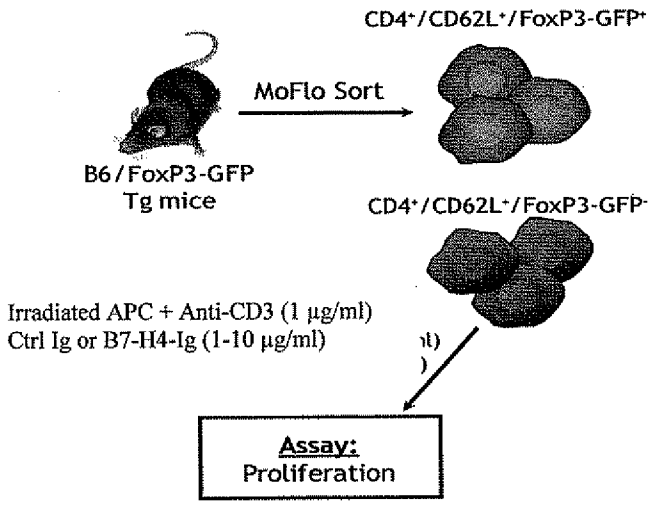


Figure 34

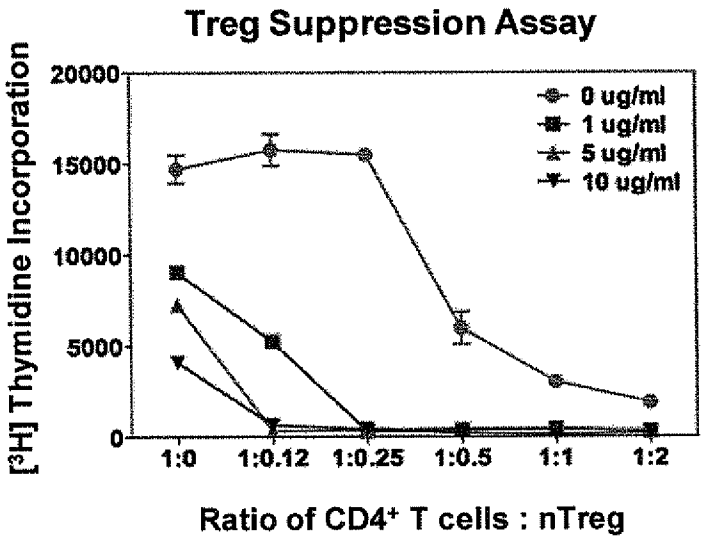


Figure 35

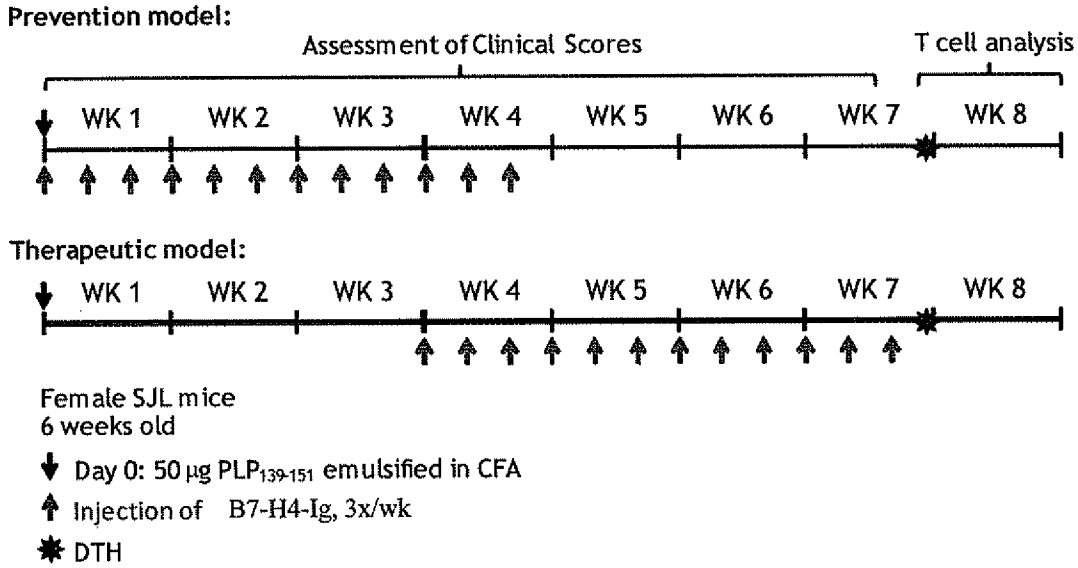


Figure 36

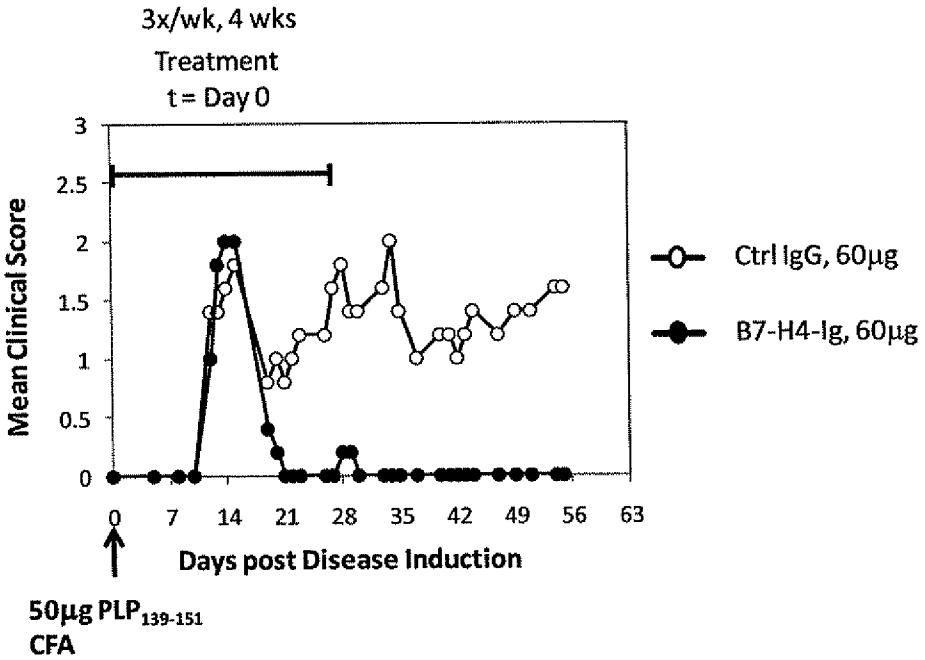


Figure 37

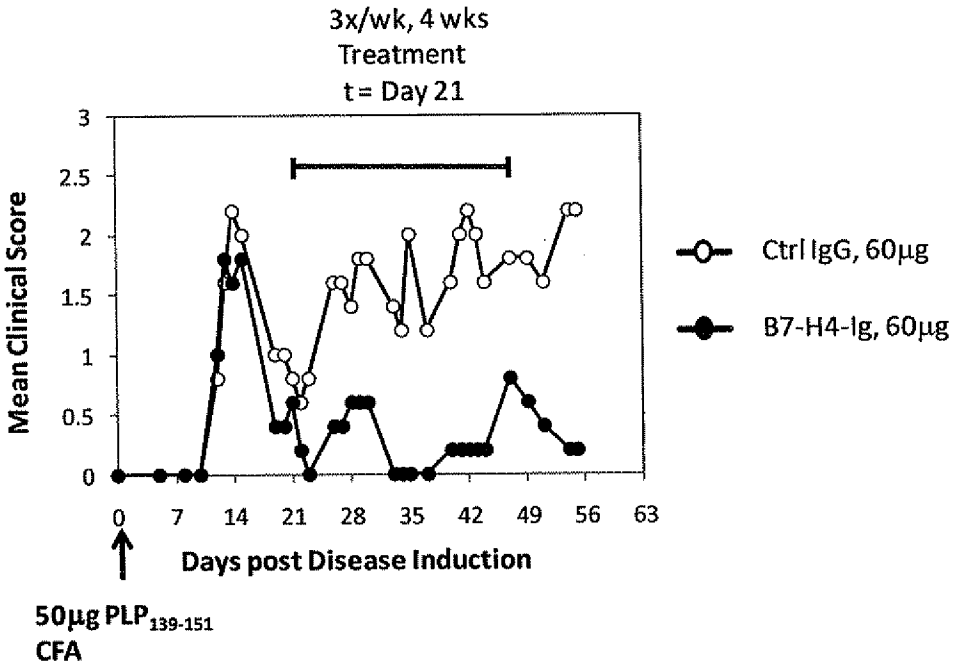


Figure 38

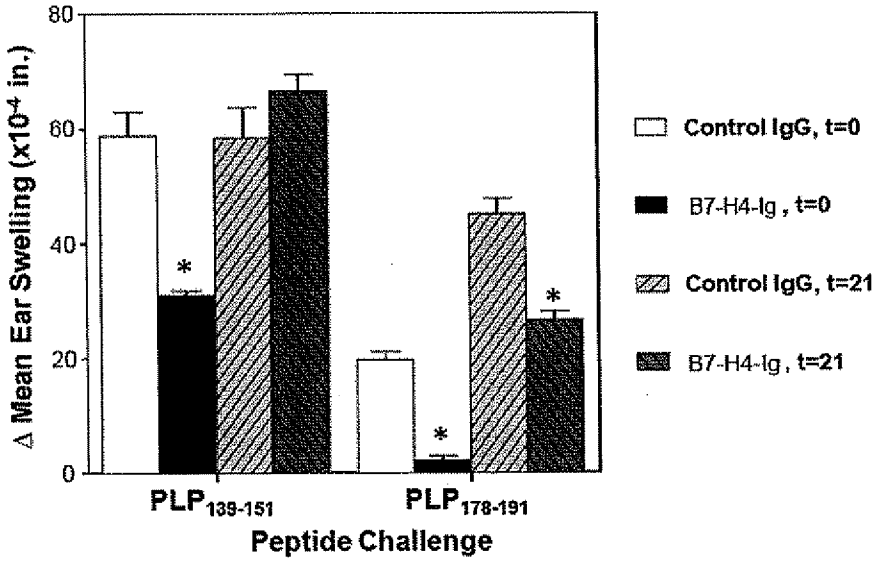


