INTERLEUKIN-2 FUSION PROTEINS AND USES THEREOF

Applicant: Hoffmann-La Roche Inc., Nutley, NJ (US)

Inventors: Ralf Hosse, Cham (CH); Christian Klein, Bonstetten (CH); Ekkhard Moessner, Krenzlingen (CH); Laurence Bernard Peterson, Cambridge (GB); Pablo Umana, Wollerau (CH); Linda Wicker, Cambridge (GB)

Assignee: Roche Glycart AG, Schlieren (CH)

Filed: Aug. 6, 2013

Provisional application No. 61/681,676, filed on Aug. 10, 2012.

The present invention generally relates to fusion proteins of immunoglobulins and interleukin-2 (IL-2). In addition, the present invention relates to polynucleotides encoding such fusion proteins, and vectors and host cells comprising such polynucleotides. The invention further relates to methods for producing the fusion proteins of the invention, and to methods of using them in the treatment of disease.
Figure 3
INTERLEUKIN-2 FUSION PROTEINS AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention generally relates to fusion proteins of immunoglobulins and interleukin-2 (IL-2). In addition, the present invention relates to polynucleotides encoding such fusion proteins, and vectors and host cells comprising such polynucleotides. The invention further relates to methods for producing the fusion proteins of the invention, and to methods of using them in the treatment of disease.

BACKGROUND

[0002] Regulatory T cells (Treg) represent specific subsets of T lymphocytes that are crucial for the maintenance of self-tolerance. These CD4+CD25+ cells with suppressor function can be distinguished from effector T cells by the intracellular expression of the transcription factor Foxp3, as well as other cell markers such as CD127+, CTLA-4+, LAP, CD39+, PD-1+, GARP, etc. Foxp3 is critical for Treg differentiation and function, and Foxp3 gene deficiency and mutations, both in scurvy mice and patients with immune dysregulation polyendocrinopathy, enteropathy, X-chromosome linked syndrome (IPEX) result in the breakdown of self-tolerance and the development of autoimmune diseases due to Treg deficiency or lack of function.

[0003] The autoimmune responses in type 1 diabetes, Systemic Lupus Erythematosus (SLE), multiple sclerosis, and many others are correlated with a deficiency in Treg. Data from animal models support the hypothesis that autoimmune responses are facilitated by a failure of Treg to control the destructive immune response to self. Type 1 diabetes is an autoimmune disease that occurs after the destruction of a majority of the insulin producing β cells in the pancreas. The frequency of type 1 diabetes is ~0.3% of the population in the US and its incidence continues to increase in the US, Europe, and in particular Scandinavia (nearly 1%) and is expected to double within the next twenty years.

[0004] The cytokine IL-2 plays a major role in the activation and function of both Treg as well as effector T cells (Teff). A deficiency in IL-2 production or lack of responsiveness preferentially results in a loss of Treg function and an increase in the probability of autoimmunity. Because Treg constitutively express the high affinity IL-2 receptor at higher levels than Teff, low doses of IL-2 preferentially support the maintenance of Treg as compared to Teff cells.

[0005] With the preferential effect of IL-2 for activating Treg in vitro and in vivo, the potential for low dose, long-lived IL-2 therapy would seem to have a high prospect for success in autoimmune diseases. A 200 patient, double blind, placebo controlled type 1 diabetes clinical trial with IL-2 (Proleukin®) is set to begin in late 2013. Recent clinical trials with daily low dose Proleukin ameliorated some of the signs and symptoms of chronic graft-versus-host disease (GVHD) and hepatitis C virus-induced vasculitis (Koreth et al., New Engl J Med 365, 2055-2066 (2011), Saadoun et al., New Engl J Med 365, 2067-2077 (2011)). In both studies low dose Proleukin induced Treg and increased the Treg/Teff ratio. However, Proleukin’s poor PK properties make it suboptimal for maintaining low, consistent levels of IL-2 in man. Other methods being tested in clinical trials are personalized expansion of Treg ex vivo followed by reinfusion, but this approach is less than ideal and represents a challenging set of quality control issues.

[0006] Thus, a new therapeutic approach that re-establishes the natural regulatory T cell (Treg) mediated dominant immune tolerance would greatly enhance the ability to treat patients with autoimmune diseases such as type 1 diabetes, multiple sclerosis, systemic lupus erythematosus, Crohn’s disease as well as other immune-based pro-inflammatory diseases such as chronic graft versus host disease, asthma, pulmonary fibrosis, chronic obstructive pulmonary disease, and transplant rejection, both solid organ and bone marrow.

[0007] The IL-2 fusion proteins of the present invention preferentially activate Treg, tipping the balance toward a higher Treg/Teff ratio and reduce the autoimmune response. They are long-lived, allowing convenient dosing schedules, and devoid of effector functions, reducing potential side effects and impairment of efficacy.

SUMMARY OF THE INVENTION

[0008] In one aspect, the invention provides a fusion protein comprising (i) an immunoglobulin molecule comprising a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification, and (ii) two interleukin-2 (IL-2) molecules.

[0009] In one embodiment, said immunoglobulin molecule is an IgG-class immunoglobulin molecule, particularly an IgG1 subclass immunoglobulin molecule. In one embodiment, said immunoglobulin molecule is a human immunoglobulin molecule. In one embodiment, said immunoglobulin molecule is capable of specific binding to an antigen. In one embodiment, said immunoglobulin molecule is a monoclonal antibody. In one embodiment, said immunoglobulin molecule is not capable of specific binding to an antigen. In one embodiment, said immunoglobulin molecule comprises a heavy chain variable region sequence based on the human Vh3-23 germline sequence. In a specific embodiment, said immunoglobulin molecule comprises the heavy chain variable region sequence of SEQ ID NO: 9.

In one embodiment, said immunoglobulin molecule comprises a light chain variable region sequence based on the human Vk3-20 germline sequence. In a specific embodiment, said immunoglobulin molecule comprises the light chain variable region sequence of SEQ ID NO: 11.

In one embodiment, said immunoglobulin molecule comprises the heavy chain variable region sequence of SEQ ID NO: 9 and the light chain variable region sequence of SEQ ID NO: 11.

[0010] In one embodiment, said Fc receptor is an Fcy receptor, particularly a human Fcy receptor. In one embodiment, said Fc receptor is an activating Fc receptor. In one embodiment, said Fc receptor is selected from the group of FcγRIIa (CD16a), FcγRI (CD64), FcγRIIa (CD32) and FcεRI (CD89). In a specific embodiment, said Fc receptor is FcγRIIa, particularly human FcγRIIa. In one embodiment, said modification reduces effector function of the immunoglobulin molecule. In a specific embodiment, said effector function is antibody-dependent cell-mediated cytotoxicity (ADCC). In one embodiment, said modification is in the Fc region, particularly the CH2 region, of said immunoglobulin molecule. In one embodiment, said immunoglobulin molecule comprises an amino acid substitution at position 329 (EU numbering) of the immunoglobulin heavy chains. In a specific embodiment, said amino acid substitution is P329G.
In one embodiment, said immunoglobulin molecule comprises amino acid substitutions at positions 234 and 235 (EU numbering) of the immunoglobulin heavy chains. In a specific embodiment, said amino acid substitutions are L234A and L235A (L Ala). In a particular embodiment, said immunoglobulin molecule comprises the amino acid substitutions L234A, L235A and P529G (EU numbering) in the immunoglobulin heavy chains.

In one embodiment, said IL-2 molecules are wild-type IL-2 molecules. In one embodiment, said IL-2 molecules are human IL-2 molecules. In a specific embodiment, said IL-2 molecules comprise the sequence of SEQ ID NO: 1 or SEQ ID NO: 3, particularly the sequence of SEQ ID NO: 3. In one embodiment, said IL-2 molecules are each fused at their N-terminal amino acid to the C-terminal amino acid of one of the immunoglobulin heavy chains of said immunoglobulin molecule, optionally through a peptide linker.

In a specific embodiment, said fusion protein comprises the polypeptide sequences of SEQ ID NO: 17 and SEQ ID NO: 19. In one embodiment, said fusion protein essentially consists of an immunoglobulin molecule comprising a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification, two interleukin-2 (IL-2) molecules, and optionally one or more peptide linker.

The invention further provides a polynucleotide encoding the fusion protein of the invention. Further provided is a vector, particularly an expression vector, comprising the polynucleotide of the invention. In another aspect, the invention provides a host cell comprising the polynucleotide or the vector of the invention. The invention also provides a method for producing a fusion protein of the invention, comprising the steps of (i) culturing the host cell of the invention under conditions suitable for expression of the fusion protein, and (ii) recovering the fusion protein. Also provided is a fusion protein comprising (i) an immunoglobulin molecule comprising a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification, and (ii) two interleukin-2 (IL-2) molecules, produced by said method.

In one aspect, the invention provides a pharmaceutical composition comprising the fusion protein of the invention and a pharmaceutically acceptable carrier. The fusion protein or the pharmaceutical composition of the invention is also provided for use as a medicament, and for use in the treatment or prophylaxis of an autoimmune disease, specifically type 1 diabetes, multiple sclerosis (MS), systemic lupus erythematosus (SLE) or Crohn’s disease, most specifically type 1 diabetes, or graft-versus-host disease or transplant rejection. Further provided is the use of the fusion protein of the invention for the manufacture of a medicament for the treatment of a disease in an individual in need thereof, and a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the fusion protein of the invention in a pharmaceutically acceptable form. In one embodiment, said disease is an autoimmune disease. In a more specific embodiment, said autoimmune disease is type 1 diabetes, multiple sclerosis (MS), systemic lupus erythematosus (SLE) or Crohn’s disease. In an even more specific embodiment, said autoimmune disease is type 1 diabetes. In another embodiment said disease is transplant rejection or graft-versus-host disease. In one embodiment, said individual is a mammal, particularly a human.

Further provided the fusion protein of the invention for use in selective activation of regulatory T cells in vitro or in vivo. In one embodiment, said activation comprises induction of proliferation and/or induction of IL-2 receptor signaling. In one embodiment, said use is in vitro and said fusion protein is used at a concentration of about 1 ng/mL or less, particularly about 0.1 ng/mL or less. In another embodiment, said use is in vivo and said fusion protein is used at a dose of about 20 μg/kg body weight or less, particularly about 12 μg/kg body weight or less.

The invention also provides a method for selective activation of regulatory T cells in vitro or in vivo, comprising contacting said regulatory T cells with the fusion protein of the invention. In one embodiment, said activation comprises induction of proliferation and/or induction of IL-2 receptor signaling. In one embodiment, said method is in vitro and said fusion protein is used at a concentration of about 1 ng/mL or less, particularly about 0.1 ng/mL or less. In another embodiment, said method is in vivo and said fusion protein is used at a dose of about 20 μg/kg body weight or less, particularly about 12 μg/kg body weight or less.

**BRIEF DESCRIPTION OF THE DRAWINGS**

**Fig. 1.** Purification of DP47GS IgG-IL-2 fusion protein (see SEQ ID Nos 13, 15, 19). (A) Elution profile of the Protein A affinity chromatography step. (B) Elution profile of the size exclusion chromatography step. Yield 4 mg/L. (C) Analytical capillary electrophoresis SDS (Caliper) of the final product. The following band were observed: non-reduced—7.5% area at 111 kDa; 92.5% area at 174 kDa; reduced—23.6% area at 29 kDa; 23.5% area at 67 kDa. The product contains about 7.5% “half IgG”. (D) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (91% monomer content).

**Fig. 2.** Purification of DP47GS IgG-(IL-2), fusion protein (see SEQ ID Nos 17, 19). (A) Elution profile of the Protein A affinity chromatography step. (B) Elution profile of the size exclusion chromatography step. Yield 13 mg/L. (C) Analytical capillary electrophoresis SDS (Caliper) of the final product. The following band were observed: non-reduced—2.3% area at 172.5 kDa; 97.7% area at 185 kDa; reduced—18.3% area at 27.3 kDa; 0.6% area at 29.2 kDa; 81.1% area at 78.3 kDa. (D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (100% monomer content).

