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(54) Benævnelse: **PROTEINER, DER ER SPECIFIKKE FOR BAFF OG B7RP1, OG ANVENDELSER DERAF**

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DESCRIPTION

FIELD

[0001] The bispecific molecules described herein are within the field of protein therapeutics.

BACKGROUND

[0002] Most therapeutic proteins bind to a single target protein with high specificity, thereby interfering with the activity of this single target protein. That protein may be a part of one or more biological pathways that mediate a human disease being treated, and the therapeutic protein may therefore inhibit disease progression. However, efficacy of therapeutic proteins is rarely complete for all patients. Incomplete efficacy of therapeutic proteins could be due in some cases to the complexity of a disease. For example, some diseases may be mediated by multiple biological pathways, or different biological pathways may play a predominant role in mediating disease activity in different patients having the same clinically-defined condition. Hence, in some diseases it may be advantageous to simultaneously inhibit at least two biological pathways.

SUMMARY

[0003] Herein is disclosed a bispecific protein that can bind to and inhibit the biological activity of both human B7-related protein 1 (B7RP1, also known as GL50 and T-cell co-stimulator ligand (ICOSLG)) and human B-cell activating factor (BAFF, also known as tumor necrosis factor superfamily, member 13b (TNFSF13B)). BAFF plays a role in B cell survival, and B7RP1 plays a role in T cell costimulation. Sifuentes Giraldo et al. (Reumatologia Clinica 2012, Vol. 8, No. 5, pp. 263-269) discloses drug targets for treating systemic lupus erythematosus, inter alia, BAFF antagonists and ICOS-L/B7RP1/CD275 antagonists but does not suggest combining inhibitors of BAFF and B7RP1. Thus, a protein that inhibits the activity of both proteins interferes with the activity of both B and T cells.

[0004] The present invention is defined by the appended claims. In particular, the present invention provides:

1. 1. A host cell comprising a nucleic acid encoding a bispecific protein, and/or a vector comprising a nucleic acid encoding a bispecifc protein, wherein the bispecific protein comprises:
    1. (a) a polypeptide comprising an amino acid sequence having the following formula:
    A-L1-P-L2-P, wherein A is an immunoglobulin heavy chain of an IgG antibody, L1 is a first peptide linker ef-that is absent or is 3 to 40 amino acids long, P is a BAFF-binding peptide that is 10 to 40 amino acids long, and L2 is a second peptide linker that is absent or is 5 to 50 amino acids long, and
    2. (b) an immunoglobulin light chain of an IgG antibody,

    wherein the immunoglobulin heavy chain of (a) and the immunoglobulin light chain of (b) form an IgG antibody, comprising two molecules of the polypeptide of (a) and two molecules of the light chain of (b), that can bind B7RP1, and

    wherein the protein can inhibit BAFF-mediated proliferation of human B cells, and

    wherein the protein can inhibit B7RP1-mediated proliferation of human T cells, and

    wherein the host cell is a bacterial cell, a yeast cell, an insect cell, a plant cell, or a mammalian cell.

2. 2. The host cell of item 1, wherein the bispecific protein comprises an immunoglobulin heavy chain that is missing a lysine at its C-terminal end just upstream of L1.
3. 3. The host cell of item 1, wherein the IgG antibody is a human or humanized anti-B7RP1 IgG1 antibody.
4. 4. The host cell of item 1 or 2, wherein the anti-B7RP1 antibody is a human or humanized IgG2 antibody, or a human or humanized IgG4 antibody.
5. 5. The host cell of any of items 1-4, wherein P has the amino acid sequence of SEQ ID NO:1 (LPGCKWDLLIKQWVCDPL).
6. 6. The host cell of any of items 1-5, wherein L1 has the amino acid sequence of SEQ ID NO:40 (GGGGG).
7. 7. The host cell of any of items 1-6, wherein L2 has the amino acid sequence of SEQ ID NO:5, preferably wherein L2 has the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7.
8. 8. The host cell of any of items 1-7, encoding a light chain CDR1 comprising the amino acid sequence of SEQ ID NO:8 (RASQGISNWLA), a light chain CDR2 comprising the amino acid sequence of SEQ ID NO:9 (AASSLQS), a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:10 (QQYDSYPRT), a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:11 (SYWMS), a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:12 (YIKQDGNEKYYVDSVKG), and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:13 (EGILWFGDLPTF).
9. 9. The host cell of any of items 1-8, wherein the encoded bispecific protein comprises an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:14.
10. 10. The host cell of any of items 1-9, wherein the encoded bispecific protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:15.
11. 11. The host cell of any of items 5-10, wherein the immunoglobulin light chain of (b) comprises the amino acid sequence of SEQ ID NO:19.
12. 12. The host cell of any of items 5-11, wherein the polypeptide of (a) comprises the amino acid sequence of SEQ ID NO:17 or 18.
13. 13. The host cell of item 1, wherein polypeptide (a) comprises the amino acid sequence of SEQ ID NO:17 or SEQ ID NO:18, and polypeptide (b) comprises the amino acid sequence of SEQ ID NO:19.
14. 14. The host cell of item 1, wherein the bispecific protein comprises the amino acid sequences of SEQ ID NO:1, SEQ ID NO:14 and SEQ ID NO:15, preferably, wherein the protein further comprises a linker comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7.
15. 15. The host cell of any of items 1-14, wherein the host cell is a mammalian cell selected from the group consisting of a Chinese hamster ovary (CHO) cell, a baby hamster kidney (BHK) cell, a monkey kidney cell, a HeLa cell, a human hepatocellular carcinoma cell and a 293 cell.
16. 16. A method for making a bispecific protein comprising culturing the host cell of any of items 1-14 under conditions such that the nucleic acid is expressed and recovering the protein from the cell mass or the culture medium, wherein the host cell is a bacterial cell, a yeast cell, an insect cell, or a plant cell.
17. 17. The method of item 16, wherein the nucleic acid or vector is introduced into the cell by (a) electroporation, or calcium chloride transformation, wherein the host cell is a bacterial cell, or (b) lithium acetate transformation, or polyethylene glycol transformation, wherein the host cell is a yeast cell.

[0005] Described herein is bispecific protein, wherein the protein can inhibit BAFF-mediated proliferation of human B cells and wherein the protein can inhibit B7RP1-mediated proliferation of human T cells. The bispecific protein can comprise an IgG antibody comprising two immunoglobulin heavy chains having different amino acid sequences and two immunoglobulin light chains having different amino acid sequences. The IgG antibody can inhibit BAFF-mediated proliferation of human B cells and B7RP1-mediated proliferation of human T cells The IgG antibody can be an IgG1, IgG2, IgG3, or IgG4 antibody and can be a human or humanized IgG antibody. The bispecific protein can comprise a light chain complementarity determining region 1 (CDR1) comprising the amino acid sequence of SEQ ID NO:8, a light chain complementarity determining region 2 (CDR2) comprising the amino acid sequence of SEQ ID NO:9, a light chain complementarity determining region 3 (CDR3) comprising the amino acid sequence of SEQ ID NO:10, a heavy chain CDR1 comprising the amino

acid sequence of SEQ ID NO:11, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:12, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:13. Further, the bispecific protein can comprise a heavy chain variable region comprising SEQ ID NO:15 or a variant thereof and a light chain variable region comprising SEQ ID NO:14 or a variant thereof. Such variant sequences can comprise not more than 10 deletions, insertions of substitutions of a single amino acid per 100 amino acids relative to a reference sequence.

[0006] Further disclosed herein is a bispecific protein that can inhibit BAFF-mediated proliferation of human B cells and that can inhibit B7RP1-mediated proliferation of human T cells can comprise: (a) a polypeptide comprising an amino acid sequence having the following formula: A-L1-P-L2-P, wherein A is an immunoglobulin heavy chain of an IgG antibody, L1 is a first linker of that is absent or is 3 to 40 amino acids long, P is a BAFF-binding peptide that is 10 to 40 amino acids long, and L2 is a peptide linker that is absent or is 5 to 50 amino acids long; and (b) an immunoglobulin light chain. The immunoglobulin heavy chain of (a) and the immunoglobulin light chain of (b) can form an IgG antibody, comprising two molecules of the polypeptide of (a) and two molecules of the light chain of (b), that can bind B7RP1 and/or can inhibit B7RP1-mediated proliferation of human T cells. The immunoglobulin heavy chain may be missing a lysine at its C-terminal end just upstream of L1. The IgG antibody can be a human or humanized IgG1, IgG2, IgG3, or IgG4 antibody. The BAFF-binding peptide P can have the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3. L1 can have the amino acid sequence of SEQ ID NO:4, 37, 38, 39, or 40. L2 can have the amino acid sequence of SEQ ID NO:5, 6, or 7. The bispecific protein can comprise a light chain CDR1 comprising the amino acid sequence of SEQ ID NO:8 (RASQGISNWLA), a light chain CDR2 comprising the amino acid sequence of SEQ ID NO:9 (AASSLQS), a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:10 (QQYDSYPRT), a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:11 (SYWMS), a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:12 (YIKQDGNEKYYVDSVKG), and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:13 (EGILWFGDLPTF). The bispecific protein can comprise an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:14 and/or an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:15. The bispecific protein can comprise the amino acid sequence of SEQ ID NO:19 or a variant thereof and the amino acid sequence of SEQ ID NO:17 or 18 or variants thereof. Such variant sequences can comprise not more than 10 deletions, insertions of substitutions of a single amino acid per 100 amino acids relative to the reference sequence.

[0007] In a further aspect, herein is described a bispecific protein comprising: (a) a polypeptide comprising the amino acid sequence of SEQ ID NO:17 or SEQ ID NO:18 or variants thereof; and (b) another polypeptide comprising the amino acid sequence of SEQ ID NO:19 or a variant thereof. Such variant sequences can comprise not more than 10 deletions, insertions of substitutions of a single amino acid per 100 amino acids relative to the reference sequence. The bispecific protein can inhibit BAFF-mediated proliferation of human B cells and B7RP1-mediated proliferation of human T cells. The bispecific protein can be a tetramer comprising two molecules of the polypeptide of (a) and two molecules of the polypeptide of (b).

[0008] Further disclosed herein is a protein comprising a linker comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7. This protein can inhibit BAFF-mediated proliferation of human B cells and/or B7RP1-mediated proliferation of human T cells. Such a protein can comprise the amino acid sequences of SEQ ID NO:1, SEQ ID NO:14, and/or SEQ ID NO:15. Such a protein can comprise an amino acid sequence comprising at least two copies of SEQ ID NO:1 separated by SEQ ID NO:6 or 7. Such a protein can include an immunoglobulin light chain and an immunoglobulin heavy, and SEQ ID NO:6 or 7 can be downstream from the C-terminus of the heavy chain. SEQ ID NO:6 or 7 can be flanked by peptides that bind to a protein other than that bound by the heavy and light chains.

[0009] Further, herein is described a pharmaceutical composition comprising any of the bispecific proteins herein described or the protein comprising the amino acid sequence of SEQ ID NO:6 or 7 and a physiologically acceptable excipient.

[0010] Also described herein is a nucleic acid encoding any polypeptide included in one of bispecific proteins or the proteins comprising SEQ ID NO:6 or SEQ ID NO:7 herein described. Exemplary nucleic acids encoding a polypeptide included in a bispecific protein include, for example, SEQ ID NOs: 55, 56, 60, 61, 62, and 63, among others. Vectors comprising such nucleic acids and host cells containing such vectors and/or nucleic acids are described. Further described herein is method for making a bispecific protein comprising culturing the host cell containing a nucleic acid encoding any of the bispecific proteins described herein under conditions such that the nucleic acid is expressed and recovering the protein from the cell mass or the culture medium. The host cell can be a mammalian cell, for example, a CHO cell, or a bacterial cell such as *Escherichia coli*

[0011] In another aspect, described herein is a method for treating systemic lupus erythematosus, including lupus nephritis, comprising administering to a patient a therapeutically effective amount of any of the bispecific proteins described herein or a pharmaceutical composition comprising such a bispecific protein. Another therapeutic can be administered to the patient before, after, or concurrently with the bispecific protein. The other therapeutic can be a corticosteroid, an antimalarial, retinoic acid, an NSAID, cyclophosphamide, dehydroepiandrosterone, mycophenolate mofetil, azathioprine, chlorambucil, methotrexate, tacrolimus, dapsone, thalidomide, leflunomide, or cyclosporine.

[0012] In a further aspect, herein is described a method of treatment comprising administering to a patient a therapeutically effective amount of any of the bispecific proteins described herein or a pharmaceutical composition comprising a bispecific protein described herein, wherein the patient has a disease selected from the group consisting of: ANCA-positive vasculitis, rheumatoid arthritis (RA), Crohn's disease, ulcerative colitis, celiac disease, pemphigus, pemphigoid, subacute cutaneous lupus erythematosus (SCLE), multiple sclerosis, chronic inflammatory demyelinating polyneuropathy (CIDP), myasthenia gravis, Goodpasture's syndrome, glomerulonephritis, autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), chronic active hepatitis, primary billiary cirrhosis, Sjogren's syndrome, systemic sclerosis, Hashimoto's thyroiditis, Graves' disease, Addison's disease, and multiple endocrine neoplasia (MEN).

[0013] In another aspect, herein is described a pharmaceutical composition comprising any of the bispecific proteins or the proteins comprising SEQ ID NO:6 or SEQ ID NO:7 herein described. The pharmaceutical composition can be, for example, for the treatment of systemic lupus erythematosus or lupus nephritis.

[0014] In another aspect, the use of any of the bispecific proteins disclosed herein as a medicament is described. However, any reference to a method of treatment practised on the human or animal body is to be understood to refer to substances and compositions for use in such treatments.

BRIEF DESCRIPTION OF THE FIGURES

[0015]

Figure 1: Diagrams of bispecific proteins that bind to BAFF and B7RP1. Across the top row are listed the identifier for each construct. Across the second row is a brief descriptive phrase relating to the structure of each construct. Across the bottom row is a diagram of the structure of each construct. The unfilled ovals represent constant regions of an immunoglobulin heavy or light chain. The ovals filled with horizontal lines represent immunoglobulin heavy or light chain variable (VH or VL) regions. The small, solidly filled squares and loops represent BAFF-binding peptides. The hinge regions are shown as heavy vertical lines, while the disulfide bridges are shown as heavy horizontal lines. The sequence of "G4S" in Figure 1 is disclosed in SEQ ID NO: 72.

Figure 2: Activity of bispecific proteins in a human B cell proliferation assay. The data shown in Figures 2A (top) and 2B (bottom) are from B cell proliferation assays performed as described in Example 1. In both panels, the x axis indicates the concentration (log[nM]) of the bispecific protein contained in the assay mixture, and the

y axis indicates the amount of ^{3}H-thymidine uptake (counts per minute (cpm)). The meaning of each symbol is indicated by an identifier for each protein assayed. Meanings of the identifiers are shown in Figure 1 and explained in Example 1.

Figure 3: Activity of bispecific proteins in a human T cell proliferation assay. The data shown is from T cell proliferation assays performed as described in Example 1. The x axis indicates the concentration (log[nM]) of the bispecific or αB7RP1 antibody in the assay mixture, and the y axis indicates percent of T cell ^{3}H-thymidine uptake in the presence of B7RP1 inhibitors at the indicated concentrations relative to T cell ^{3}H-thymidine uptake without B7RP1 inhibitors (percent of control). The identifier for each protein tested is indicated.

Figure 4: Cytokine release by human tonsil cells stimulated with Staphylococcus enterotoxin B (SEB). Methods are described in Example 1. The y axes show the levels of signal detected for the various cytokines measured using Meso Scale Discovery (Rockville, Maryland) kits according to the manufacturer's instructions. The cells were treated with either αB7RP1 (lane 1), P74293 (lane 2), CTLA4-Ig (lane 3), or human IgG (lane 4). The cytokines assayed are indicated in the figure.

Figure 5: Pharmacokinetic profile of bispecific constructs in mice. Methods for assessing the *in vivo* pharmacokinetic properties of P71617, P71619, P71621, P71622, P74293, and P74294 in mice are described in Example 1. As explained in Example 1, the bispecific proteins were detected by two different assays, one of which detected only the Fc portion of the proteins (data points indicated by filled diamonds; Fc assay) and one of which detected both the Fc and BAFF-binding portion of the proteins (data points indicated by filled squares; intact assay). The x axis indicates the time post injection (hours), and the y axis indicates the concentration of the protein detected in serum (ng/mL). The construct injected is indicated in each panel.

Figure 6A: Inhibition of murine B cell proliferation by a murine surrogate bispecific molecule (the "murine surrogate") that binds to BAFF and B7RP1. The assay was performed as described in Example 2. The murine surrogate comprises an anti-murine B7RP1 IgG antibody that has two copies of a BAFF-binding peptide attached to the C terminus of the immunoglobulin heavy chain of the antibody, as explained in Example 2. The positive control was a BAFF-binding peptibody ("αBAFF"). Data from the murine surrogate and αBAFF are indicated, respectively, by solidly filled circles and squares. The x axis indicates the concentration of these test proteins in the assay (log[pM]), and the y axis indicates ^{3}H-thymidine incorporation (cpm).

Figure 6B: Inhibition of B7RP1 binding to murine T cells by the murine surrogate. The assay was performed as described in Example 2. An anti-murine B7RP1 IgG antibody ("anti-mB7RP1") was used as a positive control. Data from the murine surrogate and anti-mB7RP1 are indicated, respectively, by solidly filled circles and squares. The x axis indicates the concentration of these test proteins in the assay (log[pM]), and the y axis indicates the percent of murine B7RP1-Fc bound to the T cells.

Figure 7: *In vivo* effects on immunological parameters of administration of sheep red blood cells to mice. All results shown in this figure are from assays described in Example 2. The proteins that the mice were treated with are indicated by the fill in each bar as follows: unfilled, anti-mB7RP1; vertical lines, αBAFF; horizontal lines, anti-mB7RP1 plus αBAFF; diagonal lines, the murine surrogate; checkerboard, mIgG1; and solid fill (in bottom panel only), mice not challenged with SBRC. *Top panel,* percentage of spleen B cells in mice challenged with sheep red blood cells (SRBC). The y axis indicates the percent of cells from the spleen that are B cells. *Middle panel,* percentage of spleen $CD4^{+}$ T cells that are memory T cells in mice challenged with SRBC. *Bottom panel,* levels of anti-SRBC antibodies in serum from mice challenged with SRBC.

Figure 8A: Proteinuria in NZB/NZW mice treated with various proteins. Methods are described in Example 2. The treatment for each group of mice is indicated as follows: filled circles, phosphate buffered saline (PBS); filled squares, murine IgG1 (an isotype control; 5 mg/kg); unfilled squares, anti-mB7RP1 (4.68 mg/kg); filled, upward-pointing triangles, αBAFF (1.88 mg/kg); unfilled, upward-pointing triangles, αBAFF (1.88 mg/kg) plus anti-mB7RP1 (4.68 mg/kg); and unfilled, downward-pointing triangles, the murine surrogate (5 mg/kg). The x axis indicates the age of the mice (months), and the y axis indicates the percent of mice that exhibited

proteinuria, *i.e.,* ≥ 300 mb/dL protein in urine.

Figure 8B: Levels of antibodies against double stranded DNA (dsDNA) in NZB/NZW mice at 8.5 months of age treated with various proteins. Methods are described in Example 2. The x axis indicates the identity of the molecule(s) that the mice were treated with as follows: 1, anti-mB7RP1 (4.68 mg/kg); 2, αBAFF (1.88 mg/kg); 3, αBAFF (1.88 mg/kg) plus anti-mB7RP1 (4.68 mg/kg); 4, the murine surrogate bispecific (5 mg/kg); and 5, mIgG1 (the isotype control; 5 mg/kg). The y axis indicates the levels of anti-dsDNA antibodies detected as a percentage of the positive control. Each dot indicates data from a single mouse.

Figure 9A: Levels of anti-dsDNA IgG in NZB/NZW mice. Methods are described in Example 2. Data from various groups of mice are identified as follows: 1, mice that received anti-mB7RP1 (14 mg/kg); 2, mice that received αBAFF (5.6 mg/kg); 3, mice that received a combination of anti-mB7RP1 (14 mg/kg) and αBAFF (5.6 mg/kg); 4, mice that received the murine surrogate (15 mg/kg); 5, mice that received the mIgG isotype control (15 mg/kg); and 6, mice that received PBS. The asterisks above lanes 1, 3, and 4 indicate a significant (*, $p<0.05$; ***, $p<0.0001$) difference between data in those lanes and data from lane 5 (mIgG).

Figure 9B: Percentages of NZB/W F_1 mice in each group having proteinuria. Methods are described in Example 2. Data from various groups of mice are identified as follows: unfilled squares, mice that received anti-mB7RP1 (14 mg/kg); filled, upward-pointing triangles, mice that received αBAFF (5.6 mg/kg); unfilled, upward-pointing triangles, mice that received a combination of anti-mB7RP1 (14 mg/kg) and αBAFF (5.6 mg/kg); unfilled, downward-pointing triangles, mice that received the murine surrogate (15 mg/kg); filled squares, mice that received the mIgG isotype control (15 mg/kg); and filled circles, mice that received PBS. Significant differences were detected between the murine surrogate versus anti-mB7RP1 ($p<0.01$), αBAFF ($p<0.0001$), and mIgG ($p<0.0001$). The time window in which treatment occurred is indicated.