Figure 39

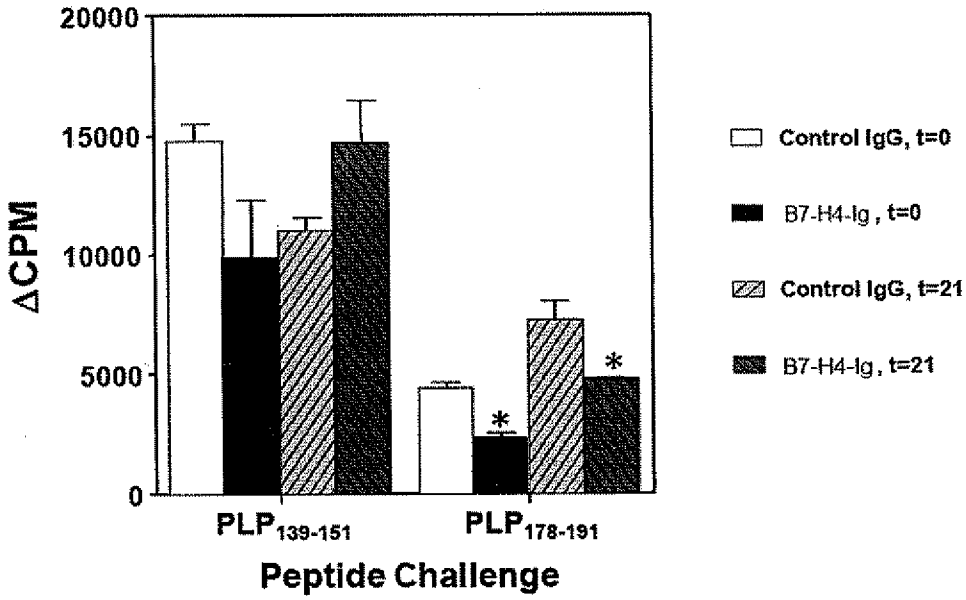


Figure 40

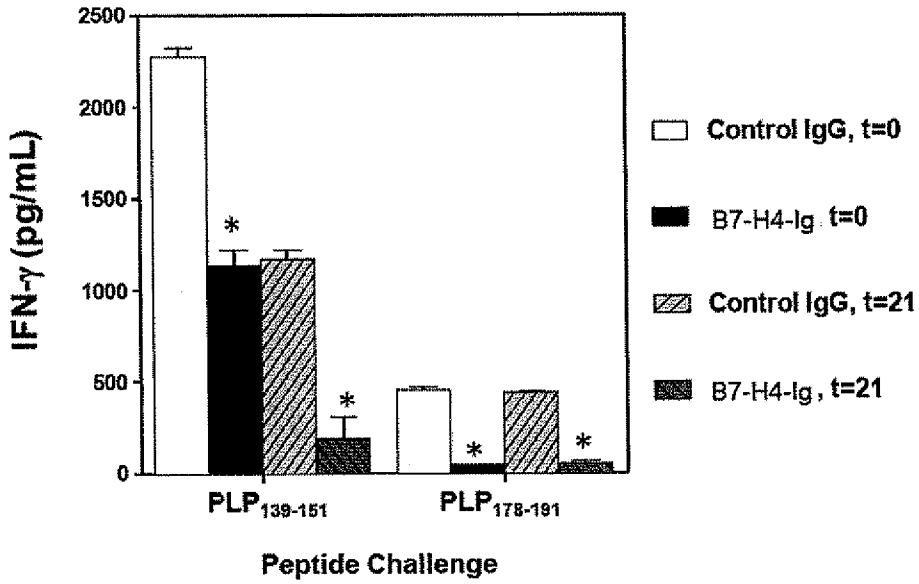


Figure 41

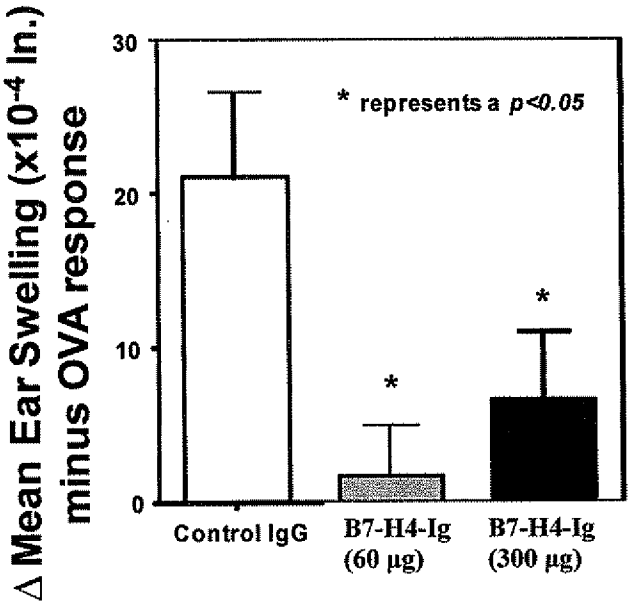


Figure 42

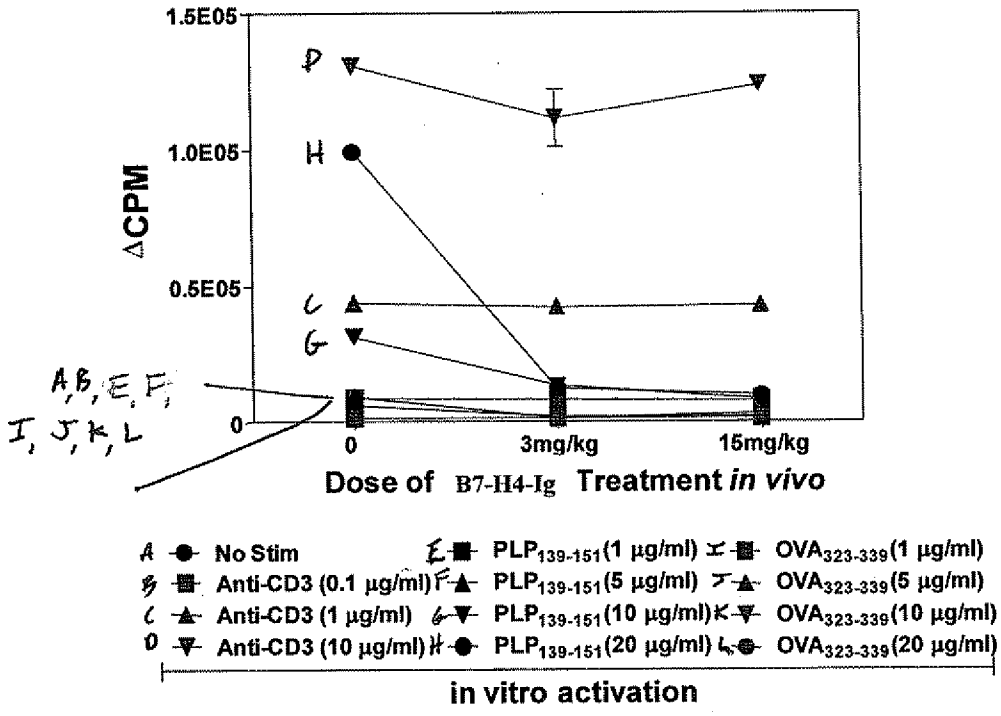

1.5E05
1.0E05
0.5E05
0
ΔCPM
D
H
C
G
A,B,E,F,
I,J,K,L
0
3mg/kg
15mg/kg
Dose of B7-H4-Ig Treatment in vivo
A No Stim
B Anti-CD3 (0.1 μg/ml)
C Anti-CD3 (1 μg/ml)
D Anti-CD3 (10 μg/ml)
E PLP139-151(1 μg/ml)
F PLP139-151(5 μg/ml)
G PLP139-151(10 μg/ml)
H PLP139-151(20 μg/ml)
I OVA323-339(1 μg/ml)
J OVA323-339(5 μg/ml)
K OVA323-339(10 μg/ml)
L OVA323-339(20 μg/ml)
in vitro activation