**Fig. 3.** CD25 (IL-2RA) and CD122 (IL-2RB) expression on CD4+ Treg subsets, NK cell subsets and NK T cells. Cell surface markers were used to define CD4+ Treg subsets, NK T cells and NK cells. In order to optimize staining for CD25 and CD122, intracellular Foxp3 staining was not performed. (A, B) Three regulatory CD4+ T cell (Treg) populations: nTreg (CD45RA-, CD25+; dotted line), memory (CD45RA-; CD25+; solid line) and activated (CD45RA-; CD25; dashed line). (C, D) NKT (dotted line), CD56bright NK cells (dashed line), CD56mid NK cells (solid line). Grey: isotype control.

**Fig. 4.** CD25 (IL-2RA) and CD122 (IL-2RB) expression on CD4+ and CD8+ conventional T cell subsets. Cell surface markers were used to define nTreg (CD45RA-; dotted line) and memory (CD45RA-; solid line) conventional CD4+ T cells (A, B), memory conventional CD8+ T cells...
**CD45RA**; solid line) and CD45RA* CD8 T cells (a combination of the naïve and TEMRA subsets; TEMRA refers to effector memory cells that have reverted to expressing CD45RA; dotted line) (C, D). Grey: isotype control.

**[0021]** FIG. 5. Induction of pSTAT5 in human peripheral blood cell subsets in response to DP47GS IgG-IL-2. The effects of various doses of DP47 IgG-IL-2 on the induction of STAT5 phosphorylation are shown in human CD4* Treg subsets, naïve and memory conventional CD4* T cells, memory conventional CD8* T cells, CD45RA* CD8 T cells, NKT cells and NK cells.

**[0022]** FIG. 6. Induction of pSTAT5 in human peripheral blood cell subsets in response to DP47GS IgG-IL-2. The effects of various doses of DP47 IgG-IL-2 immunocomplexes are shown on the induction of STAT5 phosphorylation in human CD4* Treg subsets, naïve and memory conventional CD4* T cells, memory conventional CD8* T cells, CD45RA* CD8 T cells, NKT cells and NK cells.

**[0023]** FIG. 7. Induction of pSTAT5 in human peripheral blood cell subsets: comparison of DP47GS IgG-IL-2 and DP47GS IgG-IL-2*.

**[0024]** FIG. 8. Detailed examination of Treg subset sensitivity in three donors comparing DP47GS IgG-IL-2 and DP47GS IgG-IL-2*.

**[0025]** FIG. 9. DP47GS IgG-IL-2 has a dose dependent effect in cynomolgus monkeys increasing regulatory T cells. The changes in whole blood CD4+, CD25+, Foxp3+, regulatory T cells on day 7 post treatment are shown as the absolute cell number per mm^3^ of whole blood (A) and the fold change in Treg (B); data are calculated using the mean±SEM. Shaded bars: DP47GS IgG-IL-2 (n=6); open bars: vehicle (n=3).

**[0026]** FIG. 10. Dose dependent effects of DP47 IgG-IL-2 on Natural Killer cells. NK cells were identified as CD3+, CD4* CD16* as shown. Vehicle treated monkeys (n=3, open bars) and those treated with DP47 IgG-IL-2 (n=6, shaded bars) are shown as total blood NK cells (×10^6^/μl).

**[0027]** FIG. 11. Low dose DP47GS IgG-IL-2 is more effective than high dose Proluken in Treg induction in cynomolgus monkeys. Normal healthy cynomolgus monkeys (groups of n=5) were treated with low doses of DP47GS IgG-IL-2 or high doses of Proluken and the change in regulatory T cells tested at day 10. On days 0 and 7, DP47GS IgG-IL-2 was given SC at a dose of 16,800 IU/kg. Proluken treatment was given SC 3 times per week (MWF) for a total of 5 doses at 200,000 IU/kg. The results are shown as mean±SEM for the change in total Treg (B), and the change in the ratio of Treg to conventional CD4* Foxp3* cells (C). Shaded bars: IL-2 treatment; open bars: vehicle control.

**[0028]** FIG. 12. Ex vivo whole blood pSTAT5 as a marker for DP47GS IgG-IL-2 Treg activation in vivo. One and 3 days after in vivo administration of a single low dose of DP47GS IgG-IL-2 (12 μg/kg) to healthy cynomolgus monkeys (n=5), whole blood was collected and tested for STAT5. Each monkey was bled on day 0 before treatment and the amount of STAT5 phosphorylation was measured (open bars) and used individually to assess fold-changes post treatment (shaded bars). The fold change in pSTAT5 in Treg on days 1 and 3 (A), the fold change in pSTAT5 in conventional CD4* CD45RA* memory T cells (B), and the fold change in pSTAT5 in naive T cells (C) is shown.

**[0029]** FIG. 13. Ex vivo whole blood Ki-67 as a marker for DP47GS IgG-IL-2 T cell proliferation in vivo. The cynomolgus monkeys treated with DP47GS IgG-IL-2 as described in FIG. 11 were also monitored for ex vivo changes in the intracellular marker Ki-67 to assess the extent of proliferation in vivo. The percentage of cells that were in cell cycle (Ki-67*) on day 0 (open bars) is compared to the percentage of cells Ki-67* at 2 and 7 days post treatment (shaded bars). Ki-67* Treg (A), conventional CD4* CD45RA* memory T cells (B), and naïve CD4* CD45RA* T cells (C) are shown.

**[0030]** FIG. 14. DP47GS IgG-IL-2 has enhanced PK properties compared to Proluken. NOD mice were injected IP (left panel) or SC (right panel) with the indicated doses of DP47GS IgG-IL-2 or Proluken. Human IL-2 was assessed in serum samples at the indicated times.

**[0031]** FIG. 15. Foxp3 and CD25 MFI increase in Treg after treatment with IgG-IL-2. NOD mice (3 mice/treatment group, including control cohorts at 24 and 72 h) were treated with either Proluken (Novartis, 4,000 or 40,000 IU) or DP47GS IgG-IL-2 (4,000 IU), and cell surface antigen levels on splenocytes determined after 24 and 72 h by FACS. Treg were defined as CD4*, Foxp3* from singlets within the lymphocyte gate; from this population, CD25 (right panel) and Foxp3 (left panel) mean fluorescence intensity (MFI) were calculated for all samples.

**[0032]** FIG. 16. In vivo treatment with DP47GS IgG-IL-2 suppresses murine delayed type hypersensitivity. Left panel: NOD mice, right panel: C57BL/6 mice. The magnitude of the DTH response is shown as the change in paw weight compared to non-immunized mice (A, paw weight).

**[0033]** FIG. 17. In vivo treatment with DP47GS IgG-IL-2 suppresses the murine antibody responses to KLH.

### Detailed Description of the Invention

**Definitions**

**[0034]** Terms are used herein as generally used in the art, unless otherwise defined in the following.

**[0035]** As used herein, the term “fusion protein” refers to a fusion polypeptide molecule comprising an immunoglobulin molecule and an -II-2 molecule, wherein the components of the fusion protein are linked to each other by peptide-bonds, either directly or through peptide linkers. For clarity, the individual peptide chains of the immunoglobulin component of the fusion protein may be linked non-covalently, e.g. by disulfide bonds.

**[0036]** “Fused” refers to components that are linked by peptide bonds, either directly or via one or more peptide linkers.

**[0037]** By “specific binding” is meant that the binding is selective for the antigen and can be discriminated from unwanted or non-specific interactions. The ability of an immunoglobulin to bind to a specific antigen can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. Surface Plasmon Resonance (SPR) technique (analyzed on a BIAcore instrument) (Ljiljblad et al., Glyco J 17, 323-329 (2000)), and traditional binding assays (Healey, Endocr Res 28, 217-229 (2002)). In one embodiment, the extent of binding of an immunoglobulin to an unrelated protein is less than about 10% of the binding of the immunoglobulin to the
antigen as measured, e.g., by SPR. In certain embodiments, an immunoglobulin that binds to the antigen has a dissociation constant \(K_d\) of ≤1 nM, ≤100 nM, ≤10 nM, ≤1 nM, ≤0.1 nM, or ≤0.001 nM (e.g., 10^{-5} \text{ M} \text{ or less}, e.g., from \(10^{-8} \text{ M}\) to \(10^{-11} \text{ M}\), e.g., from \(10^{-8} \text{ M}\) to \(10^{-11} \text{ M}\)).

**[0038]** “Affinity” or “binding affinity” refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g. an antibody) and its binding partner (e.g. an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g. antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant \(K_d\), which is the ratio of dissociation and association rate constants \(k_{on}\) and \(k_{off}\), respectively. Thus, equivalent affinities may comprise different rate constants, as long as the ratio of the rate constants remains the same. Affinity can be measured by common methods known in the art, including those described herein. A particular method for measuring affinity is Surface Plasmon Resonance (SPR).

**[0039]** “Reduced binding”, for example reduced binding to an Fc receptor, refers to a decrease in affinity for the respective interaction, as measured for example by SPR. For clarity the term includes also reduction of the affinity to zero (or below the detection limit of the analytic method), i.e., complete abolishment of the interaction. Conversely, “increased binding” refers to an increase in binding affinity for the respective interaction.

**[0040]** As used herein, the term “antigenic determinant” is synonymous with “antigen” and refers to a site (e.g. a contiguous stretch of amino acids or a conformational configuration made up of different regions of non-contiguous amino acids) on a polypeptide macromolecule to which an antibody binds, forming an antibody-antigen complex. Useful antigenic determinants can be found, for example, on the surfaces of cells, free in blood serum, and/or in the extracellular matrix (ECM).

**[0041]** As used herein, the term “single-chain” refers to a molecule comprising amino acid monomers linearly linked by peptide bonds.

**[0042]** The term “antibody” herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

**[0043]** An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab’, Fab’-SH, F(ab’)2, diabodies, linear antibodies, single-chain antibody molecules (e.g. scFv), and single-domain antibodies.

**[0044]** The term “immunoglobulin molecule” refers to a protein having the structure of a naturally occurring antibody. For example, immunoglobulins of the IgG class are heterotetrameric glycoproteins of about 150,000 daltons, composed of two light chains and two heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain, also called a light chain constant region. The heavy chain of an immunoglobulin may be assigned to one of five classes, called α (IgA), δ (IgD), ε (IgE), γ (IgG), or μ (IgM), some of which may be further divided into subclasses, e.g. γ1 (IgG1), γ2 (IgG2), γ3 (IgG3), γ4 (IgG4), α1 (IgA1) and α2 (IgA2). The light chain of an immunoglobulin may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain. An immunoglobulin essentially consists of two Fab molecules and an Fc domain, linked via the immunoglobulin hinge region.

**[0045]** As used herein, “Fab fragment” refers to an immunoglobulin fragment comprising a VL domain and a constant domain of a light chain (CL), and a VH domain and a first constant domain (CH1) of a heavy chain.

**[0046]** The “class” of an antibody or immunoglobulin refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

**[0047]** The term “variable region” or “variable domain” refers to the domain of an immunoglobulin or antibody heavy or light chain that is generally involved in binding the immunoglobulin or antibody to antigen. However, the immunoglobulin comprised in the fusion protein of the present invention may comprise variable regions which do not confer antigen-binding specificity. The variable domains of the heavy chain and light chain (VH and VL, respectively) of an immunoglobulin or antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). See, e.g. Kindt et al., Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL domain may be sufficient to confer antigen-binding specificity.

**[0048]** The term “hypervariable region” or “HVR”, as used herein, refers to each of the regions of an immunoglobulin or antibody variable domain which are hypervariable in sequence and/or form structurally defined loops (“hypervariable loops”). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the complementarity determining regions (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, J. Mol. Biol. 196, 901-917 (1987))). Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-53H of H1, 50-65 of H2, and 95-102 of H3 (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise “specificity determining residues;” or “SDRs,” which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviation-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-
H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-55B of H1, 50-58 of H2, and 95-102 of H3 (see Almagro and Fransson, Front. Biosci. 13, 1619-1633 (2008)). Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., supra (referred to as “Kabat numbering”).