Figure 10: Kidney scores of NZB/W F_1 mice. As explained in Example 2, kidneys were harvested when a mouse died, if that happened before the end of the study, or at the end of the study. Kidney scores were determined as described in Example 2, with higher scores indicating more severe kidney disease. Shown are averages for each group of mice plus appropriate error bars. The groups of mice received the following treatments: 1) anti-mB7RP1 (14 mg/kg), bar filled with vertical lines; 2) αBAFF (5.6 mg/kg), bar filled with horizontal lines; 3) combination of anti-mB7RP1 (14 mg/kg) and αBAFF (5.6 mg/kg), bar filled with windowpane checks; 4) the murine surrogate (15 mg/kg), bar filled with checkerboard pattern; 5) mIgG (15 mg/kg), bar filled with white dots on a black background; and 6) PBS, solidly filled bar. Asterisks indicate a significant difference from mice treated with mIgG with a p value of <0.05 (*) or <0.001 (***).

Figure 11: Effects of inhibition of BAFF and/or B7RP1 on murine collagen-induced arthritis. Methods are described in Example 4. The five groups of mice were treated with test substances indicated as follows: mIgG, filled squares connected by solid lines; PBS, filled squares connected by dashed lines; anti-mB7RP1, filled circles connected by dashed lines; α\BAFF, open circles connected by solid lines; and combination of anti-mB7RP1 and αBAFF, filled circles connected by solid lines. The top panel shows the percent incidence of arthritis of the various groups, and the bottom panel shows the average arthritic scores of the groups. The vertical, downward-pointing arrow in each panel indicates the time of the second immunization with bovine collagen.

BRIEF DESCRIPTION OF THE SEQUENCE LISTINGS

[0016]

SEQUENCE LISTING NUMBER	DESCRIPTION
SEQ ID NO:1	Amino acid sequence of a BAFF-binding peptide

SEQUENCE LISTING NUMBER	DESCRIPTION
SEQ ID NO:2	Amino acid sequence of a BAFF-binding peptide
SEQ ID NO:3	Amino acid sequence of a BAFF-binding peptide
SEQ ID NO:4	Amino acid sequence of a linker
SEQ ID NO:5	Amino acid sequence of a linker
SEQ ID NO:6	Amino acid sequence of a linker
SEQ ID NO:7	Amino acid sequence of a linker
SEQ ID NO:8	Amino acid sequence of a light chain CDR1
SEQ ID NO:9	Amino acid sequence of a light chain CDR2
SEQ ID NO:10	Amino acid sequence of a light chain CDR3
SEQ ID NO:11	Amino acid sequence of a heavy chain CDR1
SEQ ID NO:12	Amino acid sequence of a heavy chain CDR2
SEQ ID NO:13	Amino acid sequence of a heavy chain CDR3
SEQ ID NO:14	Amino acid sequence of a light chain variable region
SEQ ID NO:15	Amino acid sequence of a heavy chain variable region
SEQ ID NO:16	Amino acid sequence of a heavy chain of the P71619 BAFF/B7RP1 bispecific molecule
SEQ ID NO:17	Amino acid sequence of a heavy chain of the P74293 BAFF/B7RP1 bispecific molecule
SEQ ID NO:18	Amino acid sequence of a heavy chain of the P74294 BAFF/B7RP1 bispecific molecule
SEQ ID NO:19	Amino acid sequence of the immunoglobulin light chain of an IgG anti-huB7RP1 antibody
SEQ ID NO:20	Amino acid sequence preceding a heavy chain CDR1
SEQ ID NO:21	Amino acid sequence preceding a heavy chain CDR2
SEQ ID NO:22	Amino acid sequence following heavy chain CDR3
SEQ ID NO:23	Amino acid sequence following light chain CDR3
SEQ ID NO:24	Linker
SEQ ID NO:25	Amino acid sequence of the immunoglobulin heavy chain of an anti-B7RP1 IgG antibody
SEQ ID NO:26	Amino acid sequence of heavy chain of construct P71617
SEQ ID NO:27	Amino acid sequence of light chain of construct P71618
SEQ ID NO:28	Amino acid sequence of heavy chain of construct P71620
SEQ ID NO:29	Amino acid sequence of the heavy chain of the P71621 construct
SEQ ID NO:30	Amino acid sequence of the heavy chain of construct P71622
SEQ ID NO:31	Amino acid sequence of the heavy chain of construct P71623
SEQ ID NO:32	Amino acid sequence of αBAFF peptibody
SEQ ID NO:33	Amino acid sequence of human IgG1 Fc region
SEQ ID NO:34	Amino acid sequence of human IgG2 Fc region
SEQ ID NO:35	Amino acid sequence of human IgG3 Fc region
SEQ ID NO:36	Amino acid sequence of human IgG4 Fc region
SEQ ID NO:37	Amino acid sequence of a linker
SEQ ID NO:38	Amino acid sequence of a linker

SEQUENCE LISTING NUMBER	DESCRIPTION
SEQ ID NO:39	Amino acid sequence of a linker
SEQ ID NO:40	Amino acid sequence of a linker
SEQ ID NO:41	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1
SEQ ID NO:42	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:4
SEQ ID NO:43	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:5
SEQ ID NO:44	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:6
SEQ ID NO:45	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:7
SEQ ID NO:46	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:8
SEQ ID NO:47	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:9
SEQ ID NO:48	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:10
SEQ ID NO:49	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:11
SEQ ID NO:50	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:12
SEQ ID NO:51	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:13
SEQ ID NO:52	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:14
SEQ ID NO:53	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:15
SEQ ID NO:54	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:16
SEQ ID NO:55	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:17
SEQ ID NO:56	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:18
SEQ ID NO:57	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:19
SEQ ID NO:58	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:24
SEQ ID NO:59	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:25
SEQ ID NO:60	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:26
SEQ ID NO:61	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:27
SEQ ID NO:62	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:28
SEQ ID NO:63	Nucleic acid sequence encoding the amino acid sequence of SEQ

SEQUENCE LISTING NUMBER	DESCRIPTION
	ID NO:29
SEQ ID NO:64	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:30
SEQ ID NO:65	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:31
SEQ ID NO:66	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:32
SEQ ID NO:67	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:33
SEQ ID NO:68	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:34
SEQ ID NO:69	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:35
SEQ ID NO:70	Nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:36
SEQ ID NO:71	Amino acid sequence of a linker
SEQ ID NO:72	Amino acid sequence of a linker

DETAILED DESCRIPTION

[0017] Disclosed herein are bispecific proteins that bind to and inhibit both B cell activating factor (BAFF; also known as BLYS, TALL1, THANK, or TNFSF13B) and B7-related protein 1 (B7RP1; also known as ICOS Ligand, ICOSL, LICOS, B7 Homolog 2, B7H2, and GL50), nucleic acids encoding these bispecific proteins, and methods of making and using these proteins. The bispecific proteins can inhibit both BAFF-mediated B proliferation and B7RP1-mediated T cell proliferation. In another aspect, the bispecific proteins can inhibit B7RP1 binding to T cells. Such a bispecific protein can be an IgG antibody comprising two different immunoglobulin heavy chains and two different immunoglobulin light chains, where one heavy chain/light chain pair binds to BAFF and the other binds to B7RP1. Alternatively, the B7RP1-binding portion of the bispecific protein can comprise an IgG antibody including two identical heavy chains and two identical light chains, and the BAFF-binding portion of the bispecific protein can comprise one or more BAFF-binding peptides, which can be fused to the anti-B7RP1 antibody, optionally via the N-terminus of the immunoglobulin heavy or light chain, the carboxyterminus of the immunoglobulin heavy chain, and/or within the CH2 and/or CH3 region of the immunoglobulin heavy chain.

Definitions

[0018] An **"antibody,"** as meant herein, is a protein comprising a heavy and/or light chain immunoglobulin variable region.

[0019] A **"bispecific"** protein, as meant herein is a protein that can bind specifically to two different molecules, which can be proteins; for example, a bispecific protein that can bind to both BAFF and B7RP1.

[0020] A patient is receiving **"concurrent"** treatment with two or more therapeutics when the patient receives the two or more therapeutics during the same general timeframe, optionally at the very same time. For example, if a patient were dosed with one therapeutic daily on an ongoing basis and were also dosed with

another therapeutic once a month on an ongoing basis, the patient would be receiving these two drugs concurrently. Similarly, a patient dosed with two different therapeutics, each administered every two weeks, but not on the same day, would be receiving concurrent treatment with the two therapeutics. Further, a patient receiving one therapeutic on an ongoing basis once per week and another therapeutic once per day for only three days would be receiving treatment for a short period of time with these two therapeutics.

[0021] As meant herein, an **"Fc region"** is a dimer consisting of two polypeptide chains joined by one or more disulfide bonds, each chain comprising part or all of a hinge domain plus a CH2 and a CH3 domain. Each of the polypeptide chains is referred to as an **"Fc polypeptide chain."** More specifically, the Fc regions contemplated for use with the present invention are IgG Fc regions, which can be mammalian, for example human, IgG1, IgG2, IgG3, or IgG4 Fc regions. Among human IgG1 Fc regions, at least two allelic types are known. The amino acid sequences an Fc polypeptide chain can vary from those of a mammalian Fc polypeptide by no more than 20, 15, 12, 10, 8, 5, or 3 substitutions, insertions, or deletions of a single amino acid relative to a mammalian Fc polypeptide amino acid sequence. Alternatively or in addition, the amino acid sequence of an Fc polypeptide chain can vary from the sequence of a known or naturally occurring Fc polypeptide chain by no more thant 10 insertions, deletions, or substitutions of a single amino acid per every 100 amino acids of sequence. Such variations can be "heterodimerizing alterations" that facilitate the formation of heterodimers over homodimers. In referring to particular positions within an Fc polypeptide chain, the EU numbering system (Edelman et al (1969), Proc. Natl. Acad. Sci. 63: 78-85) is used, as illustrated in the alignment of human IgG Fc polypeptide chains in Table 1 below.

Table 1: Alignment of amino acid sequences of human IgG Fc regions

IgG1	----------					
IgG2	----------					
IgG3	ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCP					
IgG4	----------					
	225	235	245	255	265	275
	*	*	*	*	*	*
IgG1	EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVWDVSHEDPEVKF					
IgG2	ERKCCVE---CPPCPAPPVA-GPSVFLFPPKPKDTLMISRTPEVTCVWDVSHEDPEVQF					
IgG3	EPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVWDVSHEDPEVQF					
IgG4	ESKYG---PPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVWDVSQEDPEVQF					
	285	295	305	315	325	335
	*	*	*	*	*	*
IgG1	NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT					
IgG2	NWYVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT					
IgG3	KWYVDGVEVHNAKTKPREEQYNSTFRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT					
IgG4	NWYVDGVEVHNAKTKPREEQFNSTYRWSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT					
	345	355	365	375	385	395
	*	*	*	*	*	*
IgG1	ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP					
IgG2	ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP					
IgG3	ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTP					
IgG4	ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP					
	405	415	425	435	445	
	*	*	*	*	*	
IgG1	PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID					

	NO:33)
IgG2	PMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:34)
IgG3	PMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK (SEQ ID NO:35)
IgG4	PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO:36)

[0022] At some positions, naturally-occurring polymorphisms can occur. For example, the methionine at position 282 in the IgG2 sequence given above is more typically a valine in naturally occurring IgG2 sequences. Similarly, the tyrosine at position 296 in an IgG3 sequence can also be a phenylalanine.

[0023] "Heterodimerizing alterations" generally refer to alterations in the CH3 regions two different IgG heavy chains that facilitate the formation of heterodimeric heavy chain dimers, that is, dimerized heavy chains that do not have identical amino acid sequences. Heterodimerizing alterations can be asymmetric, that is, one heavy chain having a certain alteration can pair with another heavy chain having a different alteration. These alterations facilitate heterodimerization and disfavor homodimerization. One example of such paired heterodimerizing alterations are the so-called "knobs and holes" substitutions. *See, e.g.,* US Patent 7,695,936 and US Patent Application Publication 2003/0078385. As meant herein, heavy chain-heavy chain pair that contains one pair of knobs and holes substitutions, contains one substitution in one heavy chain and another substitution in the other heavy chain. For example, the following knobs and holes substitutions have been found to increase heterodimer formation as compared with that found with unmodified heavy chains: 1) Y407T in one chain and T366Y in the other; 2) Y407A in one chain and T366W in the other; 3) F405A in one chain and T394W in the other; 4) F405W in one chain and T394S in the other; 5) Y407T in one chain and T366Y in the other; 6) T366Y and F405A in one chain and T394W and Y407T in the other; 7) T366W and F405W in one chain and T394S and Y407A in the other; 8) F405W and Y407A in one chain and T366W and T394S in the other; and 9) T366W in one polypeptide of the Fc and T366S, L368A, and Y407V in the other. As meant herein, mutations in an Fc polypeptide are denoted in the following way. The amino acid (using the one letter code) normally present at a given position in the CH3 region using the EU numbering system (which is presented in Edelman et al (1969), Proc. Natl. Acad. Sci. 63: 78-85) is followed by the EU position number, which is followed by the alternate amino acid that is present at that position. For example, Y407T means that the tyrosine normally present at EU position 407 is replaced by a threonine. For the sake of clarity, the EU system of numbering is illustrated in Table 1 below. Alternatively or in addition to such alterations, substitutions creating new disulfide bridges can facilitate heterodimer formation. *See, e.g.,* US Patent Application Publication 2003/0078385. Such alterations in an IgG1 Fc region include, for example, the following substitutions: Y349C in one Fc-polypeptide chain and S354C in the other; Y349C in one Fc-polypeptide chain and E356C in the other; Y349C in one Fc-polypeptide chain and E357C in the other; L351C in one Fc-polypeptide chain and S354C in the other; T394C in one Fc-polypeptide chain and E397C in the other; or D399C in one Fc-polypeptide chain and K392C in the other. Similarly, substitutions changing the charge of a one or more residue, for example, in the CH3-CH3 interface, can enhance heterodimer formation as explained in WO 2009/089004. Such substitutions are referred to herein as "charge pair substitutions," and an Fc region containing one pair of charge pair substitutions contains one substitution in one heavy chain and a different substitution in the other. General examples of charge pair substitutions include the following: 1) R409D, R409E, K409D, or K409E in one chain plus D399K or D399R in the other; 2) N392D, N392E, K392D, or K392E in one chain plus D399K or D399R in the other; 3) K439D or K439E in one chain plus E356K, E356R, D356K, or D356R in the other; and 4) K370D or K370E in one chain plus E357K or E357R in the other. In addition, the substitutions Q355D, Q355E, R355D, R355E, K360D, or K360R in both chains can stabilize heterodimers when used with other heterodimerizing alterations. Specific charge pair substitutions can be used either alone or with other charge pair substitutions. Specific examples of single pairs of charge pair substitutions and combinations thereof include the following: 1) K409E in one chain plus D399K in the other; 2) K409E in one chain plus D399R in the

other; 3) K409D in one chain plus D399K in the other; 4) K409D in one chain plus D399R in the other; 5) K392E in one chain plus D399R in the other; 6) K392E in one chain plus D399K in the other; 7) K392D in one chain plus D399R in the other; 8) K392D in one chain plus D399K in the other; 9) K409D and K360D in one chain plus D399K and E356K in the other; 10) K409D and K370D in one chain plus D399K and E357K in the other; 11) K409D and K392D in one chain plus D399K, E356K, and E357K in the other; 12) K409D and K392D on one chain and D399K on the other; 13) K409D and K392D on one chain plus D399K and E356K on the other; 14) K409D and K392D on one chain plus D399K and D357K on the other; 15) K409D and K370D on one chain plus D399K and D357K on the other; 16) D399K on one chain plus K409D and K360D on the other; and 17) K409D and K439D on one chain plus D399K and E356K on the other. Any of these heterodimerizing alterations can be part of an immunoglobulin IgG heavy chain as described herein.

[0024] A **"human"** antibody or protein, as meant herein, is an antibody or protein encoded by a nucleic acid sequence of human origin. A human antibody or protein can be made in cultured non-human cells or *in vivo* in a transgenic organism into which a nucleic acid molecule encoding the human antibody or protein has been introduced. Alternatively, a human antibody or protein can be made in cultured human cells or in a human *in vivo*.

[0025] An **"IgG antibody,"** as meant herein, is an antibody that consists essentially of the immunoglobulin domains present in most naturally-occurring IgG antibodies, *i.e.,* a immunoglobulin heavy chain comprising a heavy chain variable (VH) region, a first heavy chain constant (CH1) region, a hinge region, a second heavy chain constant (CH2) region, and a third heavy chain constant (CH3) region and a light chain comprising a light chain variable (VL) region and a light chain constant (CL) region. Numerous sequences of such immunoglobulin domains are reported throughout the scientific literature, *e.g.,* in SEQUENCES OF IMMUNOLOGICAL INTEREST, Public Health Service, N.I.H., Bethesda, MD, 1991. An IgG antibody, as meant herein, is a tetramer consisting essentially of two heavy chains and two light chains. Naturally-occurring antibodies including only two immunoglobulin heavy chains and no immunoglobulin light chains, such as some found in camels and sharks (*see, e.g.,* Muyldermans et al., 2001, J. Biotechnol. 74:277-302; Desmyter et al., 2001, J. Biol. Chem. 276:26285-90; Streltsov et al. (2005), Protein Science 14: 2901-2909), are not "IgG antibodies" as meant herein. An IgG antibody can be human or can be from another species. In addition, an IgG antibody can contain no more than 40, 35, 30, 25, 20, 15, 10, or 5 substitutions, insertions, and/or deletions of a single amino acid relative to the amino acid sequence of the heavy or light chains of a naturally occurring IgG antibody.

[0026] An **"immunoglobulin heavy chain"** refers to a heavy chain of an IgG, IgA, IgM, IgE, or IgD antibody or variants thereof containing not more than 40, 30, 25, 20, 15, 10, or 5 insertions, deletions, or substitutions of a single amino acid relative to an immunoglobulin heavy chain encoded by nucleic acid sequences originating in nature. An **"immunoglobulin IgG heavy chain"** is limited to heavy chains from IgG antibodies or variants thereof containing not more than 40, 30, 25, 20, 15, 10, or 5 insertions, deletions, or substitutions of a single amino acid relative to the amino acid sequence of an IgG heavy chain encoded by nucleic acid sequences originating in nature. An immunoglobulin heavy chain consists essentially of a number of distinct regions or domains including a VH region, a CH1 region, a hinge region, a CH2 region, and a CH3 region. In some other isotypes, *i.e.,*IgM and IgA, additional regions are included downstream from the CH3 region. Immunoglobulin heavy chains and the regions included therein are generally described in, *e.g.,* Carayannopoulos and Capra, Immunoglobulins: Structure and Function, pp. 283-314 in FUNDAMENTAL IMMUNOLOGY, 3rd Ed, Paul, ed., Raven Press, New York, 1993. In addition, numerous sequences of subregions of immunoglobulin heavy chains are known in the art. *See, e.g.,* Kabat et al, SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, Public Health Service N.I.H., Bethesda, MD, 1991. In some cases, a polypeptide chain that includes an immunoglobulin heavy chain plus some non-immunoglobulin sequences will be referred to herein as a "heavy chain."

[0027] An **"immunoglobulin light chain,"** as meant herein, is a kappa or a lambda chain from a human antibody or an antibody from another species. Also included among immunoglobulin light chains, as meant

herein, are proteins with no more than 20, 15, 10, or 5 insertions, deletions, and/or substitutions of a single amino acid relative to an immunoglobulin light chain encoded by nucleic acid sequences of natural origin. Immunoglobulin light chains are generally described in, *e.g.,* Carayannopoulos and Capra, Immunoglobulins: Structure and Function, pp. 283-314 in FUNDAMENTAL IMMUNOLOGY, 3rd Ed, Paul, ed., Raven Press, New York, 1993. A immunoglobulin light chain contains a VL region and a CL region. Numerous sequences of these regions are known in the art. *See, e.g.,* Kabat et al, SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, Public Health Service N.I.H., Bethesda, MD, 1991. In some cases, a polypeptide chain that includes an immunoglobulin light chain plus some non-immunoglobulin sequences will be referred to herein as a "light chain."

[0028] An **"immunoglobulin variable region,"** as meant herein, is a VH or VL region, which can be of human origin or from another species. Immunoglobulin variable regions are generally described in, *e.g.*, Carayannopoulos and Capra, Immunoglobulins: Structure and Function, pp. 283-314 in FUNDAMENTAL IMMUNOLOGY, 3rd Ed, Paul, ed., Raven Press, New York, 1993. Also included among immunoglobulin variable regions, as meant herein, are proteins with no more than 20, 15, 10, or 5 insertions, deletions, and/or substitutions of a single amino acid relative to an immunoglobulin variable region encoded by nucleic acid sequences of natural origin. An immunoglobulin variable region contains three hypervariable regions, known as complementarity determining region 1 (CDR1), complementarity determining region 2 (CDR2), and complementarity determining region 3 (CDR3). These regions form the antigen binding site of an antibody. The CDRs are embedded within the less variable framework regions (FR1-FR4). The order of these subregions within a variable region is as follows: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. Numerous sequences of immunoglobulin variable regions are known in the art. *See, e.g.,* Kabat et al, SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, Public Health Service N.I.H., Bethesda, MD, 1991.