Figure 43

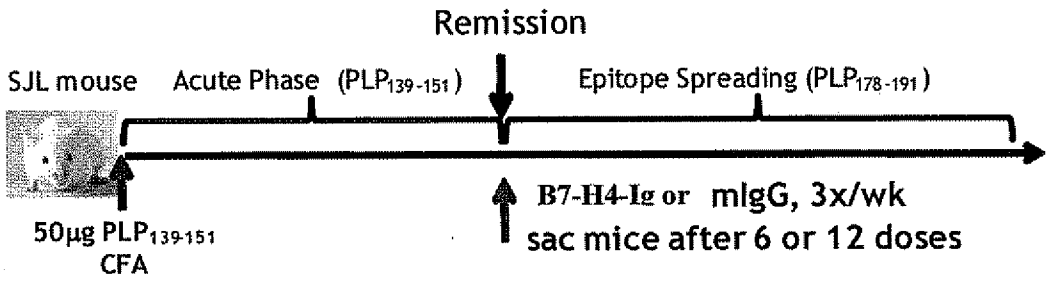


Figure 44

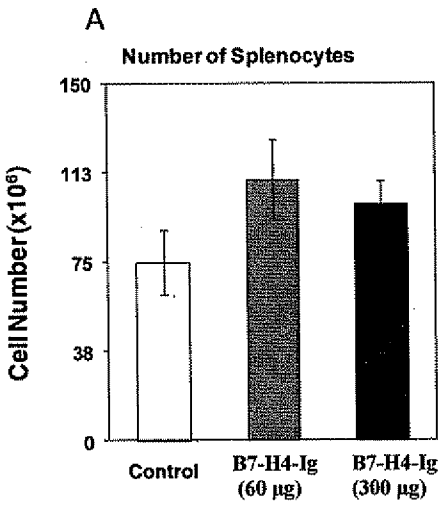


Figure 45A

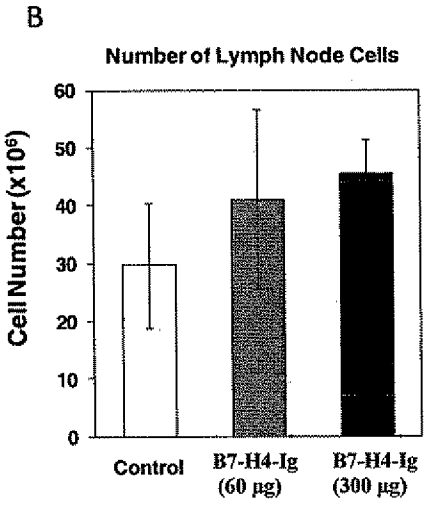


Figure 45B

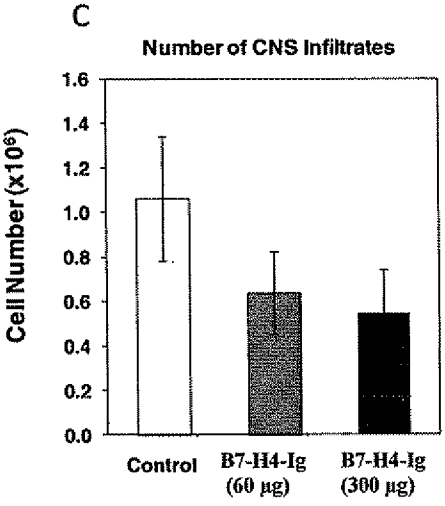


Figure 45C

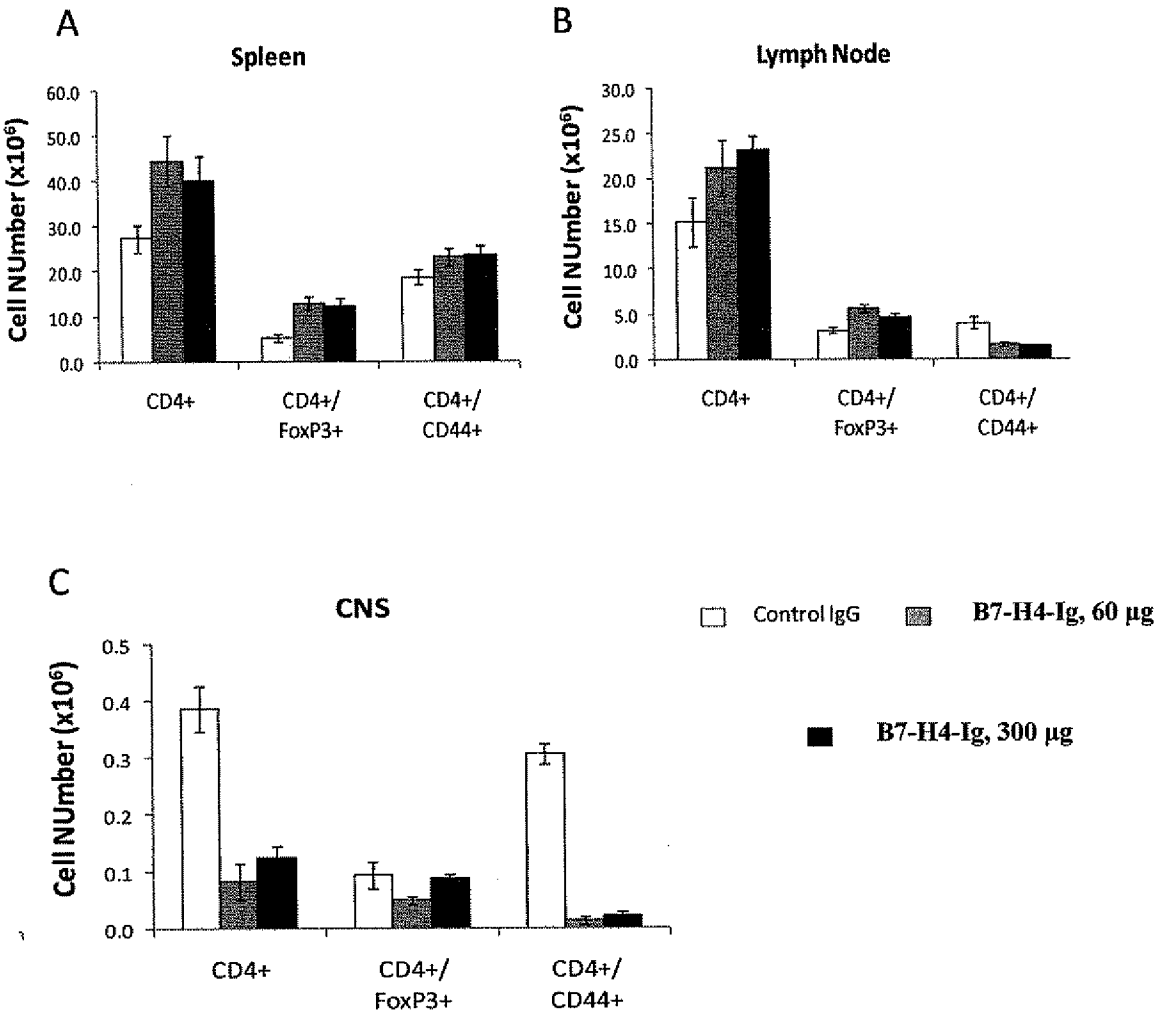


Figure 46A, Figure 46B, Figure 46C

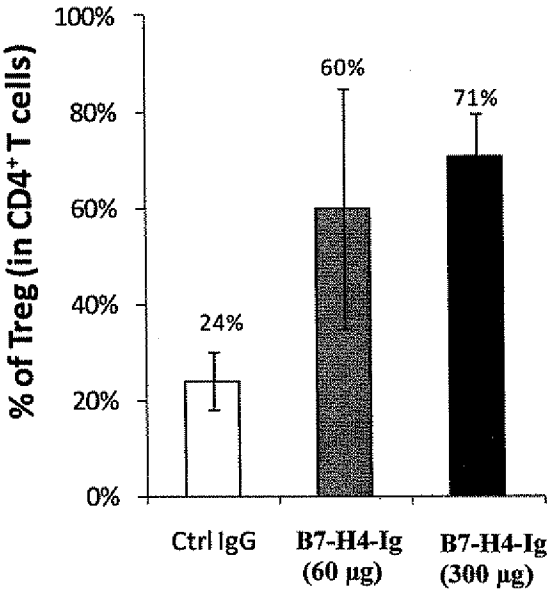