[0049] “Framework” or “FR” refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[0050] A “human immunoglobulin” is one which possesses an amino acid sequence which corresponds to that of an immunoglobulin produced by a human or a human cell or derived from a non-human source that utilizes human immunoglobulin repertoires or other human immunoglobulin-encoding sequences. This definition of a human immunoglobulin specifically excludes a humanized immunoglobulin comprising non-human antigen-binding residues.

[0051] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by a particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

[0052] The term “Fc domain” or “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. An IgG Fc region comprises an IgG CH2 and an IgG CH3 domain. The “CH2 domain” of a human IgG Fc region usually extends from an amino acid residue at position 231 to an amino acid residue at position 340. In one embodiment, a carbohydrate chain is attached to the CH2 domain. The CH2 domain herein may be a native sequence CH2 domain or variant CH2 domain. The “CH3 domain” comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (i.e., from an amino acid residue at about position 341 to an amino acid residue at about position 447 of an IgG). The CH3 region herein may be a native sequence CH3 domain or a variant CH3 domain (e.g., a CH3 domain with an introduced “protuberance” (“knob”) in one chain thereof and a corresponding introduced “cavity” (“hole”) in the other chain thereof; see U.S. Pat. No. 5,821,333, expressly incorporated herein by reference). Such variant CH3 domains may be used to promote heterodimerization of two non-identical immunoglobulin heavy chains as herein described. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, Md., 1991.

[0053] The term “effector functions” refers to those biological activities attributable to the Fc region of an immunoglobulin, which vary with the immunoglobulin isotype. Examples of immunoglobulin effector functions include: C1q binding and complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, immune complex-mediated antigen uptake by antigen presenting cells, down regulation of cell surface receptors (e.g., B cell receptor), and B cell activation.

[0054] An “activating Fc receptor” is an Fc receptor that following engagement by an Fc region of an immunoglobulin elicits signaling events that stimulate the receptor-bearing cell to perform effector functions. Activating Fc receptors include FcγRIIa (CD16a), FcγRI (CD64), FcγRIIA (CD32), and FcεRI (CD89). A particular activating Fc receptor is human FcγRIIa (see UniProt accession no. P08637 (version 141)).

[0055] The term “interleukin-2” or “IL-2” as used herein, refers to any native IL-2 from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses unprocessed IL-2 as well as any form of IL-2 that results from processing in the cell. The term also encompasses naturally occurring variants of IL-2, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human IL-2 is shown in SEQ ID NO: 1. Unprocessed human IL-2 additionally comprises an N-terminal 20 amino acid signal peptide, which is absent in the mature IL-2 molecule.

[0056] By a “native IL-2”, also termed “wild-type IL-2”, is meant a naturally occurring IL-2. The sequence of a native human IL-2 molecule is shown in SEQ ID NO: 1. For the purpose of the present invention, the term wild-type also encompasses forms of IL-2 comprising one or more amino acid mutation that does not affect IL-2 receptor binding compared to the naturally occurring, native IL-2, such as e.g. a substitution of cysteine at a position corresponding to residue 125 of human IL-2 to alanine. In some embodiments wildtype IL-2 for the purpose of the present invention comprises the amino acid substitution C125A (see SEQ ID NO: 3).

[0057] The term “CD25” or “II-2 receptor c” as used herein, refers to any native CD25 from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses “full-length”, unprocessed CD25 as well as any form of CD25 that results from processing in the cell. The term also encompasses naturally occurring variants of CD25, e.g. splice variants or allelic variants. In certain embodiments CD25 is human CD25. The amino acid...
sequence of an exemplary human CD25 (with signal sequence, Avi-tag and His-tag) is shown in SEQ ID NO: 25. The term “high-affinity IL-2 receptor” as used herein refers to the heterotrimeric form of the IL-2 receptor, consisting of the receptor γ-subunit (also known as common cytokine receptor γ-subunit, γc or CD 132), the receptor β-subunit (also known as CD 122 or p70) and the receptor α-subunit (also known as CD25 or p55). The term “intermediate-affinity IL-2 receptor” or “IL-2 receptor βγ” by contrast refers to the IL-2 receptor including only the γ-subunit and the β-subunit, without the α-subunit (for a review see e.g. Olejniczak and Kasprzak, Med Sci Monit 14, RA170-189 (2008)). The amino acid sequences of exemplary human CD122 and CD132 (fused to an Fc region with a His-tag) are shown in SEQ ID Nos 21 and 23, respectively.

By “regulatory T cell” or “Treg cell” is meant a specialized type of CD4+ T cell that can suppress the responses of other T cells. Treg cells are characterized by expression of CD4, the α-subunit of the IL-2 receptor (CD25), and the transcription factor forkhead box P3 (FOXP3) (Shakuguchi, Annu Rev Immunol 22, 531-62 (2004)) and play a critical role in the induction and maintenance of peripheral self-tolerance to antigens, including those expressed by tumors.

By “selective activation of Treg cells” is meant activation of Treg cells essentially without concomitant activation of other T cell subsets (such as CD4+ T helper cells, CD8+ cytotoxic T cells, NK T cells) or natural killer (NK) cells. Methods for identifying and distinguishing these cell types are described in the Examples. Activation may include induction of IL-2 receptor signaling (as measured e.g. by detection of phosphorylated STAT5a), induction of proliferation (as measured e.g. by detection of Ki-67) and/or up-regulation of expression of activation markers (such as e.g. CD25).

The term “peptide linker” refers to a peptide comprising one or more amino acids, typically about 2-20 amino acids. Peptide linkers are known in the art or are described herein. Suitable, non-immunogenic linker peptides include, for example, G2S7n, SGa4n, or G4(SGa)n, peptide linkers. “n” is generally a number between 1 and 10, and preferably between 2 and 4.

The term “modification” refers to any manipulation of the peptide backbone (e.g. amino acid sequence) or the post-translational modifications (e.g. glycosylation) of a polypeptide.

A “knob-into-hole modification” refers to a modification within the interface between two immunoglobulin heavy chains in the CH3 domain, wherein i) in the CH3 domain of one heavy chain, an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance (“knob”) within the interface in the CH3 domain of one heavy chain which is positionable in a cavity (“hole”) within the interface in the CH3 domain of the other heavy chain, and ii) in the CH3 domain of the other heavy chain, an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity (“hole”) within the interface in the second CH3 domain within which a protuberance (“knob”) within the interface in the first CH3 domain is positionable. In one embodiment, the “knob-into-hole modification” comprises the amino acid substitution T366W and optionally the amino acid substitution S354C in one of the antibody heavy chains, and the amino acid substitutions T366S, I368A, V407Y and optionally Y349C in the other one of the antibody heavy chains. The knob-into-hole technology is described e.g. in U.S. Pat. No. 5,731,168; U.S. Pat. No. 7,695,936; Ridgway et al., Prot Eng 9, 617-621 (1996) and Carter, J Immunol Meth 248, 7-15 (2001). Generally, the method involves introducing a protuberance (“knob”) at the interface of a first polypeptide and a corresponding cavity (“hole”) in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). Introduction of two cysteine residues at position S354 and Y349, respectively, results in formation of a disulfide bridge between the two antibody heavy chains in the Fc region, further stabilizing the dimer (Carter, J Immunol Meth 248, 7-15 (2001)).

An amino acid “substitution” refers to the replacement in a polypeptide of one amino acid with another amino acid. In one embodiment, an amino acid is replaced with another amino acid having similar structural and/or chemical properties, e.g. conservative amino acid replacements. “Conservative” amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophlicity, and/or the amphiphatic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Non-conservative substitutions will entail exchanging a member of one of these classes for another class. For example, amino acid substitutions can also result in replacing one amino acid with another amino acid having different structural and/or chemical properties, for example, replacing an amino acid from one group (e.g., polar) with another amino acid from a different group (e.g. basic). Amino acid substitutions can be generated using genetic or chemical methods well known in the art. Genetic methods may include site-directed mutagenesis, PCR, gene synthesis and the like. It is contemplated that methods of altering the side chain group of an amino acid by methods other than genetic engineering, such as chemical modification, may also be useful. Various designations may be used herein to indicate the same amino acid substitution. For example, a substitution from proline at position 329 of the immunoglobulin heavy chain to glycine can be indicated as 329G, G329, G329P, P329G, or Pro329Gly.

“Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software
such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, Calif., or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary. In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$\text{X} = \frac{\text{XY}}{100}$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program’s alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

0066] “Polynucleotide” or “nucleic acid” as used interchangeably herein, refers to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. A sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may comprise modification(s) made after synthesis, such as conjugation to a label.

0067] By a nucleic acid or polynucleotide having a nucleotide sequence at least, for example, 95% “identical” to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the 5’ or 3’ terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. As a practical matter, whether any particular polynucleotide sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs, such as the ones discussed above for polypeptides (e.g., ALIGN-2).

0068] The term “vector” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors”.

0069] The terms “host cell”, “host cell line”, and “host cell culture” are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein. A host cell is any type of cellular system that can be used to generate the fusion proteins of the present invention. Host cells include cultured cells, e.g., mammalian cultured cells, such as CHO cells, BHK cells, N30 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue.

0070] An “effective amount” of an agent refers to the amount that is necessary to result in a physiological change in the cell or tissue to which it is administered.

0071] A “therapeutically effective amount” of an agent, e.g., a pharmaceutical composition, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A therapeutically effective amount of an agent for example eliminates, decreases, delays, minimizes or prevents adverse effects of a disease.

0072] An “individual” or “subject” is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). Particularly, the individual or subject is a human.

0073] The term “pharmaceutical composition” refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unconscionably toxic to a subject to which the formulation would be administered.

0074] A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical composition, other than an active ingredient, which is nontoxic to a subject. A pharma-
A ceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative. 0075. As herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to clinical intervention in an attempt to alter the natural course of a disease in the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

0076. “Autoimmune disease” refers to a non-malignant disease or disorder arising from and directed against an individual’s own tissues. Examples of autoimmune diseases or disorders include, but are not limited to, inflammatory responses such as inflammatory skin diseases including psoriasis and dermatitis (e.g. atopic dermatitis); responses associated with inflammatory bowel disease (such as Crohn’s disease and ulcerative colitis); dermatitis; allergic conditions such as eczema and asthma; rheumatoid arthritis; systemic lupus erythematosus (SLE) (including but not limited to lupus nephritis, cutaneous lupus; diabetes mellitus (e.g. type 1 diabetes mellitus or insulin dependent diabetes mellitus); multiple sclerosis and juvenile onset diabetes.

Fusion Proteins of the Invention

0077. The invention provides novel immunoglobulin-IL-2 fusion proteins with particularly advantageous properties for the use in therapeutic methods as described herein.

0078. In a first aspect, the invention provides a fusion protein comprising (i) an immunoglobulin molecule comprising a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification, and (ii) two interleukin-2 (IL-2) molecules.

0079. In one embodiment, said fusion protein essentially consists of an immunoglobulin molecule comprising a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification, two interleukin-2 (IL-2) molecules, and optionally one or more peptide linker.

0080. As shown in the Examples, a fusion protein comprising two IL-2 molecules surprisingly provides for greatly improved efficacy and selectivity in the activation of regulatory T cells, as compared to a corresponding fusion protein comprising a single IL-2 molecule.

0081. In one embodiment, said immunoglobulin molecule is an IgG-class immunoglobulin molecule, particularly an IgG1 subclass immunoglobulin molecule. In one embodiment, said immunoglobulin molecule is a human immunoglobulin molecule, i.e. it comprises fully human variable and constant regions. The sequence of an exemplary human IgG1 constant region is shown in SEQ ID NO: 8. An IgG-class immunoglobulin molecule comprises (i) two immunoglobulin light chains, each comprising from N- to C-terminus a light chain variable domain (VL) and a light chain constant domain (CL), and (ii) two immunoglobulin heavy chains, each comprising from N-terminus to C-terminus a heavy chain variable domain (VH), a heavy chain constant domain (CH) 1, an immunoglobulin hinge region, a CH2 domain and a CH3 domain. The latter two domains form part of the Fc region of the immunoglobulin molecule. The two heavy chains dimerize in the Fc region.