[0029] CDRs can be located in a VH region sequence in the following way. CDR1 starts at approximately residue 31 of the mature VH region and is usually about 5-7 amino acids long, and it is almost always preceded by a Cys-Xxx-Xxx-Xxx-Xxx-Xxx-Xxx-Xxx-Xxx (SEQ ID NO: 20) (where "Xxx" is any amino acid). The residue following the heavy chain CDR1 is almost always a tryptophan, often a Trp-Val, a Trp-Ile, or a Trp-Ala. Fourteen amino acids are almost always between the last residue in CDR1 and the first in CDR2, and CDR2 typically contains 16 to 19 amino acids. CDR2 may be immediately preceded by Leu-Glu-Trp-Ile-Gly (SEQ ID NO: 21) and may be immediately followed by Lys/Arg-Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala. Other amino acids may precede or follow CDR2. Thirty two amino acids are almost always between the last residue in CDR2 and the first in CDR3, and CDR3 can be from about 3 to 25 residues long. A Cys-Xxx-Xxx almost always immediately precedes CDR3, and a Trp-Gly-Xxx-Gly (SEQ ID NO: 22) almost always follows CDR3.

[0030] Light chain CDRs can be located in a VL region in the following way. CDR1 starts at approximately residue 24 of the mature antibody and is usually about 10 to 17 residues long. It is almost always preceded by a Cys. There are almost always 15 amino acids between the last residue of CDR1 and the first residue of CDR2, and CDR2 is almost always 7 residues long. CDR2 is typically preceded by Ile-Tyr, Val-Tyr, Ile-Lys, or Ile-Phe. There are almost always 32 residues between CDR2 and CDR3, and CDR3 is usually about 7 to 10 amino acids long. CDR3 is almost always preceded by Cys and usually followed by Phe-Gly-Xxx-Gly (SEQ ID NO: 23).

[0031] A **"linker,"** as meant herein, is a peptide that links two polypeptides. A linker can be from 1-80 amino acids in length. A linker can be 2-40, 3-30, or 3-20 amino acids long. A linker can be a peptide no more than 14,13, 12, 11, 10, 9, 8, 7, 6, or 5 amino acids long. A linker can be 5-25, 5-15, 10-20, or 20-30 amino acids long. A linker can be about, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids long. In many cases, linkers lack free cysteine residues (i.e. and are therefore not involved in disulfide bonds) and also do not contain N-glycosylation sites (that is, Asn - Xxx - Ser/Thr, where X can be any amino acid except proline).

[0032] A **"peptibody,"** as meant herein, is one or more biologically active peptides fused to an Fc region.

Shimamoto et al. (2012), mAbs 4(5): 586-591.

[0033] A **"peptide,"** as meant herein, is a polypeptide that consists of a short amino acid sequence, which may or may not be glycosylated and/or contain modified amino acids. A peptide can be from 2 to 75 amino acids long; *e.g.,* 3-60, 3-50, 3-40, 3-30, or 3-20 amino acids long. A peptide can be 5-25, 5-15, 10-20, or 20-30 amino acids long. A peptide can be about, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids long.

[0034] A **"therapeutically effective amount"** of a drug used to treat a disease is an amount that can reduce the severity of a disease, reduce the severity of one or more symptoms associated with the disease or its treatment, or delay the onset of more serious symptoms or a more serious disease that can occur with some frequency following the treated condition.

[0035] "Treatment" of any disease mentioned herein encompasses an alleviation of at least one symptom of the disease, a reduction in the severity of the disease, or the delay or prevention of disease progression to more serious symptoms that may, in some cases, accompany the disease or lead to at least one other disease. Treatment need not mean that the disease is totally cured. A useful therapeutic agent needs only to reduce the severity of a disease, reduce the severity of one or more symptoms associated with the disease or its treatment, or delay the onset of more serious symptoms or a more serious disease that can occur with some frequency following the treated condition. For example, if the disease were an inflammatory bowel disease, a therapeutic agent used as a treatment may reduce the number of distinct sites of inflammation in the gut or the total extent of the gut affected. It may reduce pain and/or swelling, reduce symptoms such as diarrhea, constipation, or vomiting, and/or prevent perforation of the gut. A patient's condition can be assessed by standard techniques such as an x-ray performed following a barium enema or enteroclysis, endoscopy, colonoscopy, and/or a biopsy. Suitable procedures vary according to the patient's condition and symptoms. Similarly, if the disease treated were systemic lupus erythematosus (SLE), disease activity could be evaluated using the SLEDAI index for scoring, as explained below.

Bispecific Proteins that Bind to BAFF and B7RP1

[0036] Disclosed herein are bispecific proteins that bind to B7RP1 and BAFF and/or that can inhibit B7RP1-mediated T cell proliferation and BAFF-mediated B cell proliferation *in vitro.* The BAFF and B7RP1 proteins to which a bispecific protein as described herein binds can be human proteins and/or can be proteins from another species such as cynomolgus monkey, rhesus monkey, chimpanzee, mouse, and/or rabbit, among others. A bispecific protein as described herein can, for example, bind to both human (*Homo sapiens*) and cynomolgus monkey (*Macaca fascicuiaris*) B7RP1 and BAFF proteins.

[0037] These bispecific proteins can be bispecific IgG antibodies in which the B7RP1-binding portion and the BAFF-binding portion each consists essentially of an immunoglobulin IgG heavy chain and an immunoglobulin light chain. Thus, such a bispecific antibody contains two different immunoglobulin heavy chains and two different immunoglobulin light chains. Together, these two pairs of immunoglobulin heavy and light chains form a complete bispecific IgG antibody. Bispecific IgG antibodies are known in the art, and a number of other formats for bispecific antibodies are also known. *See, e.g.,* Kontermann, Bispecific Antibodies: Developments and Current Perspectives, pp. 1-28 in BISPECIFIC ANTIBODIES, Kontermann, ed., Springer-Verlag, Berlin, Heidelburg, 2011. Antibodies that can bind to BAFF and B7RP1, regardless of format, are contemplated herein. Bispecific IgG antibodies can be human, humanized, or chimeric and can be of the IgG1, IgG2, IgG3, or IgG4 isotype. Bispecific IgG antibodies disclosed herein can be conjugated to other moieties. Amino acid sequences of anti-BAFF and anti-B7RP1 antibodies are known in the art. *See e.g.,* U.S. Patent 7,737,111 and U.S. Patent Application Publication US 2011/0117093. Such bispecific antibodies can comprise "heterodimerizing alterations," as defined above, including charge pair substitutions, that facilitate formation of a heterotetrameric

bispecific IgG antibody.

[0038] The bispecific proteins described herein can be fusion proteins comprising an antibody that binds to B7RP1, which comprises an immunoglobulin IgG heavy chain and an immunoglobulin light chain, and a peptide that binds to BAFF. The BAFF-binding peptide can be present in one or multiple copies, such as two, three, four, five, six, seven, eight, or up to 16 copies. The BAFF-binding peptide may bind to BAFF proteins from species such as mouse, cynomolgus monkey, and/or humans, among many other possible species. The antibody can be an anti-B7RP1 IgG antibody, optionally a human or humanized antibody that binds to human and/or cynomolgus monkey B7RP1. In some embodiments, a linker can be attached to the C terminus of the heavy chain of the anti-B7RP1 IgG antibody, followed by a first BAFF-binding peptide, another linker, and a second BAFF-binding peptide. A third, fourth, fifth, sixth, seventh, eighth, or up to sixteenth BAFF-binding peptide can follow these two, optionally interspersed with linkers. Alternatively or in addition, one, two, three, four, five, six, seven, or eight BAFF-binding peptides can be inserted elsewhere in the anti-B7RP1 antibody, for example at the N terminus of the immunoglobulin heavy chain or immunoglobulin light chain or in a loop region in the CH2 or CH3 region. The IgG antibody can be a mammalian antibody, such as a human or murine antibody. The anti-B7RP1 antibody can be a human or humanized IgG1, IgG2, IgG3, or IgG4 antibody. In such bispecific fusion proteins comprising an anti-B7RP1 IgG antibody, the bispecific protein can comprise a heavy chain comprising the amino acid sequence of SEQ ID NO:17 or SEQ ID NO:18 and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO:19. Variants comprising a heavy chain having an amino acid sequence containing no more than 30, 25, 20, 15, 10, 5, or 3 insertions, deletions, or substitutions of a single amino acid relative to SEQ ID NO: 17 or 18 are contemplated. Similarly, variants comprising an immunoglobulin light chain having an amino acid sequence containing no more 20, 15, 10, 8, 7, 5, or 3 insertions, deletions, or substitutions or a single amino acid relative SEQ ID NO:19 are contemplated. Such bispecific proteins can be tetramers comprising two polypeptides comprising the amino acid sequence of SEQ ID NO:17 or 18 or a variant thereof and two light chains comprising the amino acid sequence of SEQ ID NO:19 or a variant thereof.

[0039] A BAFF-binding peptide portion of a bispecific fusion protein as described above can comprise the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3. Such BAFF-binding peptides are described in U.S. Patent 7,737,111. There may be one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, or sixteen copies of such a BAFF-binding peptide present in the bispecific protein. A BAFF-binding peptide can be attached to the carboxy end of the anti-B7RP1 antibody, for example, via a linker. For example, the carboxy end of an anti-B7RP1 IgG antibody can be followed by a linker having, for example, the amino acid sequence of Gly-Gly-Gly-Gly (SEQ ID NO:4). Examples of other suitable linkers include Gly-Gly, Gly-Gly-Gly, Gly-Gly-Gly-Ser (SEQ ID NO:37), Gly-Gly-Gly-Pro (SEQ ID NO:38), Gly-Gly-Gly-Gln (SEQ ID NO:39), and Gly-Gly-Gly-Gly-Gly (SEQ ID NO:40), among many others. This linker can be followed by a BAFF-binding peptide. The BAFF-binding peptide can be followed by another linker comprising, for example, the amino acid sequence of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:24. Other linker could also be used. This linker can be followed by another BAFF-binding peptide comprising, for example, the amino acid sequence of SEQ ID NO:1.

[0040] In the bispecific fusion proteins described immediately above or in the bispecific heterotetrameric IgG antibodies described above, a VL region can contain a CDR1, a CDR2, and a CDR3 comprising the amino acid sequences of SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10, respectively. A VH region CDR1, CDR2, and CDR3 can comprise the amino acid sequences of SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13, respectively. A VL region of the IgG antibody disclosed herein can comprise the amino acid sequence of SEQ ID NO:14 or a variant thereof, and the VH region can comprise the amino acid sequence of SEQ ID NO:15 or a variant thereof. Such variant sequences can comprise not more than 10 deletions, insertions of substitutions of a single amino acid per 100 amino acids relative to a reference sequence.

Proteins Comprising a Linker

[0041] Disclosed herein are linkers having the amino acid sequences of SEQ ID NO:5, 6, or 7 that confer favorable physical properties on a protein that contains them. As shown in Example 1 below, the use of two particular linkers, *i.e.,* those having the amino acid sequences of SEQ ID NO:6 and SEQ ID NO:7, had positive effects on properties such as expression, stability, and viscosity of a bispecific molecule. Thus, a variety of proteins containing these linkers may have such favorable properties as compared to similar proteins containing other linkers.

Therapeutic Uses of Bispecific Proteins

[0042] The bispecific proteins binding to BAFF and B7RP1 described herein can be used as therapeutics for a variety of indications, particularly conditions driven by autoantibodies and/or conditions mediated by both T cells and B cells. Such conditions include, for example, SLE, lupus, nephritis, ANCA-positive vasculitis, rheumatoid arthritis (RA), dermatomyositis, polymyositis, gastrointestinal diseases such as Crohn's disease, ulcerative colitis, and celiac disease, skin conditions such as pemphigus, pemphigoid, and subacute cutaneous lupus erythematosus (SCLE), diseases of the nervous system such as multiple sclerosis and chronic inflammatory demyelinating polyneuropathy (CIDP), neuromuscular diseases such as myasthenia gravis, diseases involving the kidneys such as Goodpasture's syndrome and glomerulonephritis, hematologic conditions such as autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), and autoimmune neutropenia, liver conditions such as chronic active hepatitis and primary biliary cirrhosis, Sjogren's syndrome, systemic sclerosis, and endocrine conditions including Hashimoto's thyroiditis, Graves' disease, Addison's disease, and multiple endocrine autoimmune failure (commonly including diabetes, hypothyroidism, Addison's disease, and gonadal failure). A therapeutically effective amount of a bispecific protein as described herein can be administered to a patient suffering from any of these conditions to treat the condition.

[0043] A bispecific protein that can inhibit BAFF-mediated B cell proliferation and B7RP1-mediated T cell proliferation disclosed herein can be used to treat a patient suffering from SLE. SLE is an autoimmune disease of unknown etiology marked by autoreactivity to nuclear self antigens. Its clinical manifestations are so diverse that it is questionable whether it is truly a single disease or a group of related conditions. Kotzin (1996) Systemic lupus erythematosus. Cell 85: 303-306; Rahman and Isenberg (2008), Systemic lupus erythematosus. N. Engl. J. Med. 358: 929-939. Symptoms can include the following: constitutional symptoms such as malaise, fatigue, fevers, anorexia, and weight loss; diverse skin symptoms including acute, transient facial rashes in adults, bullous disease, and chronic and disfiguring rashes of the head and neck; arthritis; muscle pain and/or weakness; cardiovascular symptoms such as mitral valve thickening, vegetations, regurgitation, stenosis, pericarditis, and ischemic heart disease, some of which can culminate in stroke, embolic disease, heart failure, infectious endocarditis, or valve failure; nephritis, which is a major cause of morbidity in SLE; neurological symptoms including cognitive dysfunction, depression, psychosis, coma, seizure disorders, migraine, and other headache syndromes, aseptic meningitis, chorea, stroke, and cranial neuropathies; hemotologic symptoms including leucopenia, thrombocytopenia, serositis, anemia, coagulation abnormalities, splenomegaly, and lymphadenopathy; and various gastrointestinal abnormalities. *Id;* Vratsanos et al., "Systemic Lupus Erythematosus," Chapter 39 in Samter's Immunological Diseases, 6th Edition, Austen et al., eds., Lippincott Williams & Wilkins, Phiiladelphia, PA, 2001. Severity of symptoms varies widely, as does the course of the disease. SLE can be deadly.

[0044] An SLE patient can be treated with a bispecific protein that inhibits BAFF and B7RP1 before, after, or concurrently with treatment using an existing therapy for SLE. Such existing therapies for SLE include corticosteroids such as prednisone, prednisolone, and methylprednisolone, antimalarials such as hydroxychloroquine, quinacrine, and chloroquine, retinoic acid, aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), cyclophosphamide, dehydroepiandrosterone, mycophenolate mofetil, azathioprine, chlorambucil, methotrexate, tacrolimus, dapsone, thalidomide, leflunomide, cyclosporine, belimumab, anti-

CD20 antibodies such as rituximab, and fusion proteins such as abatacept.

[0045] The disease activity of SLE patients can be rated using an instrument such as the Systemic Lupus Erythrmatosus Disease Activity Index (SLEDAI), which provides a score for disease activity that takes into consideration the following symptoms, which are weighted according to severity: seizure, psychosis, organic brain syndrome, visual disturbance, cranial nerve disorder, lupus headache, vasculitis, arthritis, myositis, urinary casts, hematuria, proteinuria, pyuria, new rash, alopecia, mucosal ulcers, pleurisy, pericarditis, low complement, increased DNA binding, fever, thrombocytopenia, and leucopenia. Bombardier et al. (1992), Arthr. & Rheum. 35(6): 630-640. The treatments described herein can be useful in lessening or eliminating symptoms of SLE as measured by SLEDAI. Methods of treatment described herein can improve a patient's SLEDAI score compared to a baseline value for the same patient prior to initiation of treatment with a bispecific protein as described herein.

[0046] Another method for assessing disease activity in SLE is the British Isles Lupus Assessment Group (BILAG) index, which is a disease activity assessment system for SLE patients based on the principle of the physician's intention to treat. Stoll et al. (1996), Ann. Rheum Dis. 55: 756-760; Hay et al. (1993), Q. J. Med. 86: 447-458. A BILAG score is assigned by giving separate numeric or alphabetic disease activity scores in each of eight organ-based systems, general (such as fever and fatigue), mucocutaneous (such as rash and alopecia, among many other symptoms), neurological (such as seizures, migraine headaches, and psychosis, among many other symptoms), musculoskeletal (such as arthritis), cardiorespiratory (such as cardiac failure and decreased pulmonary function), vasculitis and thrombosis, renal (such as nephritis), and hematological. *Id.* The treatments described herein can be useful in lessening or eliminating symptoms of SLE as measured by the BILAG index or in decreasing a patient's BILAG score as compared to a baseline value prior to the initiation of treatment with a bispecific protein as described herein.

[0047] A bispecific protein as described herein, which inhibits BAFF-mediated proliferation of B cells and B7RP1-mediated proliferation of T cells, could also be used to treat rheumatoid arthritis (RA). RA is a chronic disease with systemic symptoms, as well as symptoms relating specifically to the joints. Symptoms commonly include synovitis, leading to painful and swollen joints, and various laboratory abnormalities such as higher-than-normal levels of rheumatoid factor, anti-citrulline modified protein (anti-CCP) antibodies, and C-reactive protein (CRP) and an elevated erythrocyte sedimentation rate (ESR). Less common symptoms include various extra-articular symptoms involving, *e.g.,* tendons, ligaments, blood vessels, the heart, and the lungs. Disease activity can be often measured using a variety of indices. *See, e.g.,* Anderson et al. (2012), Arthritis care & Res. 64 (5): 640-647. Elements included in such scoring indices include the number of tender joints, the number of swollen joints, functional assessments, and various laboratory findings such as CRP, ESR, etc.

[0048] A patient suffering from RA can be treated with a bispecific protein disclosed herein that inhibits BAFF-mediated B cell proliferation and B7RP1-mediated T cell proliferation before, after, or concurrently with treatment with a drug in current use for RA. Therapeutics currently in use for rheumatoid arthritis (RA) include nonsteroidal anti-inflammatory drugs (NSAIDs) (such aspirin and cyclooxygenase-2 (COX-2) inhibitors), disease modifying anti-inflammatory drugs (DMARDs, such as methotrexate, leflunomide, and sulfasalazine), anti-malarials (such as hydroxychloroquine), cyclophosphamide, D-penicillamine, azathioprine, gold salts, tumor necrosis factor inhibitors (such as etanercept, infliximab, adalimumab, golimumab, and certolizumab pegol), CD20 inhibitors such as rituximab, IL-1 antagonists such as anakinra, IL-6 inhibitors such as tocilizumab, inhibitors of Janus kinases (JAKs, such as tofacitinib), abatacept, and corticosteroids, among others.

[0049] A therapeutically effective amount of a bispecific protein as described herein, which inhibits BAFF-mediated proliferation of B cells and B7RP1-mediated proliferation of T cells, can also be used to treat an inflammatory bowel disease, such as Crohn's disease or ulcerative colitis. Crohn's disease involves an abnormal inflammation of any portion of the alimentary tract from the mouth to the anus, although in most patients abnormal inflammation is confined to the ileocolic, small-intestinal, and colonic-anorectal regions.

Typically, the inflammation is discontinuous. Common symptoms include abdominal pain, anorexia, weight loss, fever, diarrhea, fullness and/or tenderness in the right lower quadrant of the abdomen, constipation, vomiting, and perianal discomfort and discharge. Other possible symptoms include peripheral arthritis, growth retardation, episcleritis, aphthous stomatitis, erythema nodosum, pyoderma gangrenosum, kidney stones, impaired urinary dilution and alkalinization, malabsorption, and gallstones, among others. *See e.g.* Strober et al., Medical Immunology, 10th Edition, Section III, Ch. 35 (2001); Merck Manual of Diagnosis and Therapy, 17th Edition, Section 3, Ch. 31 (1999). Macrophages isolated from patients with Crohn's disease produce increased amounts of IL-12, IFNγ, TNFα, and other inflammatory cytokines.

[0050] Ulcerative colitis, though it is sometimes hard to distinguish from Crohn's disease, is distinct from Crohn's disease in several respects. First, it is generally limited to the colon while Crohn's disease may occur throughout the alimentary tract. Second, ulcerative colitis mainly involves inflammation only of the superficial layers of the bowel, unlike Crohn's disease in which the inflammation can penetrate all way through the wall of the bowel or other location in the alimentary tract. Finally, ulcerative colitis typically involves a continuous area of inflammation, rather than the discontinuous sites of inflammation typical of Crohn's disease. Like Crohn's disease, ulcerative colitis is found primarily in urban areas. Also, genetic factors likely play a role in ulcerative colitis since there is a familial aggregation of cases. Autoantibodies are observed in ulcerative colitis patients more often than Crohn's disease patients. The autoantibodies are often directed to colonic epithelial cell components. Among the most common are antineutrophil cytoplasmic antibodies with specificities for catalase, α-enolase, and lactoferrin. In some cases such antibodies cross react with colonic microorganisms.