Figure 47

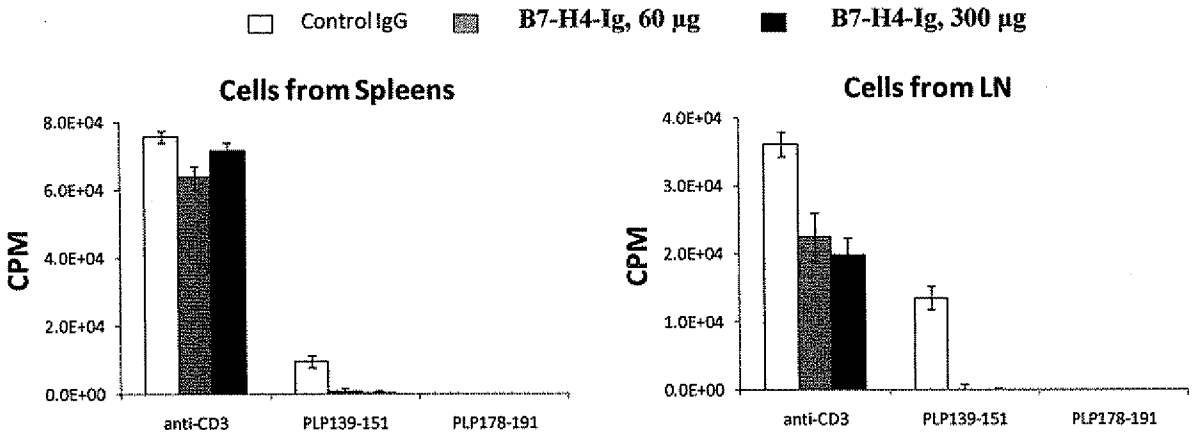


Figure 48A

Figure 48B

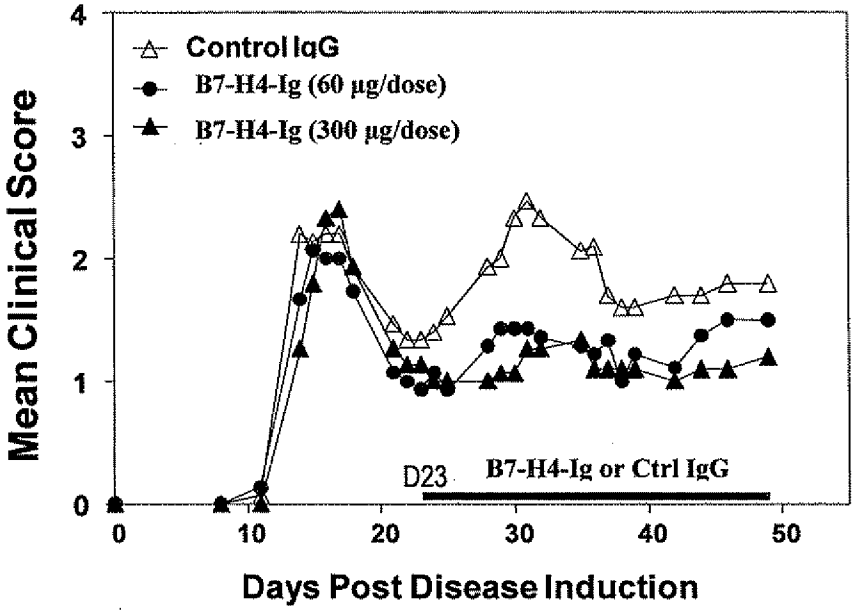


Figure 49

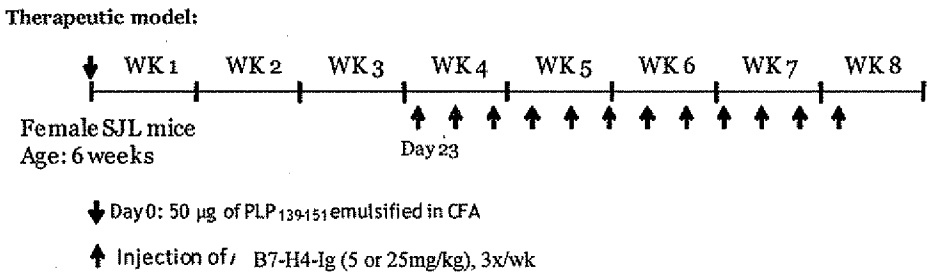


Figure 50

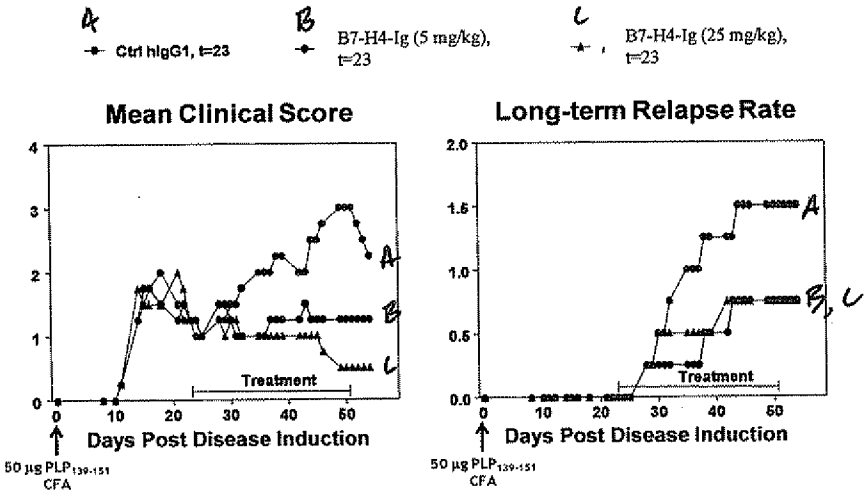
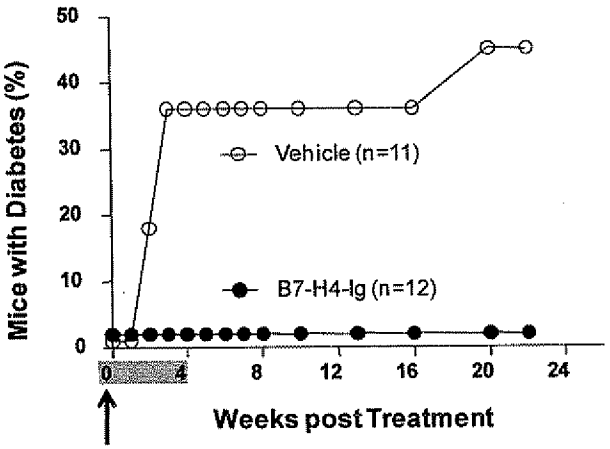


Figure 51A

Figure 51B



Mice were 2 weeks old.