0082. In one embodiment of the fusion protein according to the invention, said two IL-2 molecules are each fused at their N-terminal amino acid to the C-terminal amino acid of one of the immunoglobulin heavy chains of said immunoglobulin molecule, optionally through a peptide linker. Fusion of two (identical) IL-2 molecules to the immunoglobulin heavy chains allows for simple production of the fusion protein, avoiding the formation of undesired side products and obviating the need for modifications promoting heterodimerization of non-identical heavy chains, such as a knob-into-hole modification.

0083. Fusion of the IL-2 molecules to an immunoglobulin molecule provides for favorable pharmacokinetic properties, including a long serum half-life (due to recycling through binding to FcRn, and molecular size being well above the threshold for renal filtration), as compared to free (unfused) IL-2. Furthermore, the presence of an immunoglobulin molecule also enables simple purification of fusion proteins by e.g. protein A affinity chromatography. At the same time, however, the presence of an immunoglobulin molecule, specifically the Fc region of an immunoglobulin molecule, may lead to undesired targeting of the fusion protein to cells expressing Fc receptors rather than to the preferred IL-2 receptor bearing cells. Moreover, the engagement of Fc receptors may lead to release of (pro-inflammatory) cytokines and undesired activation of various immune cells other than regulatory T cells. Therefore, said immunoglobulin molecule comprised in the fusion protein of the invention comprises a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor, as compared to a corresponding immunoglobulin molecule without said modification. In a specific embodiment, said Fc receptor is an Fcy receptor, particularly a human Fcy receptor. Binding affinity to Fc receptors can be easily determined e.g. by ELISA, or by Surface Plasmon Resonance (SPR) using standard instrumentation such as a BIACore instrument (GE Healthcare) and Fe receptors such as may be obtained by recombinant expression. A specific illustrative and exemplary embodiment for measuring binding affinity is described in the following. According to one embodiment, Binding affinity to an Fc receptor is measured by surface plasmon resonance using a BIACORE® T100 machine (GE Healthcare) at 25°C with ligand (Fc receptor) immobilized on CM5 chips. Briefly, carboxymethylated dextran biosensor chips (CM5, GE Healthcare) are activated with N-ethyl-N-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier’s instructions. Recombinant ligand is diluted with 10 mM sodium acetate, pH 5.5, to 0.5-30 μg/ml before injection at a flow rate of 10 μl/min to achieve approximately 100-5000 response units (RU) of coupled protein. Following the injection of the ligand, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, three- to five-fold serial dilutions of antibody (range between 0.01 nM to 300 nM) are injected in HBS-EP+ (GE Healthcare, 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% Surfactant P20, pH 7.4) at 25°C at a flow rate of approximately 30-50 μl/min. Association rates (k_a) and dissociation rates (k_d) are calculated using a simple one-to-one Langmuir binding model (BIA-
In one embodiment, the modification comprises one or more amino acid mutations that reduce the binding affinity of the immunoglobulin to an Fc receptor. In one embodiment, the amino acid mutation is an amino acid substitution. Typically, the same or a more amino acid mutation is present in each of the two immunoglobulin heavy chains. In one embodiment said amino acid mutation reduces the binding affinity of the immunoglobulin to the Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold. In embodiments where there is more than one amino acid mutation that reduces the binding affinity of the immunoglobulin to the Fc receptor, the combination of these amino acid mutations may reduce the binding affinity of the immunoglobulin to the Fc receptor by at least 10-fold, at least 20-fold, or even at least 50-fold. In one embodiment said immunoglobulin molecule exhibits less than 20%, particularly less than 10%, more particularly less than 5% of the binding affinity to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification.

In another embodiment, said Fc receptor is FcyRIIa, particularly human FcyRIIa. In some embodiments binding affinity to a complement component, specifically binding affinity to C1q, is also reduced. In one embodiment binding affinity to neonatal Fc receptor (FcRn) is not reduced. Substantially similar binding to FcRn, i.e., preservation of the binding affinity of the immunoglobulin molecule to said receptor, is achieved when the immunoglobulin molecule exhibits greater than about 70% of the binding affinity of an unmodified form of the immunoglobulin molecule to FcRn. Immunoglobulin molecules comprised in the fusion proteins of the invention may exhibit greater than about 80% and even greater than about 90% of such affinity.

In one embodiment, modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor is in the Fc region, particularly the CH2 region, of the immunoglobulin molecule. In one embodiment, said immunoglobulin molecule comprises an amino acid substitution at position 329 (EU numbering) of the immunoglobulin heavy chains. In a more specific embodiment said amino acid substitution is P329A or P329G, particularly P329G. In one embodiment, said immunoglobulin molecule comprises amino acid substitutions at positions 234 and 235 (EU numbering) of the immunoglobulin heavy chains. In a specific embodiment, said amino acid substitutions are L234A and L235A (L.A.A). In one embodiment said immunoglobulin molecule comprises an amino acid substitution at position 329 (EU numbering) of the antibody heavy chains and a further amino acid substitution at a position selected from position 228, 233, 234, 235, 297 and 331 of the immunoglobulin heavy chains. In a more specific embodiment the further amino acid substitution is S228P, E233P, L234A, L235E, N297A, N297D or P331S. In a particular embodiment, said immunoglobulin molecule comprises amino acid substitutions at positions P329, L234 and L235 (EU numbering) of the immunoglobulin heavy chains. In a more particular embodiment, said immunoglobulin molecule comprises the amino acid substitutions L234A, L235A and P329G (L.A.A P329G) in the immunoglobulin heavy chains. This combination of amino acid substitutions almost completely abolishes Fc receptor binding of a human IgG-class immunoglobulin, as described in PCT patent application no. PCT/EP2012/055393, incorporated herein by reference in its entirety. PCT patent application no. PCT/EP2012/055393 also describes methods of preparing such modified immunoglobulin and methods for determining its properties such as Fc receptor binding or effector functions.

Immunoglobulins comprising modifications in the immunoglobulin heavy chains can be prepared by amino acid deletion, substitution, insertion or modification using genetic or chemical methods well known in the art. Genetic methods may include site-specific mutagenesis of the encoding DNA sequence, PCR, gene synthesis, and the like. The correct nucleotide changes can be verified for example by sequencing.

Immunoglobulins or antibodies which comprise modifications reducing Fc receptor binding generally have reduced effector functions, particularly reduced ADCC, as compared to corresponding unmodified immunoglobulins or antibodies. Hence, in one embodiment, said modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor reduces effector function of the immunoglobulin molecule. In a specific embodiment, said effector function is antibody-dependent cell-mediated cytotoxicity (ADCC). In one embodiment, ADCC is reduced to less than 20% of the ADCC induced by a corresponding immunoglobulin molecule without said modification. Effector function of an immunoglobulin or antibody can be measured by methods known in the art. Examples of in vitro assays to assess ADCC activity of a molecule of interest are described in U.S. Pat. No. 5,500,362; Hellstrom et al. Proc Natl Acad Sci USA 83, 7059-7063 (1986) and Hellstrom et al., Proc Natl Acad Sci USA 82, 1499-1502 (1985); U.S. Pat. No. 5,821,337; Bruggemann et al., J Exp Med 166, 1351-1361 (1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.); and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g. in an animal model such as that disclosed in Clynes et al., Proc Natl Acad Sci USA 95, 652-656 (1998). In some embodiments binding of the immunoglobulin molecule to a complement component, specifically to C1q, is also reduced. Accordingly, complement-dependent cytotoxicity (CDC) may also be reduced. C1q binding assays may be carried out to determine whether the immunoglobulin is able to bind C1q and hence has CDC activity. See e.g. C1q and C3b binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J Immunol Methods
202, 163 (1996); Cragg et al., Blood 101, 1045-1052 (2003); and Cragg and Glennie, Blood 103, 2738-2743 (2004)).

[0089] In addition to the immunoglobulin molecules described hereinabove and in PCT patent application no. PCT/EP2012/055393, immunoglobulins with reduced Fc receptor binding and/or effector function also include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056).

Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called “DANA” Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

[0090] IgG2 subclass immunoglobulins exhibit reduced binding affinity to Fc receptors and reduced effector functions as compared to IgG1 immunoglobulins. Hence, in some embodiments, said immunoglobulin molecule comprised in the fusion protein of the invention is an IgG2 subclass immunoglobulin, particularly a human IgG2 subclass immunoglobulin. In one embodiment said IgG2 subclass immunoglobulin comprises amino acid substitutions in the Fc region at position S228, specifically the amino acid substitution S228P. To further reduce its binding affinity to Fc receptor and/or its effector function, in one embodiment, said IgG2 subclass immunoglobulin comprises an amino acid substitution at position L235, specifically the amino acid substitution L235F. In another embodiment, said IgG2 subclass immunoglobulin comprises an amino acid substitution at position P329, specifically the amino acid substitution P329G. In a particular embodiment, said IgG2 subclass immunoglobulin comprises amino acid substitutions at positions S228, L235 and P329, specifically amino acid substitutions S228P, L235F and P329G. Such modified IgG2 subclass immunoglobulins and their Fc receptor binding properties are described in PCT patent application no. PCT/EP2012/055393, incorporated herein by reference in its entirety.

[0091] In one embodiment, said immunoglobulin molecule is capable of specific binding to an antigen. In one embodiment, said immunoglobulin molecule is a monoclonal antibody. In one embodiment, said immunoglobulin molecule is not capable of specific binding to an antigen, particularly not capable of specific binding to a human antigen. The absence of specific binding of such an immunoglobulin molecule to an antigen (i.e. the absence of any binding that can be discriminated from non-specific interaction) can be determined e.g. by ELISA or surface plasmon resonance as described herein. Such an immunoglobulin molecule is particularly useful e.g. for enhancing the serum half-life of the fusion protein, where targeting to a particular tissue is not desired.

[0092] In one embodiment, said immunoglobulin molecule comprises a heavy chain variable region sequence based on the human Vh3-23 germline sequence. In a specific embodiment, said immunoglobulin molecule comprises a heavy chain variable region sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 9. In one embodiment, said immunoglobulin molecule comprises a light chain variable region sequence based on the human Vk3-20 germline sequence. In a specific embodiment, said immunoglobulin molecule comprises a light chain variable region sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 11. In an even more specific embodiment, said immunoglobulin molecule comprises the heavy chain variable region sequence of SEQ ID NO: 9 and the light chain variable region sequence of SEQ ID NO: 11. Immunoglobulin molecules comprising these variable region sequences are not capable of specific binding to an antigen, particularly a human antigen. They lack binding to normal tissues as well as PBMCs, have no polyreactivity and show no non-specific accumulation in vivo by imaging (data not shown). The variable region sequences are entirely based on human germline sequences, with the exception of the heavy chain CDR3 wherein a GSG sequence has been introduced to generate a non-binding immunoglobulin.

[0093] In one embodiment, said IL-2 molecules are wild-type IL-2 molecules. In one embodiment, said IL-2 molecules are human IL-2 molecules. In a specific embodiment, said IL-2 molecules comprise the sequence of SEQ ID NO: 1 (native human IL-2).

[0094] In one embodiment, said IL-2 molecule comprises an amino acid substitution at a position corresponding to residue 125 of human IL-2. In one embodiment said amino acid substitution is C125A. In a specific embodiment, said IL-2 molecule comprises the sequence of SEQ ID NO: 3 (human IL-2 with the amino acid substitution C125A). Alternatively, the cysteine at position 125 may be replaced with another neutral amino acid such as serine, threonine or valine, yielding C125S IL-2, C125T IL-2 or C125V IL-2 respectively, as described in U.S. Pat. No. 4,518,584. As described therein, one may also delete the N-terminal alanine residue of IL-2 yielding such mutants as des-A1 C125S or des-A1 C125A. Alternatively or conjunctively, the IL-2 molecule may include a modification whereby methionine normally occurring at position 104 of wild-type human IL-2 is replaced by a neutral amino acid such as alanine (see U.S. Pat. No. 5,206,344). Such modifications in human IL-2 may provide additional advantages such as increased expression or stability.