[0051] In clinical trials, Crohn's disease activity is often scored using the Crohn's Disease Activity Index (CDAI). The CDAI provides a disease activity score based on eight factors including (1) the number of liquid or soft stools per day, (2) a patient rating of the amount of abdominal pain per day, (3) a patient rating of general well-being, (4) a patient report of other symptoms including arthritis, iritis, uveitis, erythema nodosum, pyoderma gangrenosum, ephthous stomatitis, anal fissure, fitula, or abscess, other fistula, or fever, (5) patient report of taking lomotil or other opiates for diarrhea, (6) abdominal mass, (7) hematocrit, and (8) body weight. *See, e.g.,* Best et al. (1976), Gastroenterol. 70: 439-444.

[0052] Symptoms of ulcerative colitis are variable. They may include diarrhea, tenesmus, abdominal cramps, blood and mucus in the stool, fever, and rectal bleeding. Toxic megacolon, a potentially life-threatening condition in which the colon is dilated beyond about 6 centimeters and may lose its muscular tone and/or perforate, may also occur. Other symptoms that may accompany ulcerative colitis include peripheral arthritis, ankylosing spondylitis, sacroiliitis, anterior uveitis, erythema nodosum, pyoderma gangrenosum, episcleritis, autoimmune hepatitis, primary sclerosing cholangitis, cirrhosis, and retarded growth and development in children.

[0053] A patient suffering from an inflammatory bowel disease (IBD), such as Crohn's disease or ulcerative colitis, can be treated with a bispecific protein that binds to BAFF and B7RP1 disclosed herein before, after, or concurrently with treatment with an existing therapy for IBD. Existing therapeutics for IBD include, for example, sulfasalazine, 5-aminosalicylic acid and its derivatives (such as olsalazine, balsalazide, and mesalamine), anti-TNF antibodies (including infliximab, adalimumab, golimumab, and certolizumab pegol), corticosteroids for oral or parenteral administration (including prednisone, methylprednisone, budesonide, or hydrocortisone), adrenocorticotropic hormone, antibiotics (including metronidazole, ciprofloxacin, or rifaximin), azathioprine, 6-mercaptopurine, methotrexate, cyclosporine, tacrolimus, and thalidomide.

Nucleic Acids Encoding Bispecific Proteins

[0054] Disclosed herein are nucleic acids encoding a bispecific protein that can inhibit B7RP1-mediated T cell proliferation and BAFF-mediated B cell proliferation. For example, SEQ ID NO:52 encodes the VL region having

the amino acid sequence of SEQ ID NO:14, and SEQ ID NO:53 encodes the VH region having the amino acid sequence of SEQ ID NO:15. Similarly, SEQ ID NOs:55 and 56 encode the amino acid sequences of SEQ ID NOs:17 and 18, respectively, which are polypeptides comprising the heavy chain of an anti-B7RP1 antibody fused to two BAFF-binding peptides. SEQ ID NO:57 encodes the light chain of an anti-B7RP1 antibody, which can be part of a hetero-tetrameric bispecific IgG antibody or a bispecific fusion protein, as described above. Any nucleic acid sequence encoding any amino acid sequence disclosed herein is contemplated. Similarly, nucleotide sequence variants including silent mutations relative to sequences disclosed herein or encoding the amino acid sequence variants described above are contemplated. More specifically, these nucleic acids can be nucleotide sequences encoding amino acid sequences that vary by no more than 10 insertions, deletions, or substitutions of a single amino acid per 100 amino acids from amino acid sequences disclosed herein.

[0055] Nucleic acid sequences encoding bispecific proteins described herein can be determined by one of skill in the art based on the amino acid sequences disclosed herein and knowledge in the art. Besides more traditional methods of producing cloned DNA segments encoding a particular amino acid sequence, companies such as DNA 2.0 (Menlo Park, CA, USA) and BlueHeron (Bothell, WA, USA), among others, now routinely produce chemically synthesized, gene-sized DNAs of any desired sequence to order, thus streamlining the process of producing such DNAs. Codon usage can be adjusted so as to optimize expression in the system of choice.

Methods of Making Bispecific Proteins that Bind to BAFF and B7RP1

[0056] Nucleic acids encoding the bispecific proteins described herein can be inserted into vectors appropriate for the host cell in which the nucleic acid will be expressed. These nucleic acids can be introduced into the host cells by any of the methods well-known in the art. Host cells that can be used include bacteria, including *Escherichia coli,* yeast, including *Saccharomyces cerevisiae or Pichia pastoris,* insect cells including *Spodoptera frugiperda* cells, plant cells, and mammalian cells, including Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, monkey kidney cells, HeLa cells, human hepatocellular carcinoma cells, and 293 cells, among many others. These host cells can be cultured under conditions such that the introduced nucleic acids will be expressed, and the bispecific protein can be recovered from the culture supernatant or the cell mass.

[0057] Generally, the procedure used to introduce the nucleic acids into the host cells may depend upon the host cell into which the nucleic acids are to be introduced. Methods of introducing nucleic acids into bacteria are well-known in the art. For example, electroporation or calcium choride transformation are commonly used. Methods for introduction of nucleic acids into yeast are also well-known in the art and include, for example, transformation methods using lithium acetate and polyethylene glycol. Methods for introducing heterologous polynucleotides into mammalian cells are well known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

[0058] Expression vectors used in any of the host cells can contain sequences necessary for DNA replication, selection of host cells containing the vector, and expression of the exogenous nucleotide sequences. Such sequences can typically include one or more of the following nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Numerous expression vectors appropriate for expression in various host cells are known in the art and are commercially available.

Pharmaceutical Compositions, Dosing, and Methods of Administration

[0059] Pharmaceutical compositions comprising the bispecific proteins described herein are disclosed. Such compositions can comprise a therapeutically effective amount of a bispecific protein with one or more additional components such as a physiologically acceptable carrier, excipient, or diluent. Such additional components can include buffers, carbohydrates, polyols, amino acids, chelating agents, stabilizers, and/or preservatives, among many possibilities. Many such additional components are described in, *e.g.,* REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Edition, (A.R. Gennaro, ed.), 1990, Mack Publishing Company.

[0060] Dosing of the bispecific proteins described herein can be adjusted to achieve the desired effects. In many cases, repeated dosing will be required because of the chronic nature of the disease being treated. For example, a bispecific protein as described herein can be dosed twice per week, once per week, once every two, three, four, five, six, seven, eight, nine, or ten weeks, or once every two, three, four, five, or six months. The amount of the bispecific protein administered on each day that it is administered can be from about 0.0036 mg to about 700 mg. Alternatively, the dose can calibrated according to the estimated skin surface of a patient, and each dose can be from about 0.002 $\mu g/m^2$ to about 350 mg/m^2. In another alternative, the dose can be calibrated according to a patient's weight, and each dose can be from about 0. 000051 mg/kg to about 10.0 mg/kg.

[0061] The bispecific proteins, or pharmaceutical compositions containing these molecules, can be administered by any feasible method. Therapeutics that comprise a protein will ordinarily be administered by a parenteral route, for example by injection, since oral administration, in the absence of some special formulation or circumstance, would lead to hydrolysis of the protein in the acid environment of the stomach. Subcutaneous, intramuscular, intravenous, intraarterial, intralesional, and peritoneal bolus injections are possible routes of administration. The bispecific proteins can also be administered via infusion, for example intravenous or subcutaneous infusion. Topical administration is also possible, especially for diseases involving the skin. Alternatively, the bispecific proteins can be administered through contact with a mucus membrane, for example by intra-nasal, sublingual, vaginal, or rectal administration or administration as an inhalant. Alternatively, certain appropriate pharmaceutical compositions comprising a bispecific protein can be administered orally.

[0062] Having described the invention in general terms above, the following examples are offered by way of illustration and not limitation.

EXAMPLES

Example 1: Designing and testing a BAFF/B7RP1 bispecific molecule for human therapeutic use

[0063] The object of this series of experiments was to find a bispecific molecule that (1) inhibits BAFF-mediated B cell proliferation and B7RP1-mediated T cell proliferation, (2) is highly active in biological assays, and (3) has favorable biophysical properties. A number of schematic designs for the fusion of a peptide that binds human BAFF to an anti-human B7RP1 IgG antibody (anti-huB7RP1) are illustrated in Figure 1. The sequence of the BAFF-binding peptide is provided in SEQ ID NO:1, and the sequences of the immunoglobulin heavy and light chains of anti-huB7RP1 are provided in SEQ ID NO:25 and SEQ ID NO:19, respectively.

[0064] To determine which design had the best biophysical properties, while retaining biological activity, the bispecific molecules diagrammed in Figure 1 were made and tested. In one construct, two tandem copies of the BAFF-binding peptide with an intervening linker (the "1K linker," having the amino acid sequence of SEQ ID NO:24) were fused to the N-terminus of either the immunoglobulin heavy chain (P71617) or immunoglobulin light chain (P71618) of anti-huB7RP1. *See* Figure 1. The amino acid sequence of the P71617 heavy chain is

provided in SEQ ID NO:26, and the amino acid sequence of the light chain of P71617 is the same as that of the immunoglobulin light chain of anti-huB7RP1 (SEQ ID NO:19). The amino acid sequence of the P71618 light chain is provided in SEQ ID NO:27, and the amino acid sequence of the heavy chain of P71618 is the same as the immunoglobulin heavy chain of anti-huB7RP1 (SEQ ID NO:25). Two tandem copies of the BAFF-binding peptide were also fused to the C-terminal end of the immunoglobulin heavy chain of anti-huB7RP1 (having the amino acid sequence of SEQ ID NO:25) using either the 1K linker mentioned above (having the amino acid sequence of SEQ ID NO:24; P71619) or a 5X(G4S) linker (SEQ ID NO: 71) between the two BAFF-binding peptides (P71620). The amino acid sequences of the heavy chains of these two fusion constructs are provided in SEQ ID NO:16 (P71619) and SEQ ID NO:28 (P71620). In construct P71621, two tandem copies of the BAFF-binding peptide with an intervening 1K linker were inserted into the antibody's CH3 domain between residues 358 and 359 of the amino acid sequence of SEQ ID NO:25 (the amino acid sequence of the immunoglobulin heavy chain of the anti-huB7RP1 antibody). The sequence of the heavy chain of the P71621 construct is provided in SEQ ID NO:29. In construct P71622, the BAFF-binding peptide was inserted into the CH3 domain of the immunoglobulin heavy chain of anti-huB7RP1 (between residues 358 and 359 of SEQ ID NO:25 and a second copy of the BAFF-binding peptide was fused to the C-terminal end of the heavy chain. The amino acid sequence of the heavy chain of P71622 is provided in SEQ ID NO:30. In construct P71623, one BAFF-binding peptide was inserted into the CH2 region (between residues 268 and 269 of SEQ ID NO:25), and a second BAFF-binding peptide was inserted into the CH3 region (between residues 358 and 359 of SEQ ID NO:25). SEQ ID NO:31 is the amino acid sequence of the heavy chain of P71623. Constructs P71619-P71623 all have the immunoglobulin light chain of anti-huB7RP1 (SEQ ID NO:19).

[0065] In constructs P74293 and P74294, the linker between the two tandem copies of the BAFF-binding peptides in construct P71619 was modified. The amino acid sequences of the heavy chains of P74293 and P74294 are provided in SEQ ID NO:17 and SEQ ID NO:18, respectively. The immunoglobulin light chains of these constructs also have the amino acid sequence of SEQ ID NO:19.

[0066] Nucleic acids encoding the constructs described above were made as follows. Nucleic acids encoding the N-terminal portion of the N-terminal BAFF peptide fusions (P71617 and P71618), including two copies of the BAFF-binding peptide plus an immunoglobulin heavy or light chain variable region, were generated synthetically. These were ligated, through convenient restriction endonuclease sites, to nucleic acids encoding the immunoglobulin heavy or light chain constant region in appropriate vectors. Nucleic acids encoding the heavy chain constant region C-terminal fusions (P71619 and P71620), Fc-loop insertions (P71621 and P71623), and the Fc-loop insertion/C-terminal fusion (P71622) were all generated synthetically and ligated into a vector containing the heavy chain variable region through convenient restriction endonuclease sites.

[0067] The various bispecific constructs described above were expressed in both transiently transfected 293 cells and stably transfected CHO cells. The fusion proteins were purified and tested for biological activity. No differences were observed in proteins produced in these two different kinds host cells.

[0068] The BAFF inhibitory activities of the bispecific molecules were tested in a BAFF-mediated human primary B cell proliferation assay. In brief, human B cells were purified from peripheral blood mononuclear cells (PBMCs) using negative selection using a human B cell kit II from Miltenyi Biotec (Auburn, CA). About 10^5 purified B cells were cultured in 96 well microtiter plates in Minimal Essential Media (MEM) plus 10% heat inactivated fetal bovine serum (FBS) in the presence of 50 ng/ml human BAFF protein, 2 µg/ml goat F(ab') 2 anti-human IgM (Jackson ImmunoResearch), and varying concentrations of one of the bispecific proteins described above at 37 °C in 5% CO^2 for 48 hours. An anti-BAFF peptibody was used as a positive control ("αBAFF," which is a homodimer containing two polypeptide chains, each comprising two BAFF-binding peptides fused to an Fc polypeptide). The αBAFF molecule is described in detail in US Patent 7,259,137, and the amino acid sequence of one polypeptide chain of this homodimer is provided in SEQ ID NO:32. Proliferation was measured by the uptake of radioactive ^{3}H-thymidine during the last 18 hours of incubation. Results are shown in Figures 2A and 2B.

[0069] The data in Figure 2A indicate that the two C-terminal fusion constructs (P71619 and P71620) were comparable to each other in inhibition of BAFF-mediated B cell proliferation and more potent than all of the other fusion constructs tested in this experiment. P71620 was not pursued further because it tended to aggregate, a property that is highly undesirable in a therapeutic protein. The data in Figure 2B indicate that P71619 is comparable to the two slightly modified versions of this construct described above (P74293 and P74294) and to a positive control (αBAFF) in inhibition of BAFF-mediated B cell proliferation. Thus, among the bispecific constructs tested, P71619, P71620, P74293, and P74294 had comparable activity in this assay of BAFF-mediated B cell proliferation and better activity than all other constructs tested.

[0070] The B7RP1 inhibitory activity of P71619, P74293, and P74294 was assayed using a human B7RP1-Fc-mediated T cell proliferation assay. Primary human T cells purified from PBMCs from healthy human donors using Pan T cell isolation kit from Miltenyi Biotec (Auburn, CA) and stimulated with plate-bound anti-CD3 (1 µg/mL) antibody and a B7RP1-Fc fusion protein (3 µg/mL) in the presence of varying concentrations of the bispecific proteins described above or an IgG2 anti-human B7RP1 antibody (referred to herein as "αB7RP1"). ^{3}H-thymidine was added to the cells after 48 hours, and incorporation of the ^{3}H-thymidine was measured 24 hours later. All of the bispecific antibodies that were tested had similar IC_{50}'s, which were similar to that of αB7RP1 (Figure 3). Thus, these data suggest that the conjugation of the BAFF-binding peptides to the anti-huB7RP1 antibody had little or no effect on the ability of the antibody to inhibit B7RP1 activity.

[0071] The binding affinities of the heterodimeric bispecific antibodies P74293 and P74294 to BAFF and B7RP1 were measured by Kinetic Exclusion Assay (KinExA®; Sapidyne Instruments, Boise, Idaho). Both antibodies have high binding affinities to human BAFF (having K_d's of approximately 30 pM) and to human B7RP1 (having K_d's of approximately 40 pM). *See* Table 2 below. In addition, both of these bispecifics have similar binding affinities to cynomolgus monkey BAFF compared to human BAFF and to cynomolgus monkey B7RP1 compared to human B7RP1. Table 2.

Table 2: binding affinity and cellular potency of P74293 and P74294.

	P74293	**P74294**
K_d (pM) for binding to human BAFF	29	37
K_d (pM) for binding to cynomolgus monkey BAFF	22.3	17.4
IC_{50} (nM) for inhibition of BAFF-mediated human B cell proliferation	0.86	0.96
IC_{50} (nM) for inhibition of BAFF-mediated cynomolgus monkey B cell proliferation	1.6	1.8
K_d (pM) for binding to human B7RP1	38	41
K_d (pM) for binding to cynomolgus monkey B7RP1	49.4	45.2
IC_{50} (nM) for inhibition of B7RP1-mediated human T cell proliferation	1.36	0.98
IC_{50} (nM) for inhibition of B7RP1-mediated cynomolgus monkey T cell proliferation	0.29	ND*
*ND means not determined.		

[0072] To further assess the activity of P74293 in an *in vitro* system using human cells, cytokine production by human tonsil cells activated by Staphylococcus enterotoxin B (SEB) was assessed in the presence of various test molecules. Briefly, human tonsil cells were isolated from tissue and stimulated with SEB (1µg/mL) in the presence of one of the following molecules: (1) αB7RP1, (2) P74293, (3) CTLA4-Ig (a positive control), or (4) human IgG (a negative control). After 72 hours of culture, the cell supernatant was collected, and cytokine

levels were assayed using kits from Meso Scale Discovery according to the manufacturer's instructions. Results are shown in Figure 4.

[0073] All three of αB7RP1, P74293, and CTLA4-Ig, bars 1, 2, and 3, respectively in all panels of Figure 4, inhibited release of IL-17, IL-10, IL-4, and IFNγ. Release of IL-2 was inhibited only by CTLA4-Ig. Thus, αB7RP1 and the anti-BAFF/B7RP1 bispecific P74293 had comparable and specific effects on cytokine secretion by SEB-activated human tonsil cells.

[0074] Three heterodimeric bispecific proteins, that is, P71619, P74293, and P74294, were examined for additional properties. Protein titers from cultures of host cells producing these proteins indicated that P74293 and P74294 were produced at about twice the levels at which P71619 was produced. P74293 and P74294 were also more stable than P71619 after storage for two weeks at 40 °C as assessed by size exclusion chromatography (SEC). P74293 formed a clear solution at the onset of storage and after 4 weeks of storage, whereas solutions containing P74394 were hazy at all time points. Solutions of P74293 and P74294 were less viscous than solutions of P71619. Thus, P74293 and P74294 were expressed at higher levels than P71619 and were also more stable and less viscous in the concentration range tested than P71619. The most obvious difference between these molecules lies in the linker between the two BAFF-binding peptides. These data suggest that the linkers in P74293 and P74294 (SEQ ID NOs:6 and 7) can confer improved properties upon these molecules.

[0075] The pharmacokinetic properties of the bispecific molecules described were evaluated in mice. Male CD-1 mice were given a single intravenous (IV) dose (5 mg/kg) of the bispecific fusion proteins P71617, P71619, P71621, P71622, P74293, or P74294. Serum samples were collected before dosing and at 0.5, 2, 8, 24, 48, 72, 96, 168, 240, 336, 408, 504 hours after dosing. The concentration of the bispecific molecule in the serum was determined by two ELISA methods, one registering the presence of the Fc portion and one registering the presence of both the Fc portion and the BAFF-binding peptide portion. For the Fc portion measurement, a biotinylated anti-Fc antibody was used as capture reagent, and ALEXA FLUOR® 647-labeled anti-Fc antibody was used as the detection reagent. To detect the BAFF-binding portion and the Fc portion of the bispecific, a biotinylated BAFF protein was used as the capture reagent, and ALEXA FLUOR® 647-labeled anti-Fc antibody was used as the detection reagent. The bispecific proteins with two tandem copies of BAFF-binding petides fused to the N-terminus (P71617), C-terminus (P71619, P74293 and P74294) or CH3 domain (P71621) of the heavy chain have very similar PK profiles in mice. Figure 5. The bispecific protein with one copy of BAFF-binding peptide inserted into the CH3 domain and another copy fused to the C-terminal end of the heavy chain (P71622) had lower exposure compared to the other bispecific proteins. Figure 5. In addition, the two different ELISA assays resulted in similar serum concentrations of the bispecific proteins, suggesting that no significant cleavage of the bispecific proteins occurred *in vivo*.

[0076] Pharmacokinetic and pharmacodynamic parameters of the P74293 and P74294 heterodimeric bispecific antibodies were also assessed by a single dose study in cynomolgus monkeys. Naive male cynomolgus monkeys (n=4) were given a single bolus intravenous or subcutaneous dose of P74293 (10 mg/kg), or a single subcutaneous dose of P74294 (10 mg/kg). Both bispecific molecules have PK profiles similar to that of an IgG antibody. The observed pharmacokinetic parameters for P74293 and P74294, as well as for anti-huB7RP1, are reported in Table 3 below.

Table 3: Pharmacokinetic parameters in cynomolgus monkey

	P74293		**P74294**	**Anti-huB7RP1**	
	10 mg/kg IV	**10 mg/kg SC**	**10 mg/kg SC**	**10 mg/kg IV**	**10 mg/kg SC**
Maximum drug concentration (C_{max}; µg/ml)	323	90	74	264	112
Time at which C_{max} was observed		45	51		72

	P74293		P74294	Anti-huB7RP1	
	10 mg/kg IV	10 mg/kg SC	10 mg/kg SC	10 mg/kg IV	10 mg/kg SC
(T_{max}; hr)					
Area under the curve (AUC_{0-inf}; µg*hr/mL)	33800	20300	22000	26100	23800
Mean residence time (MRT_{0-int}; hr)	136	132	148	138	144
Total clearance (CL; ml/hr/kg)	0.303	0.491	0.484	0.388	0.427
Volume of distribution at steady state (Vss; ml/kg)	42.5			52.1	

[0077] The data in Table 3 indicate that the pharmacokinetic parameters of P74293 and P75294 are comparable to each other and to those of anti-huB7RP1 antibody.