Treatment window: 15 mg/kg, 3x/wk, 4 weeks

Figure 52

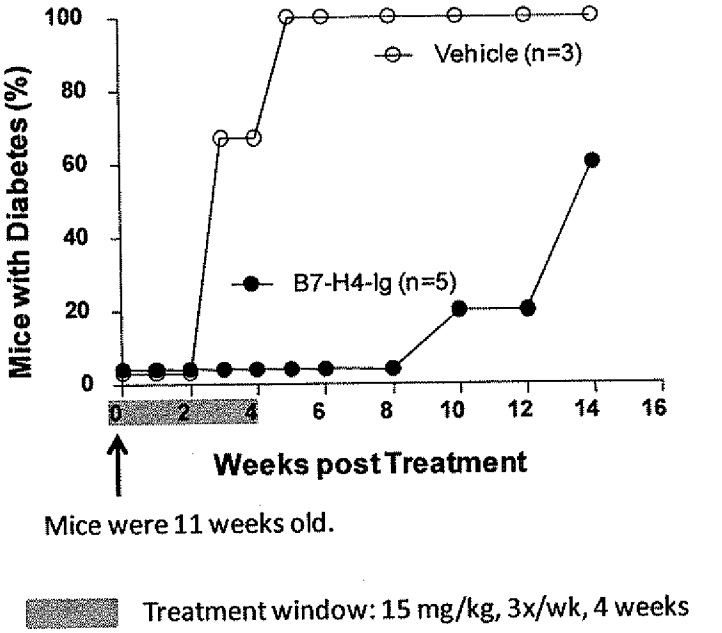


Figure 53

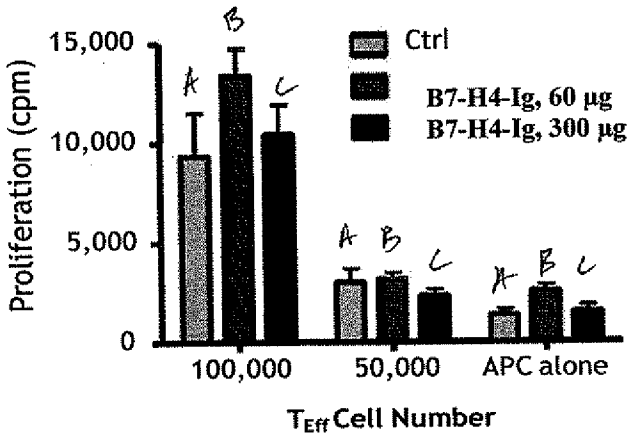


Figure 54

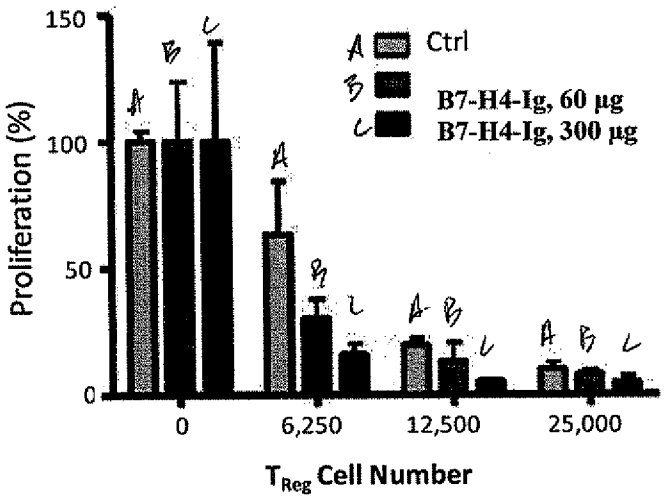


Figure 55

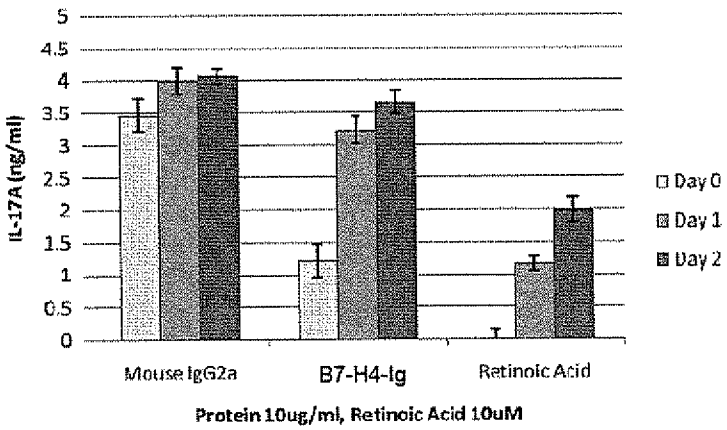


Figure 56

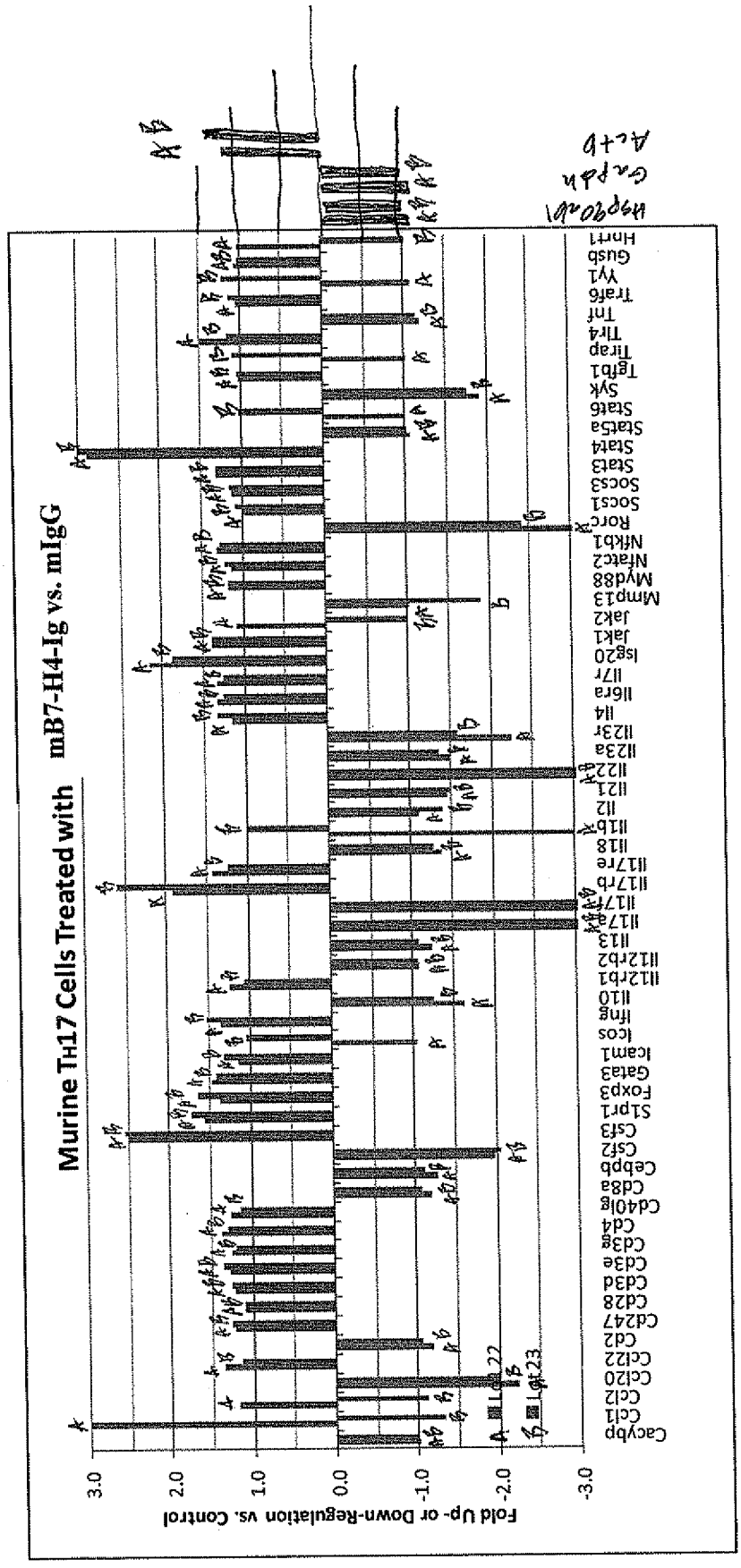

Murine TH17 Cells Treated with mB7-H4-Ig vs. mIgG
Fold Up- or Down-Regulation vs. Control
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
Cacybp
Ccl1
Ccl2
Ccl20
Ccl22
Cd2
Cd247
Cd28
Cd3d
Cd3e
Cd3g
Cd4
Cd40lg
Cd8a
Cebpb
Csf2
Csf3
Slpr1
Foxp3
Gata3
Icam1
Icos
Ifng
Il10
Il12rb1
Il12rb2
Il13
Il17a
Il17f
Il17rb
Il17re
Il18
Il1b
Il2
Il21
Il22
Il23a
Il23r
Il4
Il6ra
Il7r
Isg20
Jak1
Jak2
Mmp13