[0095] The IL-2 molecules comprised in the fusion protein of the invention may also be unglycosylated IL-2 molecules. For example, elimination of the O-glycosylation site of the IL-2 molecule results in a more homogeneous product when the fusion protein is expressed in mammalian cells such as CHO or HEK cells. Thus, in certain embodiments the IL-2 molecule comprises a modification which eliminates the O-glycosylation site of IL-2 at a position corresponding to residue 3 of human IL-2. In one embodiment said modification which eliminates the O-glycosylation site of IL-2 at a position corresponding to residue 3 of human IL-2 is an amino acid substitution. Exemplary amino acid substitutions include T3A, T3G, T3Q, T3E, T3N, T3D, T3R, T3K, and T3P. In a specific embodiment, said modification is the amino acid substitution T3A.

[0096] In one embodiment, the fusion protein is capable of binding to IL-2 receptor with an affinity constant (Kd) of smaller than 10 nM, particularly smaller than 3 nM, when measured by SPR at 25°C. In a specific embodiment, said IL-2 receptor is human IL-2 receptor. In one embodiment, the fusion protein is capable of binding to IL-2 receptor with an affinity constant (Kd) of smaller than 100 nM, particularly smaller than 20 nM, when measured by SPR at 25°C. In a specific embodiment, said IL-2 receptor is human IL-2 receptor. A method for measuring binding affinity to IL-2 receptor or IL-2 receptor is described herein. According to one embodiment, binding affinity (Kd) is measured by surface plasmon resonance using a BLAcore T100 machine (GE Healthcare) at 25°C. With IL-2 receptors immobilized on CM5 chips. Briefly, carboxymethylated dextran biosensor chips (CM5, GE Healthcare) are activated with N-ethyl-N’(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide
(NHS) according to the supplier’s instructions. Recombinant IL-2 receptor is diluted with 10 mM sodium acetate, pH 5.5, to 0.5-30 μg/mL before injection at a flow rate of 10 μL/minute to achieve approximately 200-1000 (for IL-2R α) or 500-3000 (for IL-2R βγ heterodimer) response units (RU) of coupled protein. Following the injection of IL-2 receptor, 1 M ethanolamine is injected to block unreacted groups. For kinetic measurements, three-fold serial dilutions of fusion protein (range between ~3 nM to 300 nM) are injected in HBS-EP+ (GE Healthcare, 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% Surfactant P20, pH 7.4) at 25 °C. At a flow rate of approximately 30 μL/minute. Association rates (kₐ) and dissociation rates (kₐ) are calculated using a simple one-to-one Langmuir binding model (BIACORE® T100 Evaluation Software version 1.1.1) by simultaneously fitting the association and dissociation sensograms. The equilibrium dissociation constant (Kₐ) is calculated as the ratio kₐ/kₐ. See, e.g., Chen et al., J Mol Biol 293, 865-881 (1999).

[0097] In a particular aspect, the invention provides a fusion protein comprising (i) an IgG₁ subclass immunoglobulin molecule comprising the amino acid substitutions L234A, L235A and P329G (EU numbering) in the immunoglobulin heavy chains, and (ii) two interleukin-2 (IL-2) molecules, each fused at its N-terminal amino acid to the C-terminal amino acid of one of the immunoglobulin heavy chains through a peptide linker. In a specific embodiment, said immunoglobulin molecule comprises the heavy chain variable region sequence of SEQ ID NO: 11 and the light chain variable region sequence of SEQ ID NO: 10. In a further specific embodiment, said IL-2 molecules each comprise the amino acid sequence of SEQ ID NO: 3. In an even more specific embodiment, said fusion protein comprises a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 17, and a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 19.

[0098] As shown in the Examples, the fusion protein of the invention can be used to selectively activate regulatory T cells (i.e. essentially without concomitant activation of other T cell subsets and/or NK cells). Thus, the invention particularly provides the fusion protein for use in selective activation of regulatory T cells in vitro or in vivo. In one embodiment, said use comprises contacting regulatory T cells with said fusion protein in vitro or in vivo. In one embodiment, said use further comprises contacting other (non-regulatory) T cells with said fusion protein. In one embodiment, said use is in vitro and said fusion protein is used at a concentration of about 1 mg/mL or less, particularly about 0.1 mg/mL or less. In another embodiment, said use is in vitro and said fusion protein is used at a concentration of about 1 mg/mL or less, particularly about 0.1 mg/mL or less. In another embodiment, said method is in vivo and said fusion protein is used at a dose of about 20 μg/kg body weight or less, particularly about 12 μg/kg body weight or less (wherein “body weight” refers to the body weight of the individual to whom the fusion protein is administered).

[0099] The invention also provides a method for selective activation of regulatory T cells in vitro or in vivo, comprising contacting said regulatory T cells with the fusion protein of the invention. In one embodiment, said method further comprises contacting other (non-regulatory) T cells with said fusion protein. In one embodiment, said activation comprises induction of proliferation and/or induction of IL-2 receptor signaling. In one embodiment, said method is in vitro and said fusion protein is used at a concentration of about 1 ng/mL or less, particularly about 0.1 ng/mL or less. In another embodiment, said method is in vivo and said fusion protein is used at a dose of about 20 μg/kg body weight or less, particularly about 12 μg/kg body weight or less (wherein “body weight” refers to the body weight of the individual to whom the fusion protein is administered).

[0100] According to certain embodiments of the use or method described in the preceding paragraphs, said activation comprises induction of proliferation and/or induction of IL-2 receptor signaling. Induction of proliferation can be measured e.g. by detection of the intracellular proliferation marker Ki-67, as described in the Examples. In one embodiment, proliferation of regulatory T cells activated by the fusion protein of the invention is increased at least about 1.5-fold, at least about 2-fold, or at least about 3-fold, as compared to proliferation of non-activated regulatory T cells.

In one embodiment, proliferation of other (non-regulatory) T cells and/or NK cells contacted with the fusion protein of the invention is increased less than about 1.5 fold, less than about 1.2 fold, or less than about 1.1 fold, as compared to proliferation of corresponding cells not contacted with said fusion protein. Induction of IL-2 receptor signaling can be measured e.g. by detection of phosphorylated STAT5, as described in the Examples. In one embodiment, IL-2 receptor signaling in regulatory T cells activated by the fusion protein of the invention is increased at least about 1.5-fold, at least about 2-fold, at least about 3-fold, or at least about 5-fold, as compared to IL-2 receptor signaling in non-activated regulatory T cells.

In one embodiment, IL-2 receptor signaling in other (non-regulatory) T cells and/or NK cells contacted with the fusion protein or the invention is increased less than about 1.5 fold, or less than about 1.2 fold, or less than about 1.1 fold, as compared to IL-2 receptor signaling in corresponding cells not contacted with said fusion protein.

Polynucleotides

[0101] The invention further provides polynucleotides encoding a fusion as described herein or a fragment thereof.

[0102] Polynucleotides of the invention include those that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100% identical to the sequences set forth in SEQ ID Nos. 2, 4, 5, 6, 7, 10, 12, 18 and 20 including functional fragments or variants thereof.

[0103] The polynucleotides encoding fusion proteins of the invention may be expressed as a single polynucleotide that encodes the entire fusion protein or as multiple (e.g., two or more) polynucleotides that are co-expressed. Polypeptides encoded by polynucleotides that are co-expressed may associate through, e.g., disulfide bonds or other means to form a functional fusion protein. For example, the light chain portion of an immunoglobulin may be encoded by a separate polynucleotide from the heavy chain portion of the immunoglobulin. When co-expressed, the heavy chain polypeptides will associate with the light chain polypeptides to form the immunoglobulin.

[0104] In one embodiment, the present invention is directed to a polynucleotide encoding a fusion protein of an immunoglobulin molecule and two IL-2 molecules, or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable region sequence as shown in SEQ ID NO 9 or 11. In another embodiment, the present invention is directed to a polynucleotide encoding a fusion protein of an immunoglobulin molecule and two IL-2 molecules, or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes a polypeptide sequence as shown in
SEQ ID NO 17 or 19. In another embodiment, the invention is further directed to a polynucleotide encoding a fusion protein of an immunoglobulin molecule and two IL-2 molecules, or a fragment thereof, wherein the polynucleotide comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a nucleic acid sequence shown SEQ ID NO 2, 4, 5, 6, 7, 10, 12, 18, or 20. In another embodiment, the invention is directed to a polynucleotide encoding a fusion protein of an immunoglobulin molecule and two IL-2 molecules, or a fragment thereof, wherein the polynucleotide comprises a nucleic acid sequence shown in SEQ ID NO 2, 4, 5, 6, 7, 10, 12, 18 or 20. In another embodiment, the invention is directed to a polynucleotide encoding a fusion protein of an immunoglobulin molecule and two IL-2 molecules, or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO 9 or 11. In another embodiment, the invention is directed to a polynucleotide encoding a fusion protein of an immunoglobulin molecule and two IL-2 molecules, or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes a polypeptide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO 17 or 19. The invention encompasses a polynucleotide encoding a fusion protein of an immunoglobulin molecule and two IL-2 molecules, or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes the polypeptide sequences of SEQ ID NO 17 or 19 with conservative amino acid substitutions. The invention also encompasses a polynucleotide encoding a fusion protein of an immunoglobulin molecule and two IL-2 molecules, or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes the polypeptide sequences of SEQ ID NO 17 or 19 with conservative amino acid substitutions.

[0105] In certain embodiments the polynucleotide or nucleic acid is DNA. In other embodiments, a polynucleotide of the present invention is RNA, for example, in the form of messenger RNA (mRNA). mRNA of the present invention may be single stranded or double stranded.

Recombinant Methods

[0106] Fusion proteins of the invention may be obtained, for example, by solid-phase peptide synthesis (e.g. Merrifield solid phase synthesis) or recombinant production. For recombinant production one or more polynucleotide encoding the fusion protein (fragment), e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such polynucleotide may be readily isolated and sequenced using conventional procedures. In one embodiment a vector, preferably an expression vector, comprising one or more of the polynucleotides of the invention is provided. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequence of the fusion protein (fragment) along with appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombinant genetic recombination. See, for example, the techniques described in Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. (1989); and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y (1989). The expression vector can be part of a plasmid, virus, or may be a nucleic acid fragment. The expression vector includes an expression cassette into which the polynucleotide encoding the fusion protein (fragment) (i.e. the coding region) is cloned in operable association with a promoter and/or other transcription or translation control elements. As used herein, a “coding region” is a portion of nucleic acid which consists of codons translated into amino acids. Although a “stop codon” (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, if present, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, 5′ and 3′ untranslated regions, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, e.g. on a single vector, or in separate polynucleotide constructs, e.g. on separate (different) vectors. Furthermore, any vector may contain a single coding region, or may comprise two or more coding regions, e.g. a vector of the present invention may encode one or more polypeptides, which are post- or co-translationally separated into the final proteins via proteolytic cleavage. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions, either fused or unfused to a polynucleotide encoding the fusion protein (fragment) of the invention, or variant or derivative thereof. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain. An operable association is when a coding region for a gene product, e.g. a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are “operably associated” if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription. Suitable promoters and other transcription control regions are disclosed herein. A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions, which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (e.g. the immediate early promoter, in conjunction with intron-A), simian virus 40 (e.g. the early promoter), and retroviruses such as, e.g. Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit a-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as inducible promoters (e.g. promoters
inducible tetracyclins). Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from viral systems (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence). The expression cassette may also include other features such as an origin of replication, and/or chromosome integration elements such as retroviral long terminal repeats (LTRs), or adeno-associated viral (AAV) inverted terminal repeats (ITRs).