Example 2: Designing and testing a murine bispecific surrogate molecule

[0078] To conduct preclinical studies in mice, a murine surrogate bispecific molecule that could bind to murine B7RP1 and murine BAFF (*hereinafter,* the "murine surrogate") was constructed. The anti-huB7RP1 antibody used to construct the bispecific constructs described in Example 1, does not bind to murine B7RP1, while the BAFF-binding peptide used in these constructs does bind to both human and murine BAFF. Data not shown. The murine surrogate comprises an antagonistic IgG anti-murine B7RP1 antibody (called "anti-mB7RP1" herein), which was a chimera of mouse immunoglobulin constant regions and rat anti-murine B7RP1 immunoglobulin variable regions. The use of anti-mB7RP1 is described in Hu et al. (2009), J. Immunol. 182: 1421, where it is designated 1B7-V2. The murine surrogate has two copies of a BAFF-binding peptide (SEQ ID NO:1) fused via a short linker (five amino acids long) to the C-terminus of the immunoglobulin heavy chain of anti-mB7RP1. The two copies of the BAFF-binding peptide are separated by another linker that is 23 amino acids long. Nucleic acids encoding the heavy chain of the murine surrogate were made using overlap PCR to join nucleic acids encoding the BAFF-binding portion of αBAFF to the downstream end of nucleic acids encoding the heavy chain of 1B7-V2, *i.e.,* anti-mB7RP1.

[0079] BAFF inhibition by the murine surrogate was evaluated in a BAFF-mediated B cell proliferation assay. Mouse B lymphocytes were isolated from C57BL/6 spleens by negative selection with MACS CD43 (ly-48) Microbeads according to the manufacturers instructions (Miltenyi Biotec, Auburn, CA) or from PBMC using a B cell isolation kit (Miltenyi Biotec, Auburn, CA). Purified B cells were stimulated with 0.1 µg/ml anti-IgM and 200 ng/ml BAFF in the presence of varying concentrations of the murine surrogate or αBAFF. B cell proliferation was measured by ^{3}H-thymidine incorporation at day 4. The IC_{50}'s of the murine surrogate and αBAFF were 0.59 nM and 0.73 nM, respectively.. *See* Figure 6A. Thus, the murine surrogate effectively inhibited BAFF with potency comparable to that of αBAFF.

[0080] To measure inhibition of B7RP1 binding to its receptor by the murine surrogate, mouse spleen cells were first activated to enhance their expression of the B7RP1 receptor by incubating them in microtiter wells coated with an anti-CD3 (5 µg/ml) antibody for 24 hours. The activated spleen cells were washed with phosphate buffered saline (PBS) and then incubated with 5 µg/ml biotinylated muB7RP1:Fc in the presence of varying concentrations of the murine surrogate at 4 °C for 30 minutes. The cells were washed and then stained with allophycocyanin (APC)-conjugated anti-mouse CD3 antibody and streptavidin-phycoerythrin (Streptavidin-PE) for an additional 20 minutes. The B7RP1-Fc binding to T cells was analyzed by flow cytometry. The IC_{50}'s of the murine surrogate and anti-mB7RP1 were 4.01 pM and 2.8 pM, respectively. *See* Figure 6B. Hence, the

activity of the murine surrogate was similar to that of anti-mB7RP1 in this assay. Thus, the murine surrogate inhibits both BAFF and B7RP1.

[0081] The *in vivo* pharmacodynamic effects of the murine surrogate were evaluated in mice immunized with the sheep red blood cells (SRBC). In brief, BALB/c mice (8 weeks old) received a primary immunization on day 0 and a booster immunization on day 28 with 2 X 10^8 SRBC in 0.2 ml of PBS via intraperitoneal injection. The mice (n=5 for each molecule) were treated twice per week from day 0 to day 33 with one of the following molecules at 5 mg/kg: the murine surrogate; αBAFF; anti-mB7RP1; or murine IgG1. Mice treated with SBRC, but not receiving another treatment, served as positive controls. The mice were sacrificed on day 34, and serum and spleens were collected.

[0082] To measure the proportion of B cells and memory T cells in the spleen, spleen cells were harvested by grinding the spleen tissue through a cell strainer. The spleen cells were preincubated with unlabelled anti-CD16/32 to block the nonspecific binding of antibodies to Fc gamma receptors (FcγR). The proportion of B cells was determined by staining with PE-labeled anti-B220 (which is expressed on B cells). The proportion of memory T cells cells ($CD44^{hi}CD62L^{lo}$CD4 T cells) was determined by staining with FITC-conjugated anti-CD44, PE-conjugated anti-CD62L, APC-conjugated anti-CD4 and PerCP-conjugated anti-CD3. All staining antibodies were purchased from BD Bioscience (San Diego, CA). For both B and T cell determinations, flow cytometry was performed with a FACSCALIBUR™ (BD Bioscience, San Jose, CA) flow cytometer, and the data was analyzed using FLOWJO® (TreeStar Inc., Ashland, OR) software for analysis of flow cytometry data. Results are shown in Figure 7.

[0083] To measure levels of anti-SBRC antibodies in serum, microtiter plates coated with 10 µg/ml soluble SRBC antigen were incubated for two hours at room temperature with diluted serum from treated mice. Bound SRBC-specific Ig from the serum was detected with HRP-conjugated polyclonal goat anti-mouse IgG and IgM antibodies (Southern Biotech, Birmingham, AL). The substrate reaction was performed using SUREBLUE™ TMB microwell peroxidase substrate (KPL, Gaithersburg, MD) according to the manufacturer's instructions, and the optical density was read using a Spectrum Max microplate reader (Molecular Devices). As a positive control, serial dilutions of a mixture of sera from SRBC-immunized mice without any treatment was added to each plate, and a standard curve was constructed from the readings from these wells. Levels of anti-SBRC antibodies of other samples are reported in Figure 7 as a percentage of this positive control.

[0084] The percentage of spleen cells that are B cells was reduced in mice treated with the murine surrogate as compared to the percentage observed in mice treated with murine IgG1. Figure 7 (top panel). A similar reduction was observed in mice treated with αBAFF or αBAFF plus anti-mB7RP1, but not in mice treated with anti-mB7RP1 alone. Figure 7 (top panel). With regard to memory T cells, mice treated with the murine surrogate, anti-mB7RP1, or anti-mB7RP1 plus αBAFF had reduced proportions of memory T cells compared to that observed in mice treated with mIgG1. Figure 7 (middle panel). In contrast, treatment with αBAFF did not alter the memory T cell population in spleen compared to that observed with mIgG treatment. Figure 7 (middle panel). The murine surrogate also showed potent reduction of the anti-SRBC antibody level in serum, similar to that observed upon treatment with anti-mB7RP1 or anti-mB7RP1 plus αBAFF or in mice that had not been injected with SRBC. Figure 7 (bottom panel). Moderate inhibition of anti-SRBC antibody level, compared to the level observed with mIgG1 treatment, was observed in mice treated with αBAFF alone. Figure 7 (bottom panel). These data indicate that the murine surrogate had dual inhibitory effects in B cell and T cell compartments in mice *in vivo*.

[0085] The impact of the murine surrogate on disease was evaluated in the NZB/W F_1 lupus model using two different dose amounts for each of the molecules tested. Female NZB/W F_1 mice (4.5 month old, n=20) were treated twice per week by intraperitoneal injection for 18 weeks using each of the following dosing regimes: 5 or 15 mg/kg murine surrogate (MW≅160KDa); 4.68 or 14 mg/kg anti-mB7RP1 (MW≅150KDa); 1.88 or 5.6 mg/kg

αBAFF (MW≅64KDa); a combination of αBAFF (1.88 or 5.6 mg/kg) and anti-mB7RP1 (4.68 or 14 mg/kg); murine IgG1 (15 mg/kg; an isotype control); or phosphate buffered saline (PBS) (a negative control). Proteinuria was measured in urine using ALBUSTIX® (Bayer, Elkhart, IN) every two weeks starting at 5 months of age. The incidence of proteinuria was expressed as the percentage of mice with urine protein at a concentration of at least 300 mg/dl in two consecutive measurements. Serum anti-dsDNA IgG level was measured by ELISA. Scoring for kidney disease of all mice was performed by examination of kidney tissue samples for eight different kinds of lesions, that is, glomerular capillary proliferation, mesangial cell hyperplasia, increased mesangial matrix, glomerular tuft adhesion, parietal epithelial hyperplasia, interstitial nephritis, tubular dilation/protein casts, and tubular atrophy/interstitial fibrosis. Each type of lesion was given a severity score from 0 to 5, for a maximum possible score of 32. The scores of each group of mice were averaged. Survival was monitored.

[0086] At 12 months of age, none of the mice treated with the murine surrogate at either dose level developed proteinuria. In contrast, 100% of mice treated with murine IgG1 or PBS at both dose levels tested exhibited proteinuria. Figures 8A and 9B. About 60% and 35% of mice treated with the lower dose levels of anti-mB7RP1 and αBAFF, respectively, and about 50% and 25% of mice treated with the higher dose levels of anti-mB7RP1 and αBAFF, respectively, developed proteinuria. Figures 8A and 9B. In addition, the murine surrogate treatment at both dose levels resulted in a significant reduction in serum levels of anti-dsDNA IgG as compared to the negative control treated with muIgG1. Figure 8B and 9A. The bispecific treatment also significantly improved survival compared with the mIgG and PBS control groups. Data not shown. However, no clear difference in survival was observed between the bispecific vs. the single agent treatments at the time of experiment termination.

[0087] Kidneys from all treated mice, including mice deceased before the end of study, were collected for histology scoring for severity of kidney disease. The groups of mice treated with αBAFF, the combination of αBAFF plus anti-mB7RP1, or the murine surrogate had significantly lower scores for kidney disease as compared to the control group treated with mIgG1. Figure 10. Groups treated with the surrogate bispecific or the combination also showed a trend towards reduced kidney pathology compared to the single agent treatment groups, a result that correlates well with the proteinuria results described above. *Compare* Figure 10 *to* Figures 8A and 9B. In summary, dual inhibition of BAFF and B7RP1 by the murine surrogate or by a combination treatment with αBAFF plus anti-mB7RP1 was more effective than inhibition of only BAFF (αBAFF) or only B7RP1 (anti-mB7RP1) in preventing disease onset and progression in the NZB/W F_1 lupus model.

[0088] To determine whether inhibition of both BAFF and B7RP1 could effectively inhibit the symptoms of murine collagen-induced arthritis, the following experiment was done. Male DBA mice were immunized with 100 µg of bovine type II collagen emulsified in 2 x Complete Freund's adjuvant (CFA) on day 0 and boosted with bovine type II collagen in Incomplete Freund's Adjuvant (IFA) on day 21. Mice were treated with one of the test substances twice per week during the 41 week course of the study starting on day 0. The percentage of mice in each group exhibiting arthritis symptoms and an average arthritic score for each group was assessed at each time point. Arthritis scores were determined by examining each limb and assigning a score from 0-3 for each limb, with higher scores for more swollen and/or inflamed limbs. So the maximum total arthritis score is 12. A mouse was counted as having arthritis if it had an arthritis score of at least 1 in any limb.

[0089] Results are shown in Figure 11. These data indicate that the combination of αBAFF and anti-mB7RP1 (filled circles connected by solid lines) was much more effective at suppressing arthritis symptoms than either αBAFF (open circles connected by solid lines) or anti-mB7RP1 (filled circles connected by dashed lines) alone. The negative control groups treated with mIgG (filled squares connected by solid lines) or PBS (filled squares connected by dashed lines) had the highest percent incidence of arthritis and highest arthritic scores. These results suggest that inhibiting both BAFF and B7RP1, as opposed to inhibiting only one of these pathways, could be an effective treatment of an autoimmune and/or inflammatory arthritic condition such as rheumatoid arthritis.

SEQUENCE LISTING

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Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
        195                 200                 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys
    210                 215                 220

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285
```

```
Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu

            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly
        435                 440                 445

Gly Gly Gly Gly Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln
    450                 455                 460

Trp Val Cys Asp Pro Leu Gly Ser Gly Ser Ala Thr Gly Gly Ser Gly
465                 470                 475                 480

Ser Val Ala Ser Ser Gly Ser Gly Ser Ala Thr His Leu Leu Pro Gly
                485                 490                 495

Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys Asp Pro Leu
            500                 505                 510
```

<210> 18

<211> 511

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 18

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Lys Gln Asp Gly Asn Glu Lys Tyr Tyr Val Asp Ser Val
    50                  55                  60
```

```
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Ile Leu Trp Phe Gly Asp Leu Pro Thr Phe Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser

        115                 120                 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
    130                 135                 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
        195                 200                 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys
    210                 215                 220

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
```

```
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly
        435                 440                 445

Gly Gly Gly Gly Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln
    450                 455                 460

Trp Val Cys Asp Pro Leu Gly Ser Gly Ser Ala Thr Gly Gly Ser Gly
465                 470                 475                 480

Ser Ser Ala Ser Ser Gly Ser Gly Ser Ala Thr His Leu Leu Pro Gly
                485                 490                 495

Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys Asp Pro Leu
            500                 505                 510
```

<210> 19

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 19

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Asn Trp
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
        35                  40                  45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

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

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
```

```
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys
    210
```

<210> 20
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<220>
<221> MOD_RES
<222> (2)..(9)
<223> Any amino acid

<400> 20
```
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1               5
```

<210> 21
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 21
```
Leu Glu Trp Ile Gly
1               5
```

<210> 22
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<220>
<221> MOD_RES
<222> (3)..(3)
<223> Any amino acid

<400> 22
```
Trp Gly Xaa Gly
1
```

<210> 23
<211> 4
<212> PRT

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<220>
<221> MOD_RES
<222> (3)..(3)
<223> Any amino acid

<400> 23
```
Phe Gly Xaa Gly
1
```

<210> 24
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 24
```
Gly Ser Gly Ser Ala Thr Gly Gly Ser Gly Ser Gly Ala Ser Ser Gly
1               5                   10                  15

Ser Gly Ser Ala Thr Gly Ser
            20
```

<210> 25
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 25
```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Lys Gln Asp Gly Asn Glu Lys Tyr Tyr Val Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Ile Leu Trp Phe Gly Asp Leu Pro Thr Phe Trp Gly
            100                 105                 110
```

```
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        115                 120                 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
    130                 135                 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala

                165                 170                 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
        195                 200                 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys
    210                 215                 220

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 26

<211> 508

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 26

```
Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys Asp
1               5                   10                  15

Pro Leu Gly Ser Gly Ser Ala Thr Gly Gly Ser Gly Ser Gly Ala Ser
            20                  25                  30

Ser Gly Ser Gly Ser Ala Thr Gly Ser Leu Pro Gly Cys Lys Trp Asp
        35                  40                  45

Leu Leu Ile Lys Gln Trp Val Cys Asp Pro Leu Gly Gly Glu Val Gln
    50                  55                  60

Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg
65                  70                  75                  80

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Trp Met Ser
                85                  90                  95

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Tyr Ile
            100                 105                 110

Lys Gln Asp Gly Asn Glu Lys Tyr Tyr Val Asp Ser Val Lys Gly Arg
        115                 120                 125

Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met
    130                 135                 140

Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu
145                 150                 155                 160

Gly Ile Leu Trp Phe Gly Asp Leu Pro Thr Phe Trp Gly Gln Gly Thr
                165                 170                 175

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
            180                 185                 190

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
        195                 200                 205

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
    210                 215                 220

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
225                 230                 235                 240

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
                245                 250                 255

Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
            260                 265                 270

Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys
        275                 280                 285

Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe
    290                 295                 300
```

```
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
305                 310                 315                 320

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
                325                 330                 335

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
            340                 345                 350

Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr
        355                 360                 365

Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
    370                 375                 380

Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
385                 390                 395                 400

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
                405                 410                 415

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
            420                 425                 430

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
        435                 440                 445

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser
    450                 455                 460

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
465                 470                 475                 480

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
                485                 490                 495

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
            500                 505
```

<210> 27

<211> 275

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 27

```
Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys Asp
1               5                   10                  15

Pro Leu Gly Ser Gly Ser Ala Thr Gly Gly Ser Gly Ser Gly Ala Ser
            20                  25                  30

Ser Gly Ser Gly Ser Ala Thr Gly Ser Leu Pro Gly Cys Lys Trp Asp
        35                  40                  45

Leu Leu Ile Lys Gln Trp Val Cys Asp Pro Leu Gly Gly Asp Ile Gln
    50                  55                  60

Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
65                  70                  75                  80
```

```
Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Asn Trp Leu Ala Trp
                85                  90                  95

Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile Tyr Ala Ala
            100                 105                 110

Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser
        115                 120                 125

Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe
    130                 135                 140

Ala Thr Tyr Tyr Cys Gln Gln Tyr Asp Ser Tyr Pro Arg Thr Phe Gly
145                 150                 155                 160

Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val
                165                 170                 175

Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
            180                 185                 190

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

Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val
    210                 215                 220

Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu
225                 230                 235                 240

Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
                245                 250                 255

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg
            260                 265                 270

Gly Glu Cys
        275
```

<210> 28

<211> 511

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 28

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Lys Gln Asp Gly Asn Glu Lys Tyr Tyr Val Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80
```

```
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Ile Leu Trp Phe Gly Asp Leu Pro Thr Phe Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        115                 120                 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
    130                 135                 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
        195                 200                 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys
    210                 215                 220

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430
```

```
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly
        435                 440                 445

Gly Gly Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val
    450                 455                 460

Cys Asp Pro Leu Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
465                 470                 475                 480

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Pro Gly
                485                 490                 495

Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys Asp Pro Leu
            500                 505                 510
```

<210> 29

<211> 510

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 29

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Lys Gln Asp Gly Asn Glu Lys Tyr Tyr Val Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Ile Leu Trp Phe Gly Asp Leu Pro Thr Phe Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        115                 120                 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
    130                 135                 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
        195                 200                 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys
```

```
    210                 215                 220

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr

                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Gly Gly Leu Pro Gly Cys Lys Trp Asp Leu
        355                 360                 365

Leu Ile Lys Gln Trp Val Cys Asp Pro Leu Gly Ser Gly Ser Ala Thr
    370                 375                 380

Gly Gly Ser Gly Ser Gly Ala Ser Ser Gly Ser Gly Ser Ala Thr Gly
385                 390                 395                 400

Ser Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys
                405                 410                 415

Asp Pro Leu Gly Gly Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
            420                 425                 430

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
        435                 440                 445

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp
    450                 455                 460

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
465                 470                 475                 480

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
                485                 490                 495

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
            500                 505                 510
```

<210> 30

<211> 490

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 30

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Lys Gln Asp Gly Asn Glu Lys Tyr Tyr Val Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Ile Leu Trp Phe Gly Asp Leu Pro Thr Phe Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        115                 120                 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
    130                 135                 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
        195                 200                 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys
    210                 215                 220

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335
```

```
Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Gly Gly Leu Pro Gly Cys Lys Trp Asp Leu
        355                 360                 365

Leu Ile Lys Gln Trp Val Cys Asp Pro Leu Gly Gly Thr Lys Asn Gln
    370                 375                 380

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
385                 390                 395                 400

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                405                 410                 415

Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
            420                 425                 430

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
        435                 440                 445

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    450                 455                 460

Leu Ser Pro Gly Gly Gly Gly Gly Leu Pro Gly Cys Lys Trp Asp Leu
465                 470                 475                 480

Leu Ile Lys Gln Trp Val Cys Asp Pro Leu
                485                 490
```

<210> 31

<211> 491

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 31

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Lys Gln Asp Gly Asn Glu Lys Tyr Tyr Val Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Ile Leu Trp Phe Gly Asp Leu Pro Thr Phe Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        115                 120                 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
```

130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys
210 215 220

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Gly Gly Leu Pro
260 265 270

Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys Asp Pro Leu
275 280 285

Gly Gly Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
290 295 300

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
305 310 315 320

Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
325 330 335

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
340 345 350

Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
355 360 365

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Gly Gly Leu Pro
370 375 380

Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys Asp Pro Leu
385 390 395 400

Gly Gly Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
405 410 415

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
420 425 430

Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe
435 440 445

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
450 455 460

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
465 470 475 480

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 32
<211> 293
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 32

```
Met Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys
1               5                  10                  15

Asp Pro Leu Gly Ser Gly Ser Ala Thr Gly Gly Ser Gly Ser Thr Ala
            20                  25                  30

Ser Ser Gly Ser Gly Ser Ala Thr His Met Leu Pro Gly Cys Lys Trp
        35                  40                  45

Asp Leu Leu Ile Lys Gln Trp Val Cys Asp Pro Leu Gly Gly Gly Gly
    50                  55                  60

Gly Val Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
65                  70                  75                  80

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                85                  90                  95

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            100                 105                 110

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        115                 120                 125

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    130                 135                 140

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
145                 150                 155                 160

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                165                 170                 175

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            180                 185                 190

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
        195                 200                 205

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    210                 215                 220

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
225                 230                 235                 240

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                245                 250                 255

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            260                 265                 270

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        275                 280                 285
```

```
Leu Ser Pro Gly Lys
    290
```

<210> 33

<211> 232

<212> PRT

<213> Homo sapiens

<400> 33

```
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1               5                  10                  15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
    130                 135                 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220

Ser Leu Ser Leu Ser Pro Gly Lys
225                 230
```