Myd88
Nfatc2
Nfkb1
Rorc
Socs1
Socs3
Stat3
Stat4
Stat5a
Stat6
Syk
Tgfb1
Tirap
Tnf
Tnfrsf4
Vav1
Gusb
Hnrnpa1

Figure 57

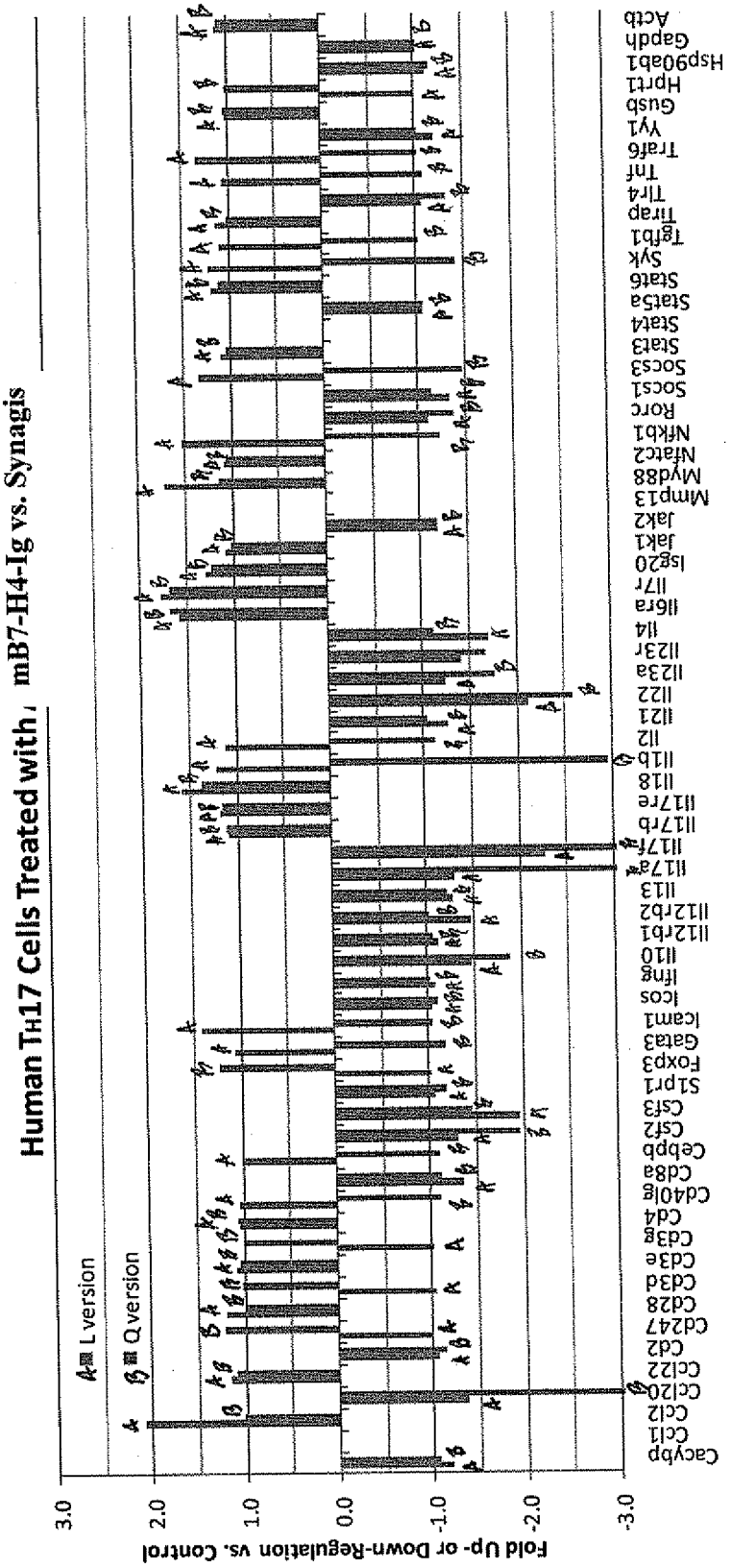

Human TH17 Cells Treated with mB7-H4-Ig vs. Synagis
L version
Q version
Fold Up- or Down-Regulation vs. Control
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
Cacybp
Ccl1
Ccl2
Ccl20
Ccl22
Cd2
Cd247
Cd28
Cd3d
Cd3e
Cd3g
Cd4
Cd40lg
Cd8a
Cebpb
Csf2
Csf3
S1pr1
Foxp3
Gata3
Icam1
Icos
Ifng
Il10
Il12rb1
Il12rb2
Il13
Il17a
Il17f
Il17rb
Il17re
Il18
Il1b
Il2
Il21
Il22
Il23a
Il23r
Il4
Il6ra
Il7r
Isg20
Jak1
Jak2
Mmp13
Myd88
Nfatc2
Nfkb1
Rorc
Socs1
Socs3
Stat3
Stat4
Stat5a
Stat6
Syk
Tgfb1
Traip
Tlr4
Tnf
Traf6
Vyl
Gusb
Hprt1
Hsp90ab1
Gapdh
Actb