[0107] Polynucleotide and nucleic acid coding regions of the present invention may be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention. For example, if secretion of the fusion is desired, DNA encoding a signal sequence may be placed upstream of the nucleic acid encoding a fusion protein of the invention or a fragment thereof. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the translated polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, e.g., an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, may be used. For example, the wild-type leader sequence may be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse β-glucuronidase. The amino acid and nucleotide sequences of exemplary secretory signal peptides are shown in SEQ ID NOS 39-47.

[0108] DNA encoding a short protein sequence that could be used to facilitate later purification (e.g. a histidine tag) or assist in labeling the fusion protein may be included within or at the ends of the fusion protein (fragment) encoding polynucleotide.

[0109] In a further embodiment, a host cell comprising one or more polynucleotides of the invention is provided. In certain embodiments a host cell comprising one or more vectors of the invention is provided. The polynucleotides and vectors may incorporate any of the features, singly or in combination, described herein in relation to polynucleotides and vectors, respectively. In one such embodiment a host cell comprises (e.g. has been transformed or transfected with) a vector comprising a polynucleotide that encodes (part of) a fusion protein of the invention. As used herein, the term "host cell" refers to any kind of cellular system which can be engineered to generate the fusion proteins of the invention or fragments thereof. Host cells suitable for replicating and for supporting expression of fusion proteins are well known in the art. Such cells may be transfected or transduced as appropriate with the particular expression vector and large quantities of vector containing cells can be grown for feeding large scale fermenters to obtain sufficient quantities of the fusion protein for clinical applications. Suitable host cells include prokaryotic microorganisms, such as E. coli, or various eukaryotic cells, such as Chinese hamster ovary cells (CHO), insect cells, or the like. For example, polypeptides may be produced in bacteria in particular when glycosylation is not needed. After expression, the polypeptide may be isolated from the bacterial cell paste in a soluble fraction and can be further purified. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for polypeptide-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized", resulting in the production of a polypeptide with a partially or fully human glycosylation pattern. See Gerngross, Nat Biotech 22, 1409-1414 (2004), and Li et al., Nat Biotech 24, 210-215 (2006). Suitable host cells for the expression of (glycosylated) polypeptides are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of Spodoptera frugiperda cells. Plant cell cultures can also be utilized as hosts. See e.g. U.S. Pat. Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTABODIES™ technology for producing antibodies in transgenic plants). Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line 293 or 293T cells as described, e.g., in Graham et al., J Gen Virol 36, 59 (1977)), baby hamster kidney cells (BHK), mouse sertoli cells (TM4 cells as described, e.g., in Mather, Biol Reprod 23, 243-251 (1980), monkey kidney cells (CV1), African green monkey kidney cells (VERO-76), human cervical carcinoma cells (HFLA), canine kidney cells (MDCK), buffalo rat liver cells (BRK 3A), human lung cells (W138), human liver cells (Hep G2), mouse mammary tumor cells (MMS 060562), TR1 cells (as described, e.g., in Mather et al., Annals N.Y. Acad Sci 383, 44-68 (1982)), MRC 5 cells, and F54 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including dlf M, CHO cells (Urlaub et al., Proc Natl Acad Sci USA 77, 4216 (1980)); and myeloma cell lines such as YO, N50, P3X63 and Sp2/0. For a review of certain mammalian host cell lines suitable for protein production, see, e.g., Yazuki and Wu, Methods in Molecular Biology, Vol. 248 (B. K. C. Lo, ed., Humana Press, Totowa, N.J.), pp. 255-268 (2003). Host cells include cultured cells, e.g., mammalian cultured cells, yeast cells, insect cells, bacterial cells and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue. In one embodiment, the host cell is a eukaryotic cell, preferably a mammalian cell, such as a Chinese Hamster Ovary (CHO) cell, a human embryonic kidney (HEK) cell or a lymphoid cell (e.g., YO, N50, Sp2/0 cell).

[0110] Standard technologies are known in the art to express foreign genes in these systems. Cells expressing a polypeptide comprising either the heavy or the light chain of an immunoglobulin, may be engineered so as to also express the other of the immunoglobulin chains such that the expressed product is an immunoglobulin that has both a heavy and a light chain.

[0111] In one embodiment, a method of producing a fusion protein according to the invention is provided, wherein the method comprises culturing a host cell comprising a polynucleotide encoding the fusion protein, as provided herein,
under conditions suitable for expression of the fusion protein, and recovering the fusion protein from the host cell (or host cell culture medium).

[0112] In the fusion proteins of the invention, the components (immunoglobulin molecule and IL-2 molecule) are genetically fused to each other. Fusion proteins can be designed such that its components are fused directly to each other or indirectly through a linker sequence. The composition and length of the linker may be determined in accordance with methods well known in the art and may be tested for efficacy. Additional sequences may also be included to incorporate a cleavage site to separate the individual components of the fusion protein if desired, for example an endopeptidase recognition sequence.

[0113] In certain embodiments the fusion proteins of the invention comprise at least one immunoglobulin variable region capable of binding to an antigen. Variable regions can form part of and be derived from naturally or non-naturally occurring antibodies and fragments thereof. Methods to produce polyclonal antibodies and monoclonal antibodies are well known in the art (see e.g. Harlow and Lane, “Antibodies, a laboratory manual”, Cold Spring Harbor Laboratory, 1988). Non-naturally occurring antibodies can be constructed using solid phase-peptide synthesis, can be produced recombinantly (e.g. as described in U.S. Pat. No. 4,186,567) or can be obtained, for example, by screening combinatorial libraries comprising variable heavy chains and variable light chains (see e.g. U.S. Pat. No. 5,969,108 to McCafferty).

[0114] Any animal species of immunoglobulin can be used in the invention. Non-limiting immunoglobulins useful in the present invention can be of murine, primate, or human origin. If the fusion protein is intended for human use, a chimeric form of immunoglobulin may be used wherein the constant regions of the immunoglobulin are from a human. A humanized or fully human form of the immunoglobulin can also be prepared in accordance with methods well known in the art (see e.g. U.S. Pat. No. 5,565,332 to Winter). Humanization may be achieved by various methods including, but not limited to (a) grafting the non-human (e.g., donor antibody) CDRs onto human (e.g. recipient antibody) framework and constant regions with or without retention of critical framework residues (e.g. those that are important for retaining good antigen binding affinity or antibody functions), (b) grafting only the non-human specificity-determining regions (SDRs or α-CDRs; the residues critical for the antibody-antigen interaction) onto human framework and constant regions, or (c) transplanting the entire non-human variable domains, but “cloaking” them with a human-like section by replacement of surface residues. Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, Front Biosci 13, 1619-1633 (2008), and are further described, e.g., in Riechmann et al., Nature 323, 323-329 (1986); Queen et al., Proc Natl Acad Sci USA 86, 10029-10033 (1989); U.S. Pat. Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Jones et al., Nature 321, 522-525 (1986); Morrison et al., Proc Natl Acad Sci 81, 6851-6855 (1984); Morrison and Oi, Adv Immunol 44, 65-92 (1988); Verhoeyen et al., Science 239, 1534-1536 (1988); Padlan, Mol Immunol 31(3), 169-217 (1994); Kashmiri et al., Methods 36, 25-34 (2005) (describing SOR (α-CDR) grafting); Padlan, Mol Immunol 28, 489-498 (1991) (describing “resurfacing”); Dal’Acqua et al., Methods 36, 43-60 (2005) (describing “FR shuffling”); and Osbourn et al., Methods 36, 61-68 (2005) and Klimka et al., Br J Cancer 83, 252-260 (2000) (describing the “guided selection” approach to FR shuffling). Particular immunoglobulins according to the invention are human immunoglobulins. Human antibodies and human variable regions can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, Curr Opin Pharmacol 5, 368-74 (2001) and Lonberg, Curr Opin Immunol 20, 450-459 (2008). Human variable regions can form part of and be derived from human monoclonal antibodies made by the hybridoma method (see e.g. Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)). Human antibodies and human variable regions may also be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge (see e.g. Lonberg, Nat Biotech 23, 1117-1125 (2005)). Human antibodies and human variable regions may also be generated by isolating Fv clone variable region sequences selected from human-derived phage display libraries (see e.g., Hoogenboom et al. in Methods in Molecular Biology 178, 1-37 (O’Brien et al., ed., Human Press, Totowa, N.J., 2001); and McCafferty et al., Nature 348, 552-554; Clackson et al., Nature 352, 624-628 (1991)). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments.

[0115] In certain embodiments, the immunoglobulins comprised in the fusion proteins of the present invention are engineered to have enhanced binding affinity according to, for example, the methods disclosed in PCT publication WO 2012/020006 (see Examples relating to affinity maturation) or U.S. Pat. Appl. Publ. No. 2004/0132066, the entire contents of which are hereby incorporated by reference. The ability of the fusion proteins of the invention to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance technique (Liljeblad et al., Glyco J 17, 323-329 (2000)), and traditional binding assays (Hseeley, Endocr Rev 28, 217-229 (2002)). Competition assays may be used to identify an antibody that competes with a reference antibody for binding to a particular antigen. In certain embodiments, such a competing antibody binds to the same epitope (e.g. a linear or a conformational epitope) that is bound by the reference antibody. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) “Epitope Mapping Protocols”, in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, N.J.). In an exemplary competition assay, immobilized antigen is incubated in a solution comprising a first labeled antibody that binds to the antigen and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to the antigen. The second antibody may be present in a hybridoma supernatant. As a control, immobilized antigen is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to the antigen, excess unbound antibody is removed, and the amount of label associated with immobilized antigen is measured. If the amount of label associated with immobilized antigen is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to the antigen. See Harlow and Lane
Fusion proteins prepared as described herein may be purified by art-known techniques such as high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, affinity chromatography, size exclusion chromatography, and the like. The actual conditions used to purify a particular protein will depend, in part, on factors such as net charge, hydrophobicity, hydrophilicity etc., and will be apparent to those skilled in the art. For affinity chromatography purification an antibody, ligand, receptor or antigen can be used to which the fusion protein binds. For example, for affinity chromatography purification of fusion proteins of the invention, a matrix with protein A or protein G may be used. Sequential Protein A or G affinity chromatography and size exclusion chromatography can be used to isolate a fusion protein essentially as described in the Examples. The purity of the fusion protein can be determined by any of a variety of well-known analytical methods including gel electrophoresis, high pressure liquid chromatography, and the like. For example, the fusion proteins expressed as described in the Examples were shown to be intact and properly assembled as demonstrated by reducing and non-reducing SDS-PAGE (see e.g., FIG. 2).

Compositions, Formulations, and Routes of Administration

In a further aspect, the invention provides pharmaceutical compositions comprising any of the fusion proteins provided herein, e.g., for use in any of the below therapeutic methods. In one embodiment, a pharmaceutical composition comprises any of the fusion proteins provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical composition comprises any of the fusion proteins provided herein and at least one additional therapeutic agent, e.g., as described below.

Further provided is a method of producing a fusion protein of the invention in a form suitable for administration in vivo, the method comprising (a) obtaining a fusion protein according to the invention, and (b) formulating the fusion protein with at least one pharmaceutically acceptable carrier, whereby a preparation of fusion protein is formulated for administration in vivo.

Pharmaceutical compositions of the present invention comprise a pharmaceutically effective amount of one or more fusion protein dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmaceutically acceptable" refers to molecules and compositions that are generally non-toxic to recipients at the dosages and concentrations employed, i.e., do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate.