<210> 34

<211> 228

<212> PRT

<213> Homo sapiens

<400> 34

```
Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val
1               5                  10                  15
```

```
Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Met Glu
    50                  55                  60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
65                  70                  75                  80

Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn
                85                  90                  95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro
            100                 105                 110

Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
    130                 135                 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

Ser Pro Gly Lys
225
```

<210> 35

<211> 279

<212> PRT

<213> Homo sapiens

<400> 35

```
Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro Arg Cys
1               5                   10                  15

Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro

            20                  25                  30

Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Glu
        35                  40                  45

Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Ala Pro
    50                  55                  60

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
65                  70                  75                  80

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
```

```
                85                  90                  95

Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp
            100                 105                 110

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
        115                 120                 125

Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
    130                 135                 140

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
145                 150                 155                 160

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg
                165                 170                 175

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
            180                 185                 190

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
        195                 200                 205

Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Asn
    210                 215                 220

Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
225                 230                 235                 240

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser
                245                 250                 255

Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser
            260                 265                 270

Leu Ser Leu Ser Pro Gly Lys
        275
```

<210> 36

<211> 229

<212> PRT

<213> Homo sapiens

<400> 36

```
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe
1               5                   10                  15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
            20                  25                  30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
        35                  40                  45

Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
    50                  55                  60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
65                  70                  75                  80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
                85                  90                  95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
            100                 105                 110
```

```
Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
        115                 120                 125

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
    130                 135                 140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
145                 150                 155                 160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                165                 170                 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
            180                 185                 190

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
        195                 200                 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser

      210                 215                 220

  Leu Ser Leu Gly Lys
  225
```

<210> 37
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 37
```
Gly Gly Gly Ser
1
```

<210> 38
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 38
```
Gly Gly Gly Pro
1
```

<210> 39
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 39

Gly Gly Gly Gln
1

<210> 40
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 40
Gly Gly Gly Gly Gly
1 5

<210> 41
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 41
ctgccgggtt gtaaatggga cctgctgatc aaacagtggg tttgtgaccc gctg 54

<210> 42
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 42
ggtggtggtg gt 12

<210> 43
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
<221> modified_base
<222> (36) .. (36)
<223> a, c, t, g, unknown or other

<400> 43
ggatccggtt ctgctactgg tggttccggc tccdbngcaa gctctggttc aggcagtgcg 60

actcatctg 69

<210> 44
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 44
ggatccggtt ctgctactgg tggttccggc tccgtcgcaa gctctggttc aggcagtgcg 60

actcatctg 69

<210> 45
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 45
ggatccggtt ctgctactgg tggttccggc tcctcggcaa gctctggttc aggcagtgcg 60

actcatctg 69

<210> 46
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 46
cgggcgagtc agggtattag caactggtta gcc 33

<210> 47
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 47
gctgcatcca gtttgcaaag t 21

<210> 48
<211> 27
<212> DNA
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 48
caacagtatg atagttaccc tcggacg 27

<210> 49
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 49
agttattgga tgagt 15

<210> 50
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 50
tacataaagc aagatggaaa tgagaaatac tatgtggact ctgtgaaggg c 51

<210> 51
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 51
gaagggatac tttggttcgg ggacttaccg acgttc 36

<210> 52
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 52
gacatccaga tgacccagtc tccatcctca ctgtctgcat ctgtaggaga cagagtcacc 60

atcacttgtc gggcgagtca gggtattagc aactggttag cctggtatca gcagaaacca 120

gagaaagccc ctaagtccct gatctatgct gcatccagtt tgcaaagtgg ggtcccatca 180

aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240

gaagattttg caacttatta ctgccaacag tatgatagtt accctcggac gttcggccaa 300

gggaccaagg tggaaatcaa acga 324

<210> 53
<211> 363
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 53
gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggGtc cctgagactc 60

tcctgtgcag cttctggatt tacctttagt agttattgga tgagttgggt ccgccaggct 120

ccagggaaag ggctggagtg ggtggcctac ataaagcaag atggaaatga gaaatactat 180

gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctcattgtat 240

ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagggaaggg 300

atactttggt tcggggactt accgacgttc tggggccagg gaaccctggt caccgtctct 360

agt 363

<210> 54
<211> 1527
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 54
gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggGtc cctgagactc 60

tcctgtgcag cttctggatt tacctttagt agttattgga tgagttgggt ccgccaggct 120

ccagggaaag ggctggagtg ggtggcctac ataaagcaag atggaaatga gaaatactat 180

gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctcattgtat 240

ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagggaaggg 300

atactttggt tcggggactt accgacgttc tggggccagg gaaccctggt caccgtctct 360

agtgcctcca ccaagggccc atcggtcttc cccctggcgc cctgctccag gagcacctcc 420

gagagcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 480

tcgtggaact caggcgctct gaccagcggc gtgcacacct tcccagctgt cctacagtcc 540

tcaggactct actccctcag cagcgtggta acggtgccct cctcaaattt cgggacgcag 600

acatatacat gcaatgtgga tcataagcct tccaacacga aggtggacaa gactgtggag 660

cggaagtgtt gcgtcgagtg cccaccgtgt cccgctcctc cggtcgctgg cccatcagta 720

tttctcttcc ctcccaagcc aaaagataca ctcatgatct caagaacccc agaagtgact 780

tgtgtggtcg tggacgtgtc gcatgaggat ccggaggtgc agtttaactg gtatgtggat 840

ggcgtagaag tccacaacgc caagaccaag cctagagagg aacaattcaa ctcgacgttc 900

agggtggtca gcgtgttgac agtagtccac caggactggc ttaatgggaa ggaatacaaa 960

tgtaaggtct caaacaaagg gctcccggca cccattgaga agacaatttc caaaaccaag 1020

ggagagggca gggaagggga agtgtataag ctgggcgcaa gcgcgggagga aatgaggaaa 1080

ggacagccca gggaacccca agtgtatacg ctgccccaa gccgggagga aatgacgaaa 1080

aatcaggtca gcctcacgtg tctcgtaaag ggattttacc cgtcggacat cgcggtggag 1140

tgggagtcaa atggacagcc cgaaaacaac tataagacca caccaccgat gctcgactcc 1200

gacggaagct tctttttgta ctcgaaactg acggtggaca aatcgcgctg gcaacagggg 1260

aatgtcttta gctgctcggt catgcacgag gccctccaca atcattacac tcagaaaagc 1320

ttgtcgctct cgccgggtgg gggtggagga ctgcccggtt gcaaatggga tctgttgatc 1380

aaacagtggg tatgcgaccc tttgggaagc ggctcggcga cgggtgggtc ggggtcgggt 1440

gcgtccagcg gatcgggctc ggccactggg tcactccctg gatgcaagtg ggatcttctt 1500

atcaagcaat gggtgtgcga tcccctc 1527

<210> 55
<211> 1533
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 55
gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggggtc cctgagactc 60

tcctgtgcag cttctggatt tacctttagt agttattgga tgagttgggt ccgccaggct 120

ccagggaaag ggctggagtg ggtggcctac ataaagcaag atggaaatga gaaatactat 180

gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctcattgtat 240

ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagggaaggg 300

atactttggt tcggggactt accgacgttc tggggccagg gaaccctggt caccgtctct 360

agtgcctcca ccaagggccc atcggtcttc cccctggcgc cctgctccag gagcacctcc 420

gagagcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 480

tcgtggaact caggcgctct gaccagcggc gtgcacacct tcccagctgt cctacagtcc 540

tcaggactct actccctcag cagcgtggta acggtgccct cctcaaattt cgggacgcag 600

acatatacat gcaatgtgga tcataagcct tccaacacga aggtggacaa gactgtggag 660

cggaagtgtt gcgtcgagtg cccaccgtgt cccgctcctc cggtcgctgg cccatcagta 720

tttctcttcc ctcccaagcc aaaagataca ctcatgatct caagaacccc agaagtgact 780

tgtgtggtcg tggacgtgtc gcatgaggat ccggaggtgc agtttaactg gtatgtggat 840

ggcgtagaag tccacaacgc caagaccaag cctagagagg aacaattcaa ctcgacgttc 900

agggtggtca gcgtgttgac agtagtccac caggactggc ttaatgggaa ggaatacaaa 960

tgtaaggtct caaacaaagg gctcccggca cccattgaga agacaatttc caaaaccaag 1020

ggacagccca gggaacccca agtgtatacg ctgccccaa gccgggagga aatgacgaaa 1080

aatcaggtca gcctcacgtg tctcgtaaag ggattttacc cgtcggacat cgcggtggag 1140

tgggagtcaa atggacagcc cgaaaacaac tataagacca caccaccgat gctcgactcc 1200

gacggaagct tctttttgta ctcgaaactg acggtggaca aatcgcgctg gcaacagggg 1260

aatgtcttta gctgctcggt catgcacgag gccctccaca atcattacac tcagaaaagc 1320

ttgtcgctct cgccgggtaa aggtggtggt ggtggtctgc cgggttgtaa atgggacctg 1380

ctgatcaaac agtgggtttg tgacccgctg ggatccggtt ctgctactgg tggttccggc 1440

tccgtcgcaa gctctggttc aggcagtgcg actcatctgc tgccgggttg taaatgggac 1500

ctgctgatca aacagtgggt ttgtgacccg ctg 1533

<210> 56
<211> 1533
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 56
gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggGtc cctgagactc 60
tcctgtgcag cttctggatt tacctttagt agttattgga tgagttgggt ccgccaggct 120
ccagggaaag ggctggagtg ggtggcctac ataaagcaag atggaaatga gaaatactat 180
gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctcattgtat 240
ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagggaaggg 300
atactttggt tcggggactt accgacgttc tggggccagg gaaccctggt caccgtctct 360
agtgcctcca ccaagggccc atcggtcttc cccctggcgc cctgctccag gagcacctcc 420
gagagcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 480
tcgtggaact caggcgctct gaccagcggc gtgcacacct tcccagctgt cctacagtcc 540
tcaggactct actccctcag cagcgtggta acggtgccct cctcaaattt cgggacgcag 600

acatatacat gcaatgtgga tcataagcct tccaacacga aggtggacaa gactgtggag 660
cggaagtgtt gcgtcgagtg cccaccgtgt cccgctcctc cggtcgctgg cccatcagta 720
tttctcttcc ctcccaagcc aaaagataca ctcatgatct caagaacccc agaagtgact 780
tgtgtggtcg tggacgtgtc gcatgaggat ccggaggtgc agtttaactg gtatgtggat 840
ggcgtagaag tccacaacgc caagaccaag cctagagagg aacaattcaa ctcgacgttc 900
agggtggtca gcgtgttgac agtagtccac caggactggc ttaatgggaa ggaatacaaa 960
tgtaaggtct caaacaaagg gctcccggca cccattgaga agacaatttc caaaaccaag 1020
ggacagccca gggaacccca agtgtatacg ctgcccccaa gccgggagga aatgacgaaa 1080
aatcaggtca gcctcacgtg tctcgtaaag ggattttacc cgtcggacat cgcggtggag 1140
tgggagtcaa atggacagcc cgaaaacaac tataagacca caccaccgat gctcgactcc 1200
gacggaagct tcttttgta ctcgaaactg acggtggaca aatcgcgctg gcaacagggg 1260
aatgtcttta gctgctcggt catgcacgag gccctccaca atcattacac tcagaaaagc 1320
ttgtcgctct cgccgggtaa aggtggtggt ggtggtctgc cgggttgtaa atgggacctg 1380
ctgatcaaac agtgggtttg tgacccgctg ggatccggtt ctgctactgg tggttccggc 1440
tcctcggcaa gctctggttc aggcagtgcg actcatctgc tgccgggttg taaatgggac 1500
ctgctgatca aacagtgggt ttgtgacccg ctg 1533

<210> 57
<211> 642
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 57
gacatccaga tgacccagtc tccatcctca ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgtc gggcgagtca gggtattagc aactggttag cctggtatca gcagaaacca 120

atcacttgtc gggcgagtca gggtattagc aactggttag cctggtatca gcagaaacca 120

gagaaagccc ctaagtccct gatctatgct gcatccagtt tgcaaagtgg ggtcccatca 180

aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240

gaagattttg caacttatta ctgccaacag tatgatagtt accctcggac gttcggccaa 300

gggaccaagg tggaaatcaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcca 360

tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat 420

cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag 480

gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540

ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 600

ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gt 642

<210> 58
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 58
ggaagcggct cggcgacggg tgggtcgggg tcgggtgcgt ccagcggatc gggctcggcc 60

actgggtca 69

<210> 59
<211> 1341
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 59
gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggGtc cctgagactc 60

tcctgtgcag cttctggatt tacctttagt agttattgga tgagttgggt ccgccaggct 120

ccagggaaag ggctggagtg ggtggcctac ataaagcaag atggaaatga gaaatactat 180

gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctcattgtat 240

ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagggaaggg 300

atactttggt tcggggactt accgacgttc tggggccagg gaaccctggt caccgtctct 360

agtgcctcca ccaagggccc atcggtcttc cccctggcgc cctgctccag gagcacctcc 420

gagagcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 480

tcgtggaact caggcgctct gaccagcggc gtgcacacct tcccagctgt cctacagtcc 540

tcaggactct actccctcag cagcgtggta acggtgccct cctcaaattt cgggacgcag 600

acatatacat gcaatgtgga tcataagcct tccaacacga aggtggacaa gactgtggag 660

cggaagtgtt gcgtcgagtg cccaccgtgt cccgctcctc cggtcgctgg cccatcagta 720

tttctcttcc ctcccaagcc aaaagataca ctcatgatct caagaacccc agaagtgact 780

tgtgtggtcg tggacgtgtc gcatgaggat ccggaggtgc agtttaactg gtatgtggat 840

ggcgtagaag tccacaacgc caagaccaag cctagagagg aacaattcaa ctcgacgttc 900

agggtggtca gcgtgttgac agtagtccac caggactggc ttaatgggaa ggaatacaaa 960

tgtaaggtct caaacaaagg gctcccggca cccattgaga agacaatttc caaaaccaag 1020

ggacagccca gggaacccca agtgtatacg ctgcccccaa gccgggagga aatgacgaaa 1080

aatcaggtca gcctcacgtg tctcgtaaag ggattttacc cgtcggacat cgcggtggag 1140

tgggagtcaa atggacagcc cgaaaacaac tataagacca caccaccgat gctcgactcc 1200

gacggaagct tctttttgta ctcgaaactg acggtggaca aatcgcgctg gcaacagggg 1260

aatgtcttta gctgctcggt catgcacgag gccctccaca atcattacac tcagaaaagc 1320

ttgtcgctct cgccgggtaa a 1341

<210> 60
<211> 1524
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 60
cttcccggat gcaagtggga tctgttgatc aagcaatggg tctgcgaccc tctcgggtca 60

gggtccgcga ccggtggatc ggggtcggga gcgtcatcgg gcagcggaag cgctacggga 120

tcacttcccg ggtgcaaatg ggacctcctg atcaaacaat gggtatgtga tccgctcggt 180

ggcgaggtgc agctggtgga gtctgggggа ggcttggtcc agcctggggg gtccctgaga 240

ctctcctgtg cagcttctgg atttaccttt agtagttatt ggatgagttg ggtccgccag 300

gctccaggga aagggctgga gtgggtggcc tacataaagc aagatggaaa tgagaaatac 360

tatgtggact ctgtgaaggg ccgattcacc atctccagag acaacgccaa gaactcattg 420

tatctgcaaa tgaacagcct gagagccgag gacacggctg tgtattactg tgcgagggaa 480

gggatacttt ggttcgggga cttaccgacg ttctggggcc agggaaccct ggtcaccgtc 540

tctagtgcct ccaccaaggg cccatcggtc ttcccctgg cgccctgctc caggagcacc 600

tccgagagca cagcggccct gggctgcctg gtcaaggact acttccccga accggtgacg 660

gtgtcgtgga actcaggcgc tctgaccagc ggcgtgcaca ccttcccagc tgtcctacag 720

tcctcaggac tctactccct cagcagcgtg gtgaccgtgc cctccagcaa cttcggcacc 780

cagacctaca cctgcaacgt agatcacaag cccagcaaca ccaaggtgga caagacagtt 840

gagcgcaaat gttgtgtcga gtgcccaccg tgcccagcac cacctgtggc aggaccgtca 900

gtcttcctct tccccccaaa acccaaggac accctcatga tctcccggac ccctgaggtc 960

acgtgcgtgg tggtggacgt gagccacgaa gaccccgagg tccagttcaa ctggtacgtg 1020

gacggcgtgg aggtgcataa tgccaagaca aagccacggg aggagcagtt caacagcacg 1080

ttccgtgtgg tcagcgtcct caccgttgtg caccaggact ggctgaacgg caaggagtac 1140

aagtgcaagg tctccaacaa aggcctccca gccccatcg agaaaaccat ctccaaaacc 1200

aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga ggagatgacc 1260

aagaaccagg tcagcctgac ctgcctggtc aaaggcttct accccagcga catcgccgtg 1320

gagtgggaga gcaatgggca gccggagaac aactacaaga ccacacctcc catgctggac 1380

tccgacggct ccttcttcct ctacagcaag ctcaccgtgg acaagagcag gtggcagcag 1440

gggaacgtct tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacgcagaag 1500

agcctctccc tgtctccggg taaa 1524

<210> 61

<211> 825
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 61
ctccctgggt gcaaatggga cctgttgatt aagcagtggg tctgcgaccc tctcggatcg 60

ggaagcgcaa ctgggggttc aggctcaggg gctagctccg gatcggggtc ggccacaggg 120

tcgctccccg gatgtaagtg ggacctttg attaaacagt gggtgtgcga tccacttgga 180

ggtgatatcc agatgacaca gtcaccctcg tcgttgagcg ccagcgtggg agatagagtg 240

acgatcacct gtcgagccag ccagggcatc tccaactggc ttgcgtggta ccaacaaaag 300

cccgagaagg caccgaaatc gctgatctac gcggcgtcgt cactgcagtc gggtgtaccg 360

tcgcggttta gcgggtccgg gtccggaacg gacttcacgc tcacgatttc ctcattgcag 420

ccggaagatt ttgcgactta ttactgtcag caatatgact catatccccg cacattcggt 480

cagggaacca aggtcgagat caaacgtacg gtggctgcac catctgtctt catcttcccg 540

ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgcctgct gaataacttc 600

tatcccagag aggccaaagt acagtggaag gtggataacg ccctccaatc gggtaactcc 660

caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcaccctg 720

acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt cacccatcag 780

ggcctgagct cgcccgtcac aaagagcttc aacaggggag agtgt 825

<210> 62
<211> 1533
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 62
gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggggtc cctgagactc 60

tcctgtgcag cttctggatt tacctttagt agttattgga tgagttgggt ccgccaggct 120

ccagggaaag ggctggagtg ggtggcctac ataaagcaag atggaaatga gaaatactat 180

gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctcattgtat 240

ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagggaaggg 300

atactttggt tcggggactt accgacgttc tggggccagg gaaccctggt caccgtctct 360

agtgcctcca ccaagggccc atcggtcttc cccctggcgc cctgctccag gagcacctcc 420

gagagcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 480

tcgtggaact caggcgctct gaccagcggc gtgcacacct tcccagctgt cctacagtcc 540

tcaggactct actccctcag cagcgtggta acggtgccct cctcaaattt cgggacgcag 600

acatatacat gcaatgtgga tcataagcct tccaacacga aggtggacaa gactgtggag 660

cggaagtgtt gcgtcgagtg cccaccgtgt cccgctcctc cggtcgctgg cccatcagta 720

tttctcttcc ctcccaagcc aaaagataca ctcatgatct caagaacccc agaagtgact 780

tgtgtggtcg tggacgtgtc gcatgaggat ccggaggtgc agtttaactg gtatgtggat 840

ggcgtagaag tccacaacgc caagaccaag cctagagagg aacaattcaa ctcgacgttc 900

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agggtggtca gcgtgttgac agtagtccac caggactggc ttaatgggaa ggaatacaaa      960
tgtaaggtct caaacaaagg gctcccggca cccattgaga agacaatttc caaaaccaag     1020
ggacagccca gggaacccca agtgtatacg ctgcccccaa gccgggagga aatgacgaaa     1080
aatcaggtca gcctcacgtg tctcgtaaag ggattttacc cgtcggacat cgcggtggag     1140
tgggagtcaa atggacagcc cgaaaacaac tacaaaacga ccccacctat gctcgattcg     1200
gacggcagct tctttttgta ttcaaagttg acagtggaca aatcgcgatg gcagcagggc     1260
aacgtcttct catgttcagt aatgcatgag gcccttcaca accactacac gcagaagtcc     1320
ctctcattgt cgccgggtgg gggtggagga ctgcccgggt gcaagtggga cctcttgatc     1380
aaacagtggg tatgcgaccc tttgggaggg ggtgggtcag gaggggggagg ttccggtgga     1440
ggtggttccg ggggaggcgg atcaggaggt ggaggatcgt tgcccggctg taagtgggat     1500
ctgctgatca agcagtgggt ctgtgatcct ttg                                  1533
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<210> 63
<211> 1530
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 63