Figure 58

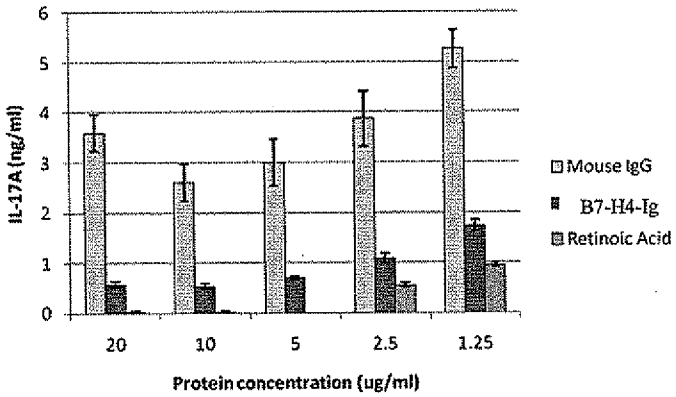


Figure 59

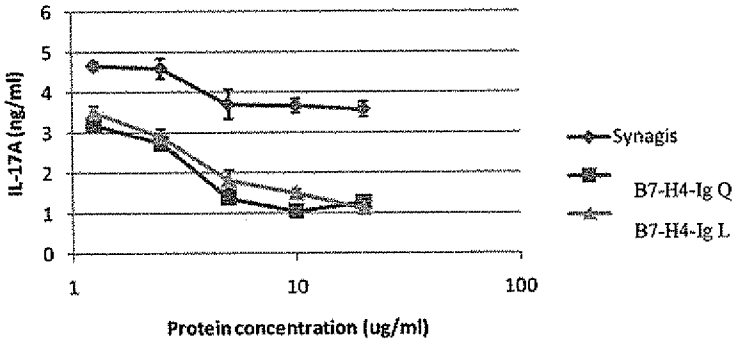


Figure 60

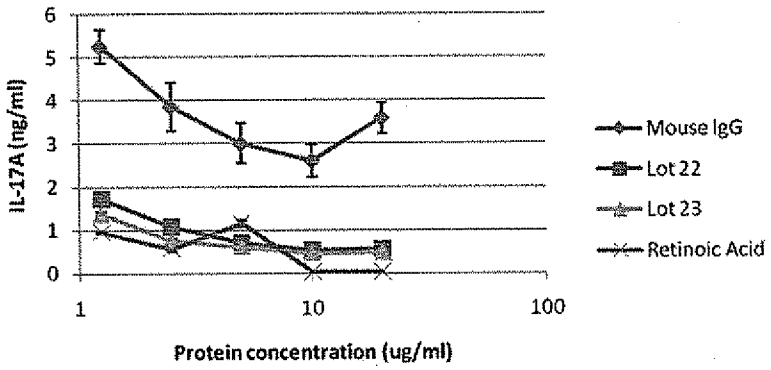


Figure 61

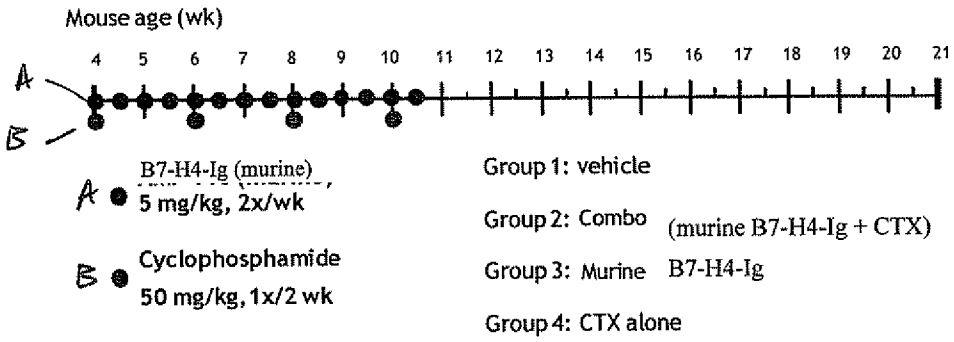


Figure 62

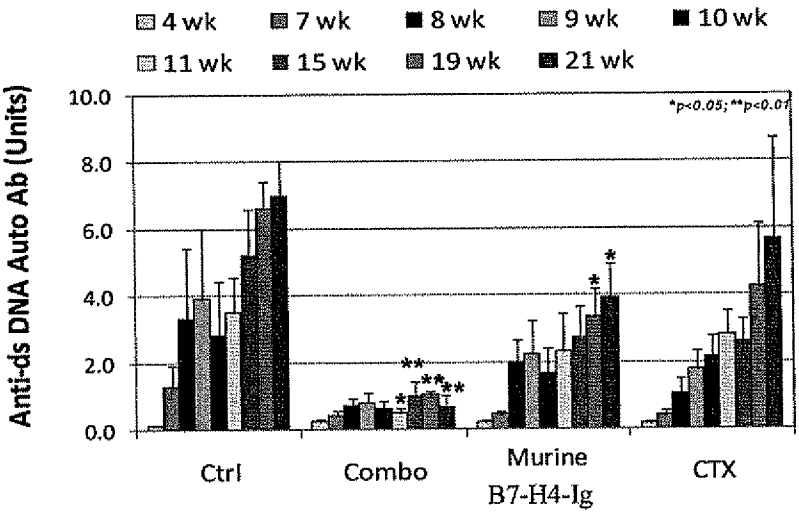


Figure 63

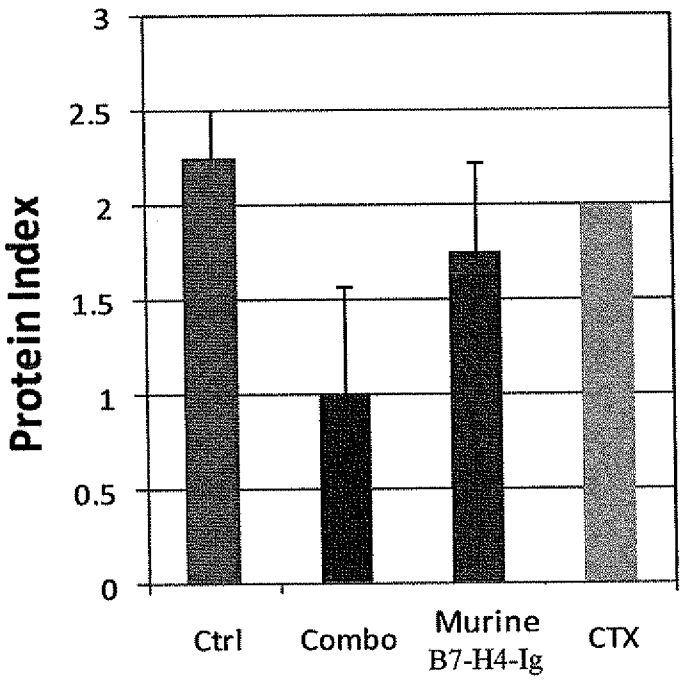


Figure 64