The preparation of a pharmaceutical composition that contains at least one fusion protein and optionally an additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington’s Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. Moreover, for animal (e.g., human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards or corresponding authorities in other countries. Preferred compositions are lyophilized formulations or aqueous solutions. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, buffers, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, antioxidants, proteins, drugs, drug stabilizers, polymers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington’s Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

The composition may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration as injection. Fusion proteins of the present invention (and any additional therapeutic agent) can be administered intravenously, intradermally, intraarticularly, intraperitoneally, intralesionally, intracranially, intrathecally, intraprostatically, intrapleurally, intramuscularly, intravenously, subcutaneously, subconjunctively, intravenously, mucosally, intrapericardially, intraumbilically, intracocularly, orally, topically, locally, by inhalation (e.g. aerosol inhalation), injection, infusion, continuous infusion, localized perfusion bathing target cells directly, via a catheter, via a lavage, in ointments, in lipid compositions (e.g. liposomes), or by other method or any combination of the foregoing as would be known to one of ordinary skill in the art (see, for example, Remington’s Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated, herein by reference). Parenteral administration, in particular intravenous injection, is most commonly used for administering polypeptide molecules such as the fusion proteins of the invention.

Parenteral compositions include those designed for administration by injection, e.g., subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intramuscular or intrarateonal injection. For injection, the fusion proteins of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks’ solution, Ringer’s solution, or physiological saline buffer. The solution may contain formulation agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the fusion proteins may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. Sterile injectable solutions are prepared by incorporating the fusion proteins of the invention in the required amount in the appropriate solvent with various of the other ingredients enumerated below, as required. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and/or the other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suspensions or emulsion, the preferred methods of preparation are vacuum-drying or freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered liquid medium thereof. The liquid medium should be suitably buffered if necessary and the liquid diluted first rendered isotonic prior to injection with sufficient saline or glucose. The composition must be stable
under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi. It will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less than 0.5 ng/mg protein. Suitable pharmaceutically acceptable carriers include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyl(dimethylbenzyl) ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

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

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidial drug delivery systems (for example, liposomes, albumin microparticles, microemulsions, nano-particles and nanoparticles) or in macromulsions. Such techniques are disclosed in Remington’s Pharmaceutical Sciences (18th Ed. Mack Printing Company, 1990). Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the polypeptide, which matrices are in the form of shaped articles, e.g. films, or microcapsules. In particular embodiments, prolonged absorption of an injectable composition can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin or combinations thereof.

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

Pharmaceutical compositions comprising the fusion proteins of the invention may be manufactured by means of conventional mixing, dissolving, emulsifying, encapsulating, entrapping or lyophilizing processes. Pharmaceutical compositions may be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries which facilitate processing of the proteins into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

Any of the fusion proteins provided herein may be used in therapeutic methods.

For use in therapeutic methods, fusion proteins of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

In one aspect, fusion proteins of the invention for use as a medicament are provided. In further aspects, fusion proteins of the invention for use in treating a disease are provided. In certain embodiments, fusion proteins of the invention for use in a method of treatment are provided. In one embodiment, the invention provides a fusion protein as described herein for use in the treatment of a disease in an individual in need thereof. In certain embodiments, the invention provides a fusion protein for use in a method of treating an individual having a disease comprising administering to the individual a therapeutically effective amount of the fusion protein. In certain embodiments the disease to be treated is an autoimmune disease. Exemplary autoimmune diseases include type 1 diabetes, psoriasis, asthma, rheumatoid arthritis, Crohn’s disease, systemic lupus erythematosus (SLE) and multiple sclerosis. In one embodiment, the disease is transplant rejection or graft-versus-host disease. In a particular embodiment the disease is selected from the group of type 1 diabetes, Crohn’s disease, SLE, and multiple sclerosis. In a more particular embodiment, the disease is type 1 diabetes. In certain embodiments the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g. an immunosuppressive agent if the disease to be treated is an autoimmune disease. An “individual” according to any of the above embodiments is a mammal, preferably a human.

In a further aspect, the invention provides for the use of a fusion protein of the invention in the manufacture or preparation of a medicament for the treatment of a disease in
an individual in need thereof. In one embodiment, the medicament is for use in a method of treating a disease comprising administering to an individual having the disease a therapeutically effective amount of the medicament. In certain embodiments the disease to be treated is an autoimmune disease. In one embodiment, the disease is transplant rejection or graft-versus-host disease. In a particular embodiment the disease is selected from the group of type 1 diabetes, Crohn’s disease, SLE, and multiple sclerosis. In one embodiment, the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g., an immunosuppressive agent if the disease to be treated is an autoimmune disease. An “individual” according to any of the above embodiments may be a mammal, preferably a human.

[0130] In a further aspect, the invention provides a method for treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a fusion protein of the invention. In one embodiment a composition is administered to said individual, comprising a fusion protein of the invention in a pharmaceutically acceptable form. In certain embodiments the disease to be treated is an autoimmune disease. In one embodiment, the disease is transplant rejection or graft-versus-host disease. In a particular embodiment the disease is selected from the group of type 1 diabetes, Crohn’s disease, SLE, and multiple sclerosis. In a more particular embodiment, the disease is type 1 diabetes. In certain embodiments the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g., an immunosuppressive agent if the disease to be treated is an autoimmune disease. An “individual” according to any of the above embodiments may be a mammal, preferably a human.

[0131] In some embodiments, an effective amount of a fusion protein of the invention is administered to a cell. In other embodiments, a therapeutically effective amount of a fusion protein of the invention is administered to an individual for the treatment of disease.

[0132] For the prevention or treatment of disease, the appropriate dosage of a fusion protein of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the route of administration, the body weight of the patient, the type of fusion protein, the severity and course of the disease, whether the fusion protein is administered for preventive or therapeutic purposes, previous or concurrent therapeutic interventions, the patient’s clinical history and response to the fusion protein, and the discretion of the attending physician. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0133] The fusion protein is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g., 0.1 mg/kg-10 mg/kg) of fusion protein can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the fusion protein would be in the range from about 0.005 mg/kg to about 10 mg/kg. In other non-limiting examples, a dose may also comprise from about 1 μg/kg body weight, about 5 μg/kg body weight, about 10 μg/kg body weight, about 50 μg/kg body weight, about 100 μg/kg body weight, about 200 μg/kg body weight, about 350 μg/kg body weight, about 500 μg/kg body weight, about 1 mg/kg body weight, about 5 mg/kg body weight, about 10 mg/kg body weight, about 50 mg/kg body weight, about 100 mg/kg body weight, about 200 mg/kg body weight, about 350 mg/kg body weight, about 500 mg/kg body weight, about 1000 mg/kg body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 μg/kg body weight to about 100 mg/kg body weight, about 5 μg/kg body weight to about 500 mg/kg body weight etc., can be administered, based on the numbers described above. Thus, on one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 5.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g., every week or every three weeks (e.g., such that the patient receives from about two to about twenty, or e.g., about six doses of the fusion protein). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

[0134] The fusion proteins of the invention will generally be used in an amount effective to achieve the intended purpose. For use to treat or prevent a disease condition, the fusion proteins of the invention, or pharmaceutical compositions thereof, are administered or applied in a therapeutically effective amount. Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0135] For systemic administration, a therapeutically effective dose can be estimated initially from in vitro assays, such as cell culture assays. A dose can then be formulated in animal models to achieve a circulating concentration range that includes the IC₅₀ as determined in cell culture. Such information can be used to more accurately determine useful doses in humans.

[0136] Initial dosages can also be estimated from in vivo data, e.g., animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

[0137] Dosage amount and interval may be adjusted individually to provide plasma levels of the fusion proteins which are sufficient to maintain therapeutic effect. Usual patient dosages for administration by injection range from about 0.1 to 50 mg/kg/day, typically from about 0.5 to 1 mg/kg/day. Therapeutically effective plasma levels may be achieved by administering multiple doses each day. Levels in plasma may be measured, for example, by HPLC.

[0138] In cases of local administration or selective uptake, the effective local concentration of the fusion protein may not be related to plasma concentration. One having skill in the art
will be able to optimize therapeutically effective local dosages without undue experimentation.

A therapeutically effective dose of the fusion proteins described herein will generally provide therapeutic benefit without causing substantial toxicity. Toxicity and therapeutic efficacy of a fusion protein can be determined by standard pharmaceutical procedures in cell culture or experimental animals. Cell culture assays and animal studies can be used to determine the LD_{50} (the dose lethal to 50% of a population) and the ED_{50} (the dose therapeutically effective in 50% of a population). The dose ratio between toxic and therapeutic effects is the therapeutic index, which can be expressed as the ratio LD_{50}/ED_{50}. Fusion proteins that exhibit large therapeutic indices are preferred. In one embodiment, the fusion protein according to the present invention exhibits a high therapeutic index. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosages suitable for use in humans. The dosage lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon a variety of factors, e.g., the dosage form employed, the route of administration utilized, the condition of the subject, and the like. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, e.g., Fingl et al., 1975, in: The Pharmacological Basis of Therapeutics, Ch. 1, p. 1, incorporated herein by reference in its entirety).

The attending physician for patients treated with fusion proteins of the invention would know how and when to terminate, interrupt, or adjust administration due to toxicity, organ dysfunction, and the like. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the disorder of interest will vary with the severity of the condition to be treated, with the route of administration, and the like. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency will also vary according to the age, body weight, and response of the individual patient.

Other Agents and Treatments

The fusion proteins of the invention may be administered in combination with one or more other agents in therapy. For instance, a fusion protein of the invention may be co-administered with at least one additional therapeutic agent. The term "therapeutic agent" encompasses any agent administered to treat a symptom or disease in an individual in need of such treatment. Such additional therapeutic agent may comprise any active ingredients suitable for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. In certain embodiments, an additional therapeutic agent is an immunosuppressive agent.

Such other agents are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of fusion protein used, the type of disorder or treatment, and other factors discussed above. The fusion proteins are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate compositions), and separate administration, in which case, administration of the fusion protein of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant.

Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a fusion protein of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a fusion protein of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer’s solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Examples

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Recombinant DNA Techniques

Standard methods were used to manipulate DNA as described in Sambrook et al., Molecular cloning: A laboratory manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989. The molecular biological reagents were used according to the manufacturer’s instructions. General information regarding the nucleotide sequences of human immunoglobulin light and heavy chains is given in: Kabat, E. A. et al., (1991) Sequences of Proteins of Immunological Interest, Fifth Ed., NIH Publication No 91-3242.
DNA Sequencing

DNA sequences were determined by double strand sequencing.

Gene Synthesis

Desired gene segments were synthesized by PCR using appropriate templates. Gene synthesis was performed by Geneart AG (Regensburg, Germany) from synthetic oligonucleotides. Desired gene segments were cloned into eukaryotic expression vectors. Endonuclease cleavage sites were included in the cloning vectors. The plasmid DNA was purified from transformed bacteria and concentration determined by UV spectrophotometry. The DNA sequence of the isolated gene fragments was confirmed by DNA sequencing. Gene segments were designed with suitable restriction sites to allow subcloning into the respective expression vectors. All constructs were designed with a 5'-end DNA sequence coding for a leader peptide which targets proteins for secretion in eukaryotic cells. SEQ ID NOs 39-47 give exemplary leader peptides and polynucleotide sequences encoding them.

Preparation of IL-2R βγ Subunit-Fc Fusions and IL-2R α Subunit Fc Fusion

To study IL-2 receptor binding affinity, a tool was generated to allow for the expression of a heterodimeric IL-2 receptor. The βγ-subunit of the IL-2 receptor was fused to an Fc molecule that was engineered to heterodimerize (Fc (hole)) (SEQ ID NOs 21 and 22 (human), SEQ ID NOs 27 and 28 (mouse) and SEQ ID NOs 33 and 34 (cytomolgus)) using the “knobs-into-holes” technology (Merchant et al., Nat. Biotechniq. 16, 677-681 (1998)). The γ-subunit of the IL-2 receptor was then fused to the Fc (knob) variant (SEQ ID NOs 23 and 24 (human), SEQ ID NOs 29 and 30 (mouse) and SEQ ID NOs 35 and 36 (cytomolgus)), which heterodimerized with Fc (hole). This heterodimeric Fc-fusion protein was then used as a substrate for analyzing the IL-2/IL-2 receptor interaction. The IL-2 α-subunit was expressed as a monocistronic chain with an AcTev cleavage site and an Avi His tag (SEQ ID NOs 25 and 26 (human), SEQ ID NOs 31 and 32 (mouse) and SEQ ID NOs 37 and 38 (cytomolgus)). The respective IL-2R α-subunits were transiently expressed in HEK EBNA 293 cells with serum for the IL-2R βγ subunit construct and without serum for the α-subunit construct. The IL-2R βγ subunit construct was purified on protein A (GE Healthcare), followed by size exclusion chromatography (GE Healthcare, Superdex 200). The IL-2R α-subunit was purified via His tag on a NTA column (Qiagen) followed by size exclusion chromatography (GE Healthcare, Superdex 75). Amino acid and corresponding nucleotide sequences of various receptor constructs are given in SEQ ID NOs 21-38.