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gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggggtc cctgagactc       60
tcctgtgcag cttctggatt tacctttagt agttattgga tgagttgggt ccgccaggct      120
ccagggaaag ggctggagtg ggtggcctac ataaagcaag atggaaatga gaaatactat      180
gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctcattgtat      240
ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagggaaggg      300
atactttggt tcggggactt accgacgttc tggggccagg gaaccctggt caccgtctct      360
agtgcctcca ccaagggccc atcggtcttc cccctggcgc cctgctccag gagcacctcc      420
gagagcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg      480
tcgtggaact caggcgctct gaccagcggc gtgcacacct tcccagctgt cctacagtcc      540
tcaggactct actccctcag cagcgtggtg accgtgccct ccagcaactt cggcacccag      600
acctacacct gcaacgtaga tcacaagccc agcaacacca aggtggacaa gacagttgag      660
cgcaaatgtt gtgtcgagtg cccaccgtgc ccagcaccac ctgtggcagg accgtcagtc      720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcacg      780
tgcgtggtgg tggacgtgag ccacgaagac cccgaggtcc agttcaactg gtacgtggac      840
ggcgtggagg tgcataatgc caagacaaag ccacgggagg agcagttcaa cagcacgttc      900
cgtgtggtca gcgtcctcac cgttgtgcac caggactggc tgaacggcaa ggagtacaag      960
tgcaaggtct ccaacaaagg cctcccagcc cccatcgaga aaaccatctc caaaaccaaa     1020
gggcagcccc gagaaccaca ggtgtacacc ctgccacctt cgcgggagga aatgggagga     1080
ctccccgggt gcaagtggga tcttcttatc aaacagtggg tatgcgaccc gctggggtca     1140
gggtcagcga caggtggatc gggtagcggc gcatcgagcg gatcagggtc cgcgacgggc     1200
tcacttcccg gatgcaaatg ggacctcttg attaagcagt gggtgtgtga cccgttgggt     1260
ggaacgaaga atcaggtctc gttgacgtgt ctggtgaagg ggttttatcc ctcggatatc     1320
gctgtcgagt gggagtcgaa tggacagccc gaaaacaact acaagaccac cccgcctatg     1380
ctggactccg atggttcctt ctttttgtac tcgaaactga ctgtggataa gagcaggtgg     1440
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cagcaaggga atgtattctc gtgttccgtc atgcacgaag ccctccataa ccactataca 1500

caaaaatcgc tttcacttag cccgggaaaa 1530

<210> 64
<211> 1470
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 64
gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggGtc cctgagactc 60

tcctgtgcag cttctggatt tacctttagt agttattgga tgagttgggt ccgccaggct 120

ccagggaaag ggctggagtg ggtggcctac ataaagcaag atggaaatga gaaatactat 180

gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctcattgtat 240

ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagggaaggg 300

atactttggt tcggggactt accgacgttc tggggccagg gaaccctggt caccgtctct 360

agtgcctcca ccaagggccc atcggtcttc cccctggcgc cctgctccag gagcacctcc 420

gagagcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 480

tcgtggaact caggcgctct gaccagcggc gtgcacacct tcccagctgt cctacagtcc 540

tcaggactct actccctcag cagcgtggtg accgtgccct ccagcaactt cggcacccag 600

acctacacct gcaacgtaga tcacaagccc agcaacacca aggtggacaa gacagttgag 660

cgcaaatgtt gtgtcgagtg cccaccgtgc ccagcaccac ctgtggcagg accgtcagtc 720

ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcacg 780

tgcgtggtgg tggacgtgag ccacgaagac cccgaggtcc agttcaactg gtacgtggac 840

ggcgtggagg tgcataatgc caagacaaag ccacgggagg agcagttcaa cagcacgttc 900

cgtgtggtca gcgtcctcac cgttgtgcac caggactggc tgaacggcaa ggagtacaag 960

tgcaaggtct ccaacaaagg cctcccagcc cccatcgaga aaaccatctc caaaaccaaa 1020

gggcagcccc gagaaccaca ggtgtacacc ctgccgccct cgagagaaga gatgggcggg 1080

ttgccggggt gtaagtggga cttgctgatt aaacaatggg tgtgcgaccc tctgggcggt 1140

accaagaatc aggtctcact gacatgtctc gtaaaaggtt tttacccgtc agatatcgcg 1200

gtcgagtggg aatccaacgg acaacccgag aataactaca agacgactcc cccaatgctc 1260

gattcggatg gatccttctt cctttatagc aaacttacag tagacaaatc acggtggcag 1320

caggggaacg tgtttagctg ttcggtgatg cacgaagcct tgcataatca ctatacgcag 1380

aagtcgcttt ccctgtcgcc gggaggggga ggtgggctcc ctggatgcaa gtgggatctt 1440

ttgatcaagc agtgggtctg cgacccccTc 1470

<210> 65
<211> 1473
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 65
gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggGtc cctgagactc 60

tcctgtgcag cttctggatt tacctttagt agttattgga tgagttgggt ccgccaggct 120

ccagggaaag ggctggagtg ggtggcctac ataaagcaag atggaaatga gaaatactat 180

gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctcattgtat 240

ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagggaaggg 300

atactttggt tcggggactt accgacgttc tggggccagg gaaccctggt caccgtctct 360

agtgcctcca ccaagggccc atcggtcttc cccctggcgc cctgctccag gagcacctcc 420

gagagcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 480

tcgtggaact caggcgctct gaccagcggc gtgcacacct tcccagctgt cctacagtcc 540

tcaggactct actccctcag cagcgtggtg accgtgccct ccagcaactt cggcacccag 600

acctacacct gcaacgtaga tcacaagccc agcaacacca aggtggacaa gacagttgag 660

cgcaaatgtt gtgtcgagtg cccaccgtgc ccagcaccac ctgtggcagg accgtcagtc 720

ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcacg 780

tgcgtggtgg tggatgtaag ccatggggga ctgcctggat gcaagtggga tcttctcatt 840

aagcaatggg tctgtgaccc tttgggcgga gaggacccgg aagtccagtt caactggtac 900

gtggacggcg tggaggtgca taatgccaag acaaagccac gggaggagca gttcaacagc 960

acgttccgtg tggtcagcgt cctcaccgtt gtgcaccagg actggctgaa cggcaaggag 1020

tacaagtgca aggtctccaa caaaggcctc ccagccccca tcgagaaaac catctccaaa 1080

accaaagggc agccccgaga accacaggtg tacaccctgc cgccctcgag agaagagatg 1140

ggcgggttgc cggggtgtaa gtgggacttg ctgattaaac aatgggtgtg cgaccctctg 1200

ggcggtacca agaatcaggt ctcactgaca tgtctcgtaa aaggttttta cccgtcagat 1260

atcgcggtcg agtgggaatc caacggacaa cccgagaata actacaagac gactccccca 1320

atgctcgatt cggatggatc cttcttcctt tatagcaaac ttacagtaga caaatcacgg 1380

tggcagcagg ggaacgtgtt tagctgttcg gtgatgcacg aagccttgca taatcactat 1440

acgcagaagt cgctttccct gtctccgggt aaa 1473

<210> 66
<211> 879
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 66
atgcttccag gttgtaaatg ggatcttctt attaaacaat gggtttgtga tccacttggt 60

tctggttctg ctactggtgg ttccggctcc accgcaagct ctggttcagg ttctgctact 120

catatgctgc cgggttgtaa atgggacctg ctgatcaaac agtgggtttg tgacccgctg 180

ggtggaggcg gtggggtcga caaaactcac acatgtccac cttgtccagc tccggaactc 240

ctggggggac cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc 300

cggacccctg aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag 360

ttcaactggt acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag 420

cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg 480

aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa 540

accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc 600

cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatccc 660

agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg 720

cctcccgtgc tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag 780

agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac 840

cactacacgc agaagagcct ctccctgtct ccgggtaaa 879

<210> 67
<211> 696
<212> DNA
<213> Homo sapiens

<400> 67
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gggggaccgt cagtcttcct cttccccccа aaacccaagg acaccctcat gatctcccgg 120

acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 180

aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 240

tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 300

ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagccccca cgagaaaacc 360

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg 420

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gtcacgtgcg tggtggtgga cgtgagccag gaagaccccg aggtccagtt caactggtac      180
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Patentkrav

1. Værtscelle omfattende en nukleinsyre, der koder for et bispecifikt protein, og/eller en vektor omfattende en nukleinsyre, der koder for et bispecifikt protein, hvor det bispecifikke protein omfatter:
(a) et polypeptid omfattende en aminosyresekvens med følgende formel:
A-L1-P-L2-P, hvor A er en immunoglobulin-tungkæde af et IgG-antistof, L1 er en første peptid-linker, der er fraværende eller er 3 til 40 aminosyrer lang, P er et BAFF-bindende peptid, der er 10 til 40 aminosyrer langt, og L2 er en anden peptid-linker, der er fraværende eller er 5 til 50 aminosyrer lang, og
(b) en immunoglobulin-letkæde af et IgG-antistof,
hvor immunoglobulin-tungkæden fra (a) og immunoglobulin-letkæden fra (b) danner et IgG-antistof, der omfatter to molekyler af polypeptidet fra (a) og to molekyler af letkæden fra (b), der kan binde sig til B7RP1, og
hvor proteinet kan hæmme BAFF-medieret proliferation af humane B-celler, og
hvor proteinet kan hæmme B7RP1-medieret proliferation af humane T-celler, og
hvor værtscellen er en bakteriecelle, en gærcelle, en insektcelle, en plantecelle eller en pattedyrscelle.

2. Værtscelle ifølge krav 1, hvor det bispecifikke protein omfatter en immunoglobulin-tungkæde, der mangler et lysin i sin C-terminale ende umiddelbart opstrøms for L1.

3. Værtscelle ifølge krav 1, hvor IgG-antistoffet er et humant eller humaniseret anti-B7RP1 IgG1-antistof.

4. Værtscelle ifølge krav 1 eller 2, hvor anti-B7RP1-antistoffet er et humant eller humaniseret IgG2-antistof eller et humant eller humaniseret IgG4-antistof.

5. Værtscelle ifølge et hvilket som helst af kravene 1-4, hvor P har aminosyresekvensen fra SEQ ID NO:1 (LPGCKWDLLIKQWVCDPL).

6. Værtscelle ifølge et hvilket som helst af kravene 1-5, hvor L1 har aminosyresekvensen fra SEQ ID NO:40 (GGGGG).

7. Værtscelle ifølge et hvilket som helst af kravene 1-6, hvor L2 har aminosyresekvensen fra SEQ ID NO:5, fortrinsvis hvor L2 har aminosyresekvensen fra SEQ ID NO:6 eller SEQ ID NO:7.

8. Værtscelle ifølge et hvilket som helst af kravene 1-7, der koder for en letkæde-CDR1 omfattende aminosyresekvensen fra SEQ ID NO:8 (RASQGISNWLA), en letkæde-CDR2 omfattende aminosyresekvensen fra SEQ ID NO:9 (AASSLQS), en letkæde-CDR3 omfattende aminosyresekvensen fra SEQ ID NO:10 (QQYDSYPRT) , en tungkæde-CDR1 omfattende aminosyresekvensen fra SEQ ID NO:11 (SYWMS), en tungkæde-CDR2 omfattende aminosyresekvensen fra SEQ ID NO:12 (YIKQDGNEKYYVDSVKG) og en tungkæde-CDR3 omfattende aminosyresekvensen fra SEQ ID NO:13 (EGILWFGDLPTF).

9. Værtscelle ifølge et hvilket som helst af kravene 1-8, hvor det bispecifikke protein, der kodes for, omfatter en variabel immunoglobulin-letkæderegion, der omfatter aminosyresekvensen fra SEQ ID NO:14.

10. Værtscelle ifølge et hvilket som helst af kravene 1-9, hvor det bispecifikke protein, der kodes for, omfatter en variabel immunoglobulin-tungkæderegion, der omfatter aminosyresekvensen fra SEQ ID NO:15.

11. Værtscelle ifølge et hvilket som helst af kravene 5-10, hvor immunoglobulin-letkæden fra (b) omfatter aminosyresekvensen fra SEQ ID NO:19.

12. Værtscelle ifølge et hvilket som helst af kravene 5-11, hvor polypeptidet fra (a) omfatter aminosyresekvensen fra SEQ ID NO:17 eller 18.

13. Værtscelle ifølge krav 1, hvor polypeptid (a) omfatter aminosyresekvensen fra SEQ ID NO:17 eller SEQ ID NO:18, og

polypeptid (b) omfatter aminosyresekvensen fra SEQ ID NO:19.

14. Værtscelle ifølge krav 1, hvor det bispecifikke protein omfatter aminosyresekvensen fra SEQ ID NO:1, SEQ ID NO:14 og SEQ ID NO:15, fortrinsvis hvor proteinet endvidere omfatter en linker, der omfatter aminosyresekvensen fra SEQ ID NO:6 eller SEQ ID NO:7.

15. Værtscelle ifølge et hvilket som helst af kravene 1-14, hvor værtscellen er en pattedyrscelle udvalgt fra gruppen bestående af en kinesisk hamster-ovariecelle (CHO-celle), en hamsterunge-nyrecelle (BHK-celle), en abe-nyrecelle, en HeLa-celle, en human hepatocellulær carcinom-celle og en 293-celle.

16. Fremgangsmåde til fremstilling af et bispecifikt protein, der omfatter dyrkning af værtscellen ifølge et hvilket som helst af kravene 1-14 under sådanne betingelser, at nukleinsyren udtrykkes, og genindvinding af proteinet fra cellemassen eller dyrkningsmediet, hvor værtscellen er en bakteriecelle, en gærcelle, en insektcelle eller en plantecelle.

17. Fremgangsmåde ifølge krav 16, hvor nukleinsyren eller vektoren indføres i cellen ved
(a) elektroporese eller calciumchloridtransformation, hvor værtscellen er en bakteriecelle, eller
(b) lithiumacetattransformation eller polyethylenglycoltransformation, hvor værtscellen er en gærcelle.

DRAWINGS

Construct name	P71617	P71618	P71619	P71620	P71621	P71622	P71523
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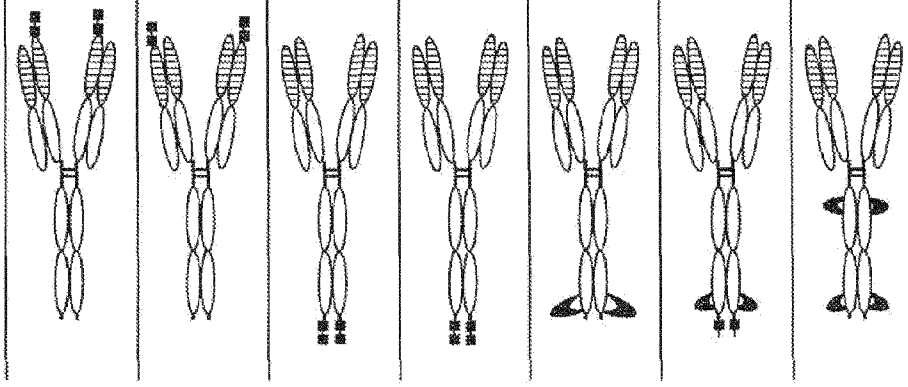
Construct design							

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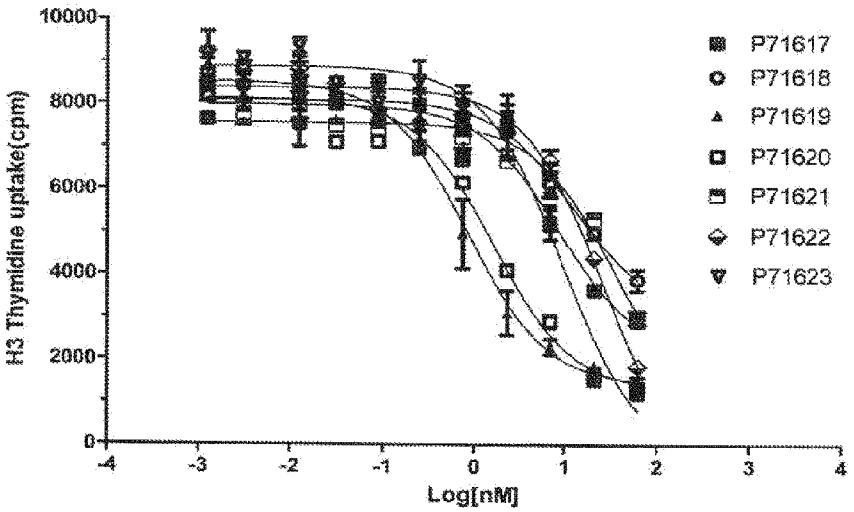

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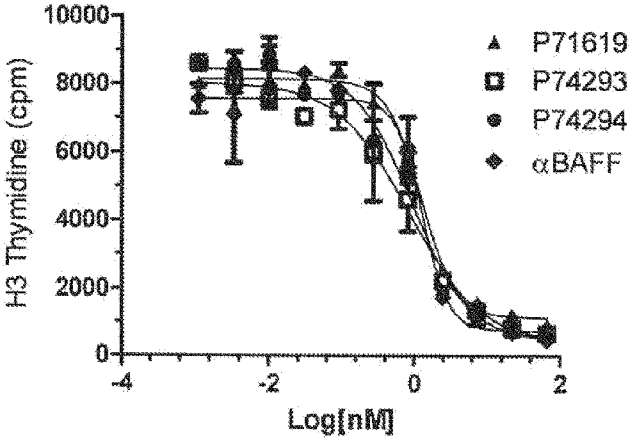

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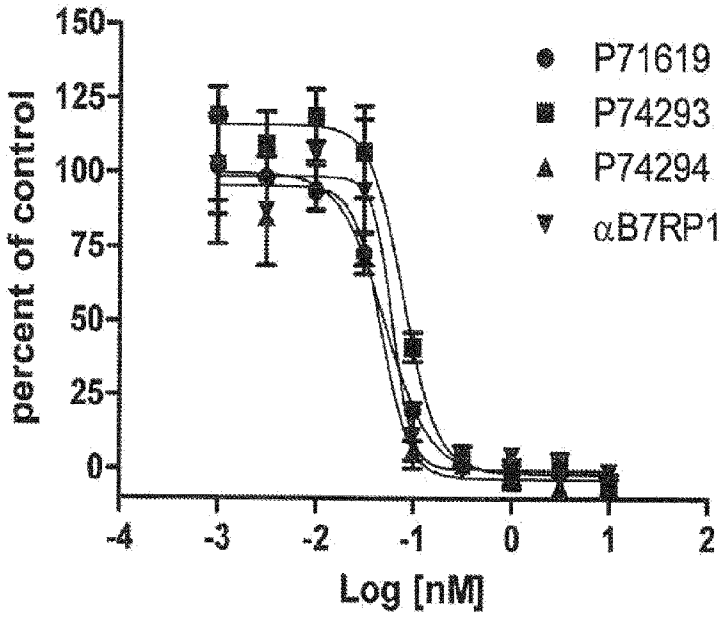
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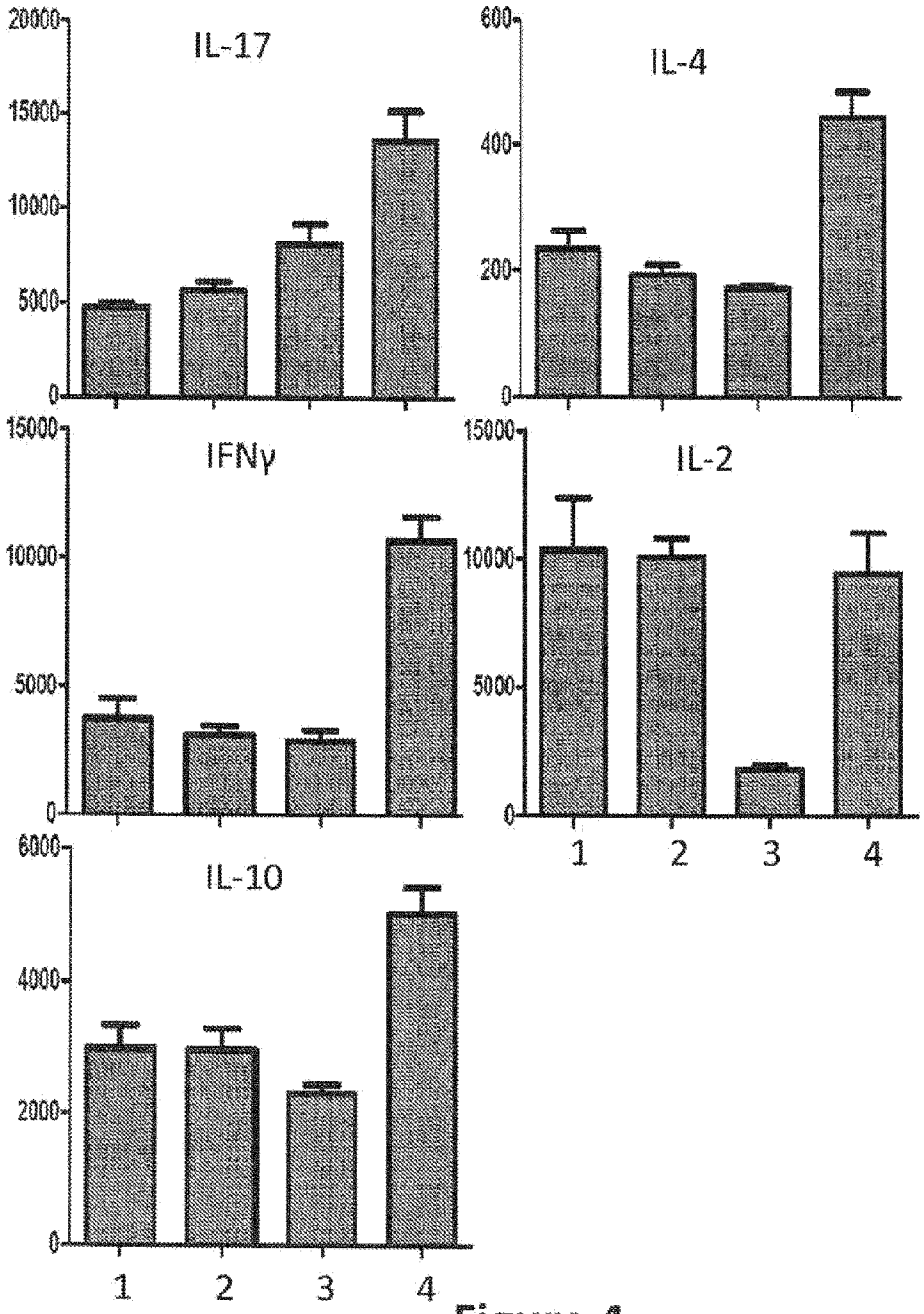
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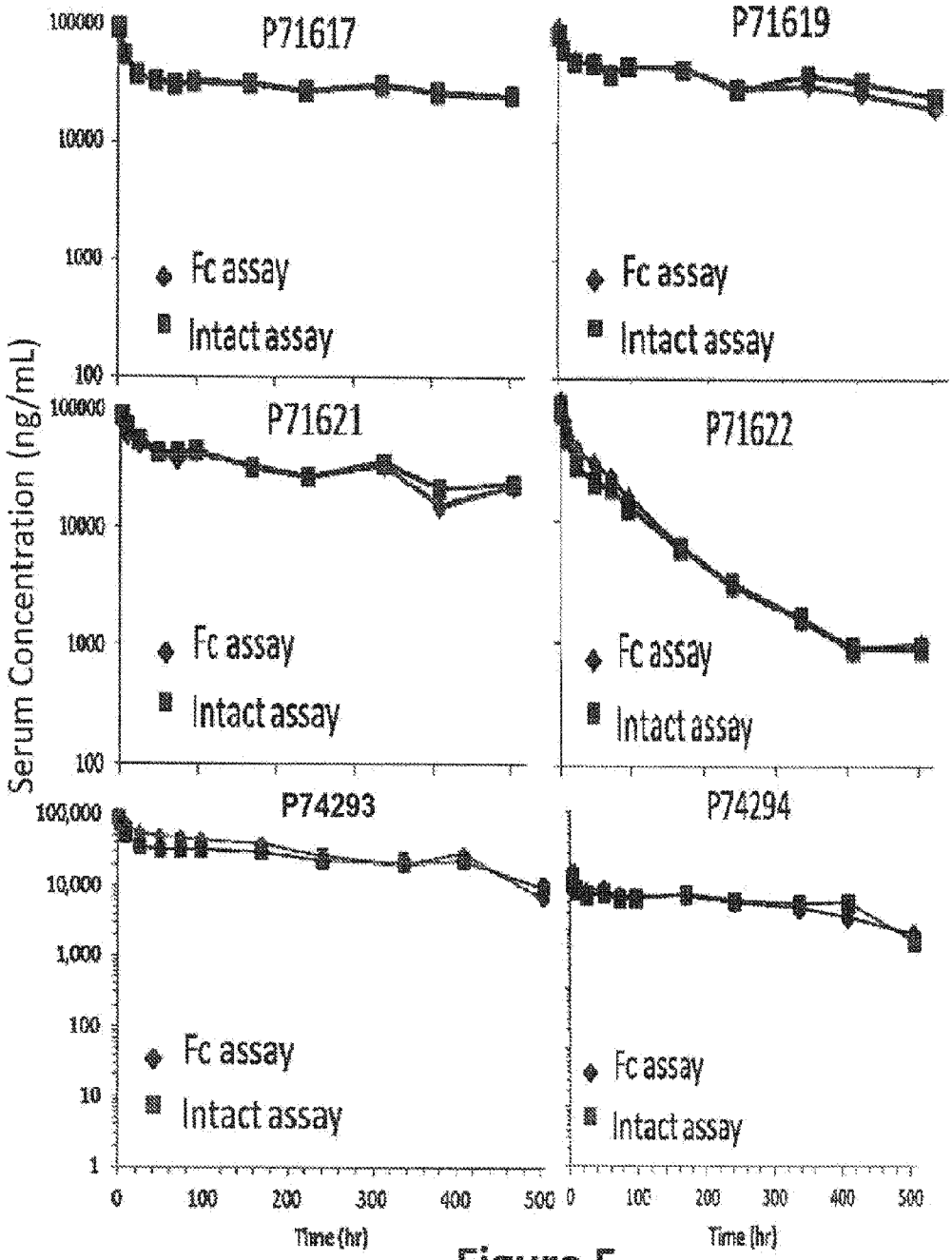
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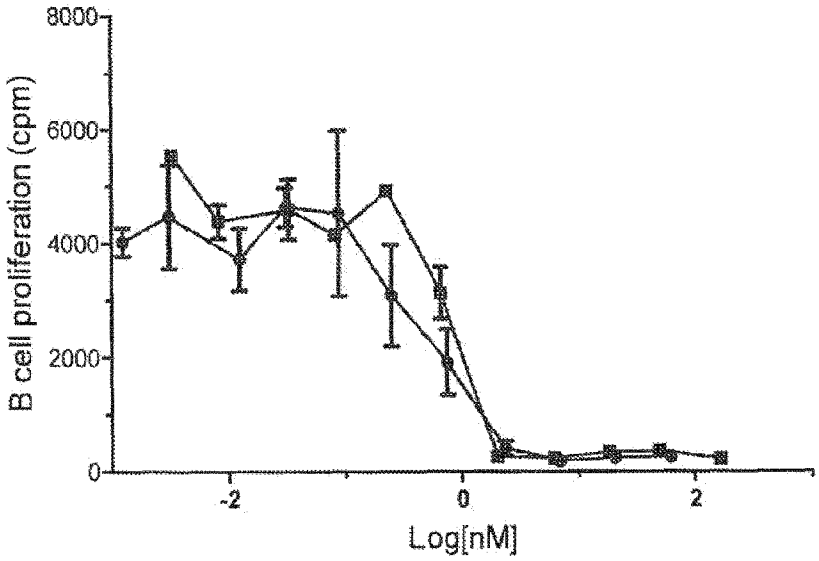

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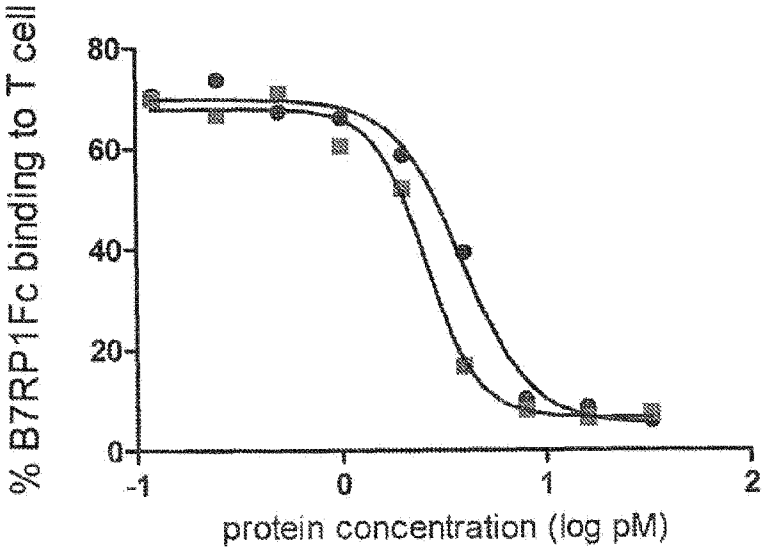

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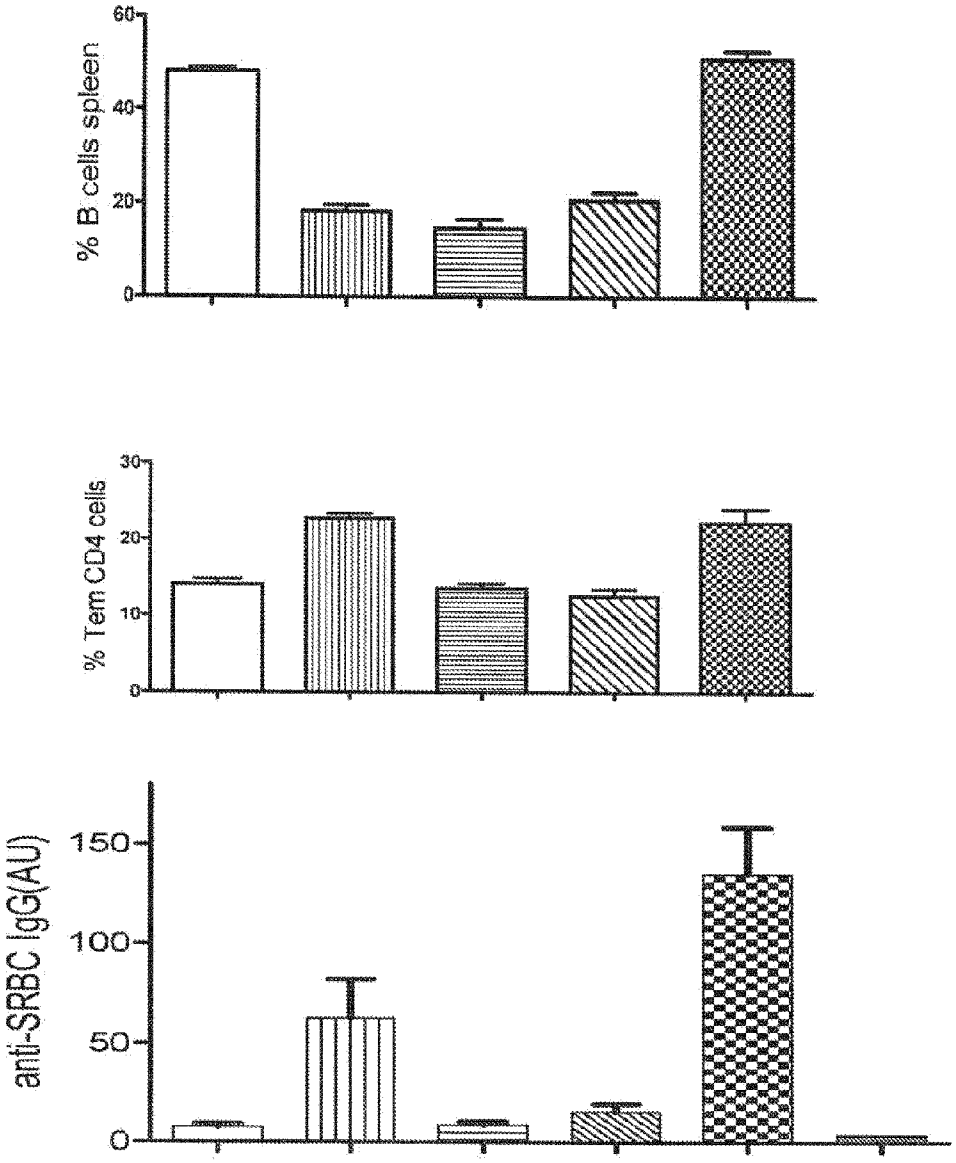
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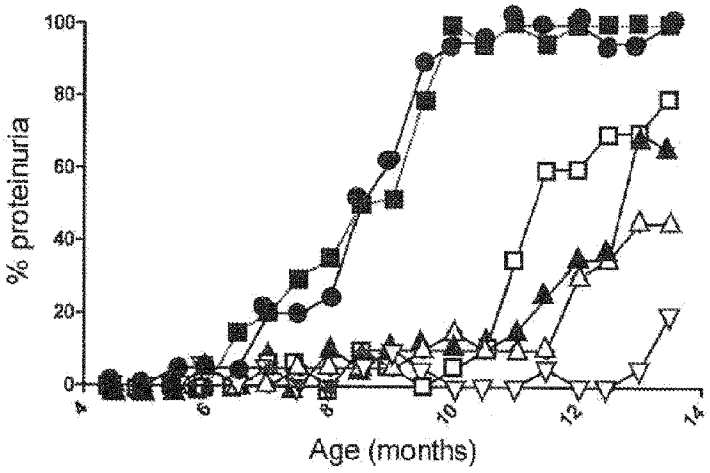

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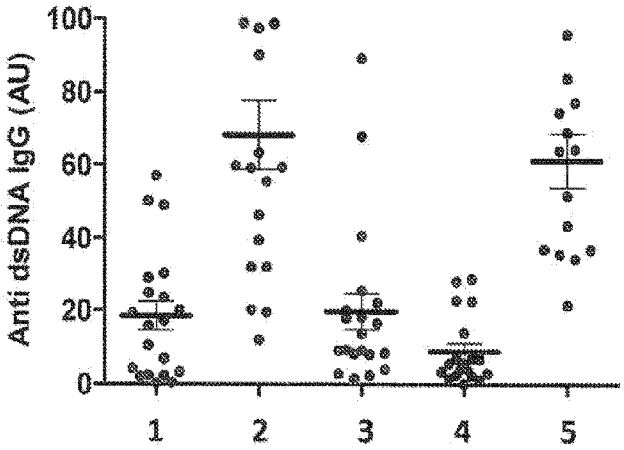

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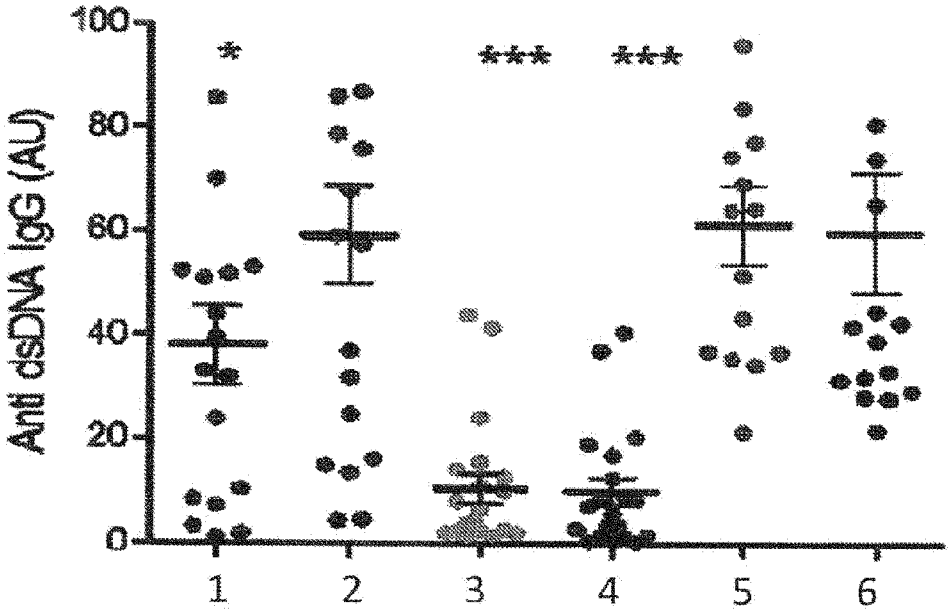

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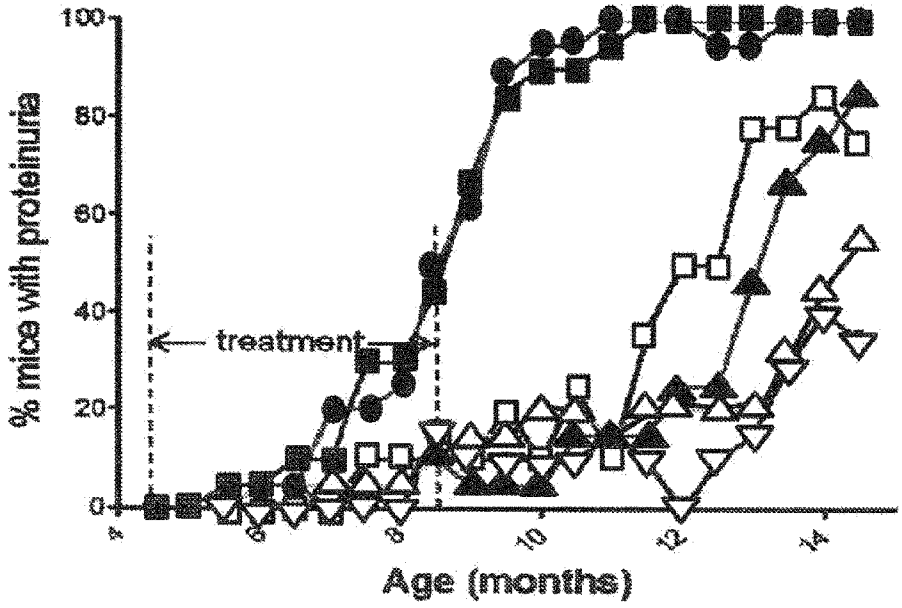

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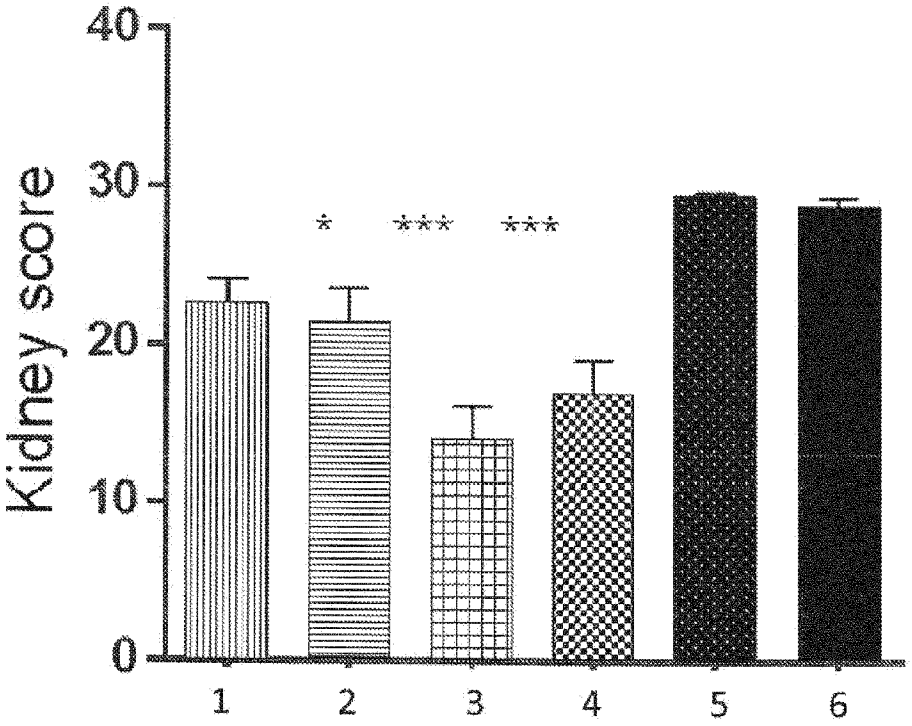

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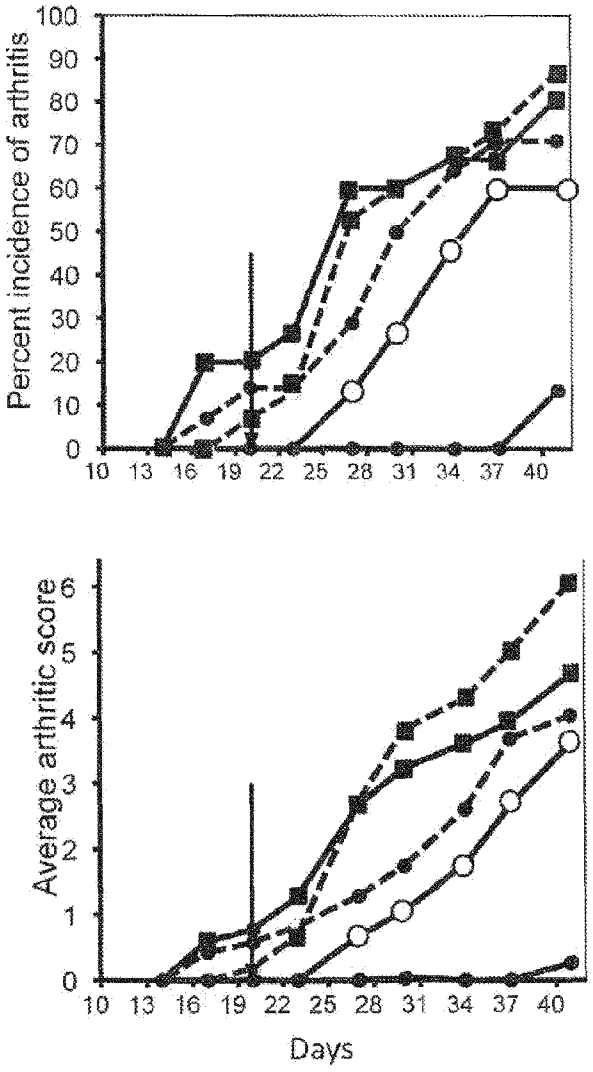
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5
4
3
2
1
0
10 13 16 19 22 25 28 31 34 37 40
Days

Figure 11