Preparation of Fusion Proteins

The DNA sequences were generated by gene synthesis and/or classical molecular biology techniques and subcloned into mammalian expression vectors under the control of an MPSV promoter and upstream of an synthetic polyA site, each vector carrying an EBV OriP sequence. Fusion proteins as applied in the examples below were produced by co-transfecting exponentially growing HEK293-EBNA cells with the mammalian expression vectors using calcium phosphate-transfection. Alternatively, HEK293 cells growing in suspension were transfected by polyethylenimine (PEI) with the respective expression vectors. Alternatively, stably transfected CHO cell pools or CHO cell clones were used for expression in serum-free media. Subsequently, the fusion proteins were purified from the supernatant. Briefly, fusion proteins were purified by a single affinity step with protein A (HiTrap ProtA, GE Healthcare) equilibrated in 20 mM sodium phosphate, 20 mM sodium citrate pH 7.5. After loading of the supernatant, the column was first washed with 20 mM sodium phosphate, 20 mM sodium citrate, pH 7.5 and subsequently washed with 13.3 mM sodium phosphate, 20 mM sodium citrate, 500 mM sodium chloride, pH 5.45. The fusion protein was eluted with 20 mM sodium citrate, 100 mM sodium chloride, 100 mM glycine, pH 3. Fractions were neutralized and pooled by size exclusion chromatography (HiLoad 16/60 Superdex 200, GE Healthcare) in final formulation buffer: 20 mM histidine, 140 mM NaCl pH 6.0. The protein concentration of purified protein samples was determined by measuring the absorption at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence. Purity and molecular weight of fusion proteins were analyzed by SDS-PAGE and in the presence and absence of a reducing agent (5 mM 1,4-dithiothreitol) and stained with Coomassie blue (Simple-Blue™ SafeStain, Invitrogen). The NuPAGE® Pre-Cast gel system (Invitrogen) was used according to the manufacturer’s instructions (4-20% Tris-glycine gels or 3-12% Bis-Tris). Alternatively, purity and molecular weight of molecules were analyzed by CE-SDS analyses in the presence and absence of a reducing agent, using the Caliper LabChip GXII system (Caliper Lifesciences) according to the manufacturer’s instructions. The aggregate content of fusion protein samples was analyzed using a Superdex 200 10/300GL analytical size-exclusion column (GE Healthcare) in 20 mM MOPS, 150 mM NaCl, 0.02% NaN₃ pH 7.3 running buffer at 25°C.

Results of the purification and characterization of the DP47GS IgG-IL-2 and DP47GS IgG-(IL-2)₂ constructs are shown in FIGS. 1 and 2.

Affinity to IL-2 Receptors

The affinity of the fusion proteins was determined by surface plasmon resonance (SPR) for the human, murine and cynomolgus IL-2R βγ heterodimer using recombinant IL-2R βγ heterodimer under the following conditions: ligand: human, murine and cynomolgus IL-2R β knob γ hole heterodimer immobilized on CM5 chip, analyte: DP47GS IgG-IL-2 (SEQ ID NOs 13, 15 and 19) or DP47GS IgG-(IL-2)₂ (SEQ ID NOs 17 and 19), temperature: 25°C, buffer: HBS-Eq, analyte concentration: 300 nM down to 3.7 nM (1:3 dilutions), flow: 30 µl/min, association: 180 s, dissociation: 300 s, regeneration: 60 s 3 M MgCl₂, fitting: 1:1 binding, R2<0, Rmax—global. The affinity of the fusion proteins was also determined by surface plasmon resonance (SPR) for the human, murine and cynomolgus IL-2R α-subunit using recombinant monomeric IL-2R α-subunit under the follow-
ing conditions: ligand: human, murine and cynomolgus IL-2R α-subunit immobilized on a CM5 chip, analyte: DP47GS IgG-IL-2 or DP47GS IgG-(IL-2), temperature: 25°C, buffer: HBS-EP; analyte concentration 100 nM down to 3.1 nM (1:3 dilutions), flow: 30 µl/min, association: 60 s, dissociation: 180 s, regeneration: none, fitting: 1:1 binding, R1=0, Rmax=global.

[0153] Results of the kinetic analysis with the IL-2R βγ heterodimer or the IL-2R α-subunit are given in Table 1.

<table>
<thead>
<tr>
<th>Binding of fusion proteins to IL-2R βγ and IL-2R α.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusion Protein</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>DP47GS</td>
</tr>
<tr>
<td>IgG-IL-2</td>
</tr>
<tr>
<td>DP47GS</td>
</tr>
<tr>
<td>IgG-(IL-2)</td>
</tr>
</tbody>
</table>

[0154] The affinity of human IL-2 to the human IL-2R βγ heterodimer is described to be around 1 nM, while the fusion proteins both have a slightly lower affinity between 2 and 3 nM. The affinity to the murine IL-2R is several times weaker than for the human and cynomolgous IL-2R.

Expression of IL-2 Receptors on Immune Cells

[0155] The high-affinity trimeric IL-2 receptor is composed of the α (IL-2RA, CD25), β (IL-2RB, CD122) and γ (IL-2RG, CD152) chains and has a K_d of ~10 nM. CD25 alone has only a low affinity (K_d~10 nM) for IL-2. The IL-2RB/IL-2RG dimer, which is expressed on some cell types in the absence of IL-2RA, also binds IL-2 but with an intermediate affinity (K_d~1 nM). Signalling via the IL-2 receptor is mediated by the IL-2RB and IL-2RG chains. From crystal structure analyses, IL-2RA does not seem to contact either IL-2RB or IL-2RG. It has been proposed that the basis of the cooperativity of the trimeric receptor is an entropy reduction when CD25 captures IL-2 at the cell surface for presentation to IL-2RB and IL-2RG, or alternatively a CD25-induced alteration in IL-2R conformation occurs, thus stabilizing the complex. In Foxp3 regulatory CD4+ T cells, there is a large stoichiometric excess of IL-2RA as compared to the β and γ chains of the receptor supporting the hypothesis that dimers, or even larger complexes, of the α chain aid in the binding of IL-2. There is also evidence that CD25 on one cell can present IL-2 to IL-2RB/IL-2RG dimers on another cell, in a high-affinity, intercellular interaction emphasizing the unique relationship amongst the three chains composing the high affinity IL-2 receptor.

[0156] CD25 (IL-2RA) and CD122 (IL-2RB) expression on CD4+ Treg subsets, NK cell subsets and NK cell subsets, as well as on CD4+ and CD8+ conventional T cell subsets was determined by FACS (Figs. 3 and 4). Cell surface markers were used to define CD4+ Treg subsets, NK cell subsets and NK cell subsets (Fig. 3). In order to optimize staining for CD25 and CD122, intracellular Foxp3 staining was not performed. Briefly, using 150 µl blood donors of a healthy volunteer, fluorescent antibodies were incubated for 45 min at room temperature in the dark (vortexed at the beginning and after 20 min). Red blood cells were lysed with BD lysis buffer (BD FACS Lysing Solution, 349202) for 9 minutes and the remaining cells were washed (2 ml of PBS with 0.1% BSA) and fixed (1% PFA). Cells were analysed on an LSRFortessa cell analyser (Becton Dickinson) and data analysed using FlowJo software (TreeStar). Treg subsets were identified using antibodies specific for TCRαβ-FTTC (IP26, BioLegend), CD4-Alexa Flour 700 (RPA-T4, BioLegend), CD127-PE/CY7 (eBioRDR5, Ebioscience), CD45RA-Pacific Blue (HI100, BioLegend), CD25-APC (2A3, M-A251, BD Bioscience) and CD122-PE (TU27, BioLegend). NK and NKT cells were stained in a separate tube with antibodies specific for TCRαβ-FTTC,
CD25<sup>hi</sup>) (FIG. 3A). On average, conventional memory CD4<sup>+</sup> T cells express approximately 10-fold less CD25 than T<sub>reg</sub>~s (FIG. 4A). The expression of CD25 on naïve CD4<sup>+</sup> T cells varies significantly amongst donors but is always lower than that observed on memory CD4<sup>+</sup> T cells (FIG. 4A). Expression of CD25 on NK, NKT and CD8 T cells is very low or not detectable except for CD56<sup>bright</sup> NK cells (FIGS. 3C and 4C). The CD56<sup>bright</sup> NK and CD56<sup>+</sup> NK cells express the highest level of IL-2R (FIG. 3D), approximately 10-fold more than any of the T cell subsets, including NKT cells (FIGS. 3B, 3D, 4B, 4D).

Induction of pSTAT5α in Human Peripheral Blood Cell Subsets

[0159] Following IL-2-induced oligomerization of the trimeric IL-2R, the Jak1 and Jak3 cytoplasmic protein tyrosine kinases, that are associated with the intracellular domains of IL-2RB and IL-2RG respectively, become activated. These kinases phosphorylate certain IL-2RB tyrosine residues that act as docking sites for STAT5α and STAT5β that are in turn phosphorylated. The IL-2-induced activation of several signalling pathways eventually results in the transcription of target genes that contribute to the various functions associated with the IL-2/IL-2R pathway. Since various cell types express different levels of the IL-2 receptor IL-2RA and IL-2RB molecules (FIGS. 3 and 4), in order to understand the integrated signalling response to IL-2 mediated by various combinations of the high and intermediate affinity receptors we measured pSTAT5α levels within individual cells by polychromatic flow cytometry.

[0160] The effects of various doses of DP47GS IgG-IL-2 or DP47GS IgG-IL-2<sub>2</sub>, on the induction of STAT5α phosphorylation were assessed in human CD4<sup>+</sup> T<sub>reg</sub> subsets, naïve and memory conventional CD4<sup>+</sup> T cells, memory conventional CD8<sup>+</sup> T cells, CD45RA<sup>+</sup>CD8<sup>+</sup> T cells, NK cells and CD56<sup>+</sup> NK cells (FIGS. 5 and 6). All subsets were characterized in a single tube for each dose. Briefly, blood from a healthy volunteer was drawn into heparinized tubes. Various concentrations of DP47GS IgG-IL-2 or DP47GS IgG-IL-2<sub>2</sub> were added to 500 μl of blood and incubated at 37°C. After 30 minutes the blood was lysed and fixed using pre-warmed lyse/fix buffer (Becton Dickinson #558049) for 10 minutes at 37°C, washed 2x with PBS containing 0.2% BSA followed by permeabilization with −20°C pre-cooled methanol (Sigma, BioTech grade #494437) for 20 minutes on ice. The cells were then extensively washed 4x with PBS containing 0.2% BSA before FACS staining was performed using a panel of fluorescent antibodies to distinguish different lymphocyte and NK cell subpopulations and pSTAT5α status. The antibodies utilized were anti-CD4-Alexa Fluor 700 (clone RPA-T4), CD3-PercpCy5.5 (UCHT1), CD45RA-PE/Cy7 (HI100), CD8-Brilliant Violet 605 (RO-T8), CD56-Brilliant Violet 421 (HC56), Foxp3-PE (259D) (all from BioLegend), CD25-APC (clones M-A251 & 2A3) and pSTAT5α-Alexa Fluor 488 (pY694) (Becton Dickinson). Samples were acquired using an LSRSFortessa cell analyser (Becton Dickinson) and data analysed using FlowJo software (TreeStar). After gating on lymphocytes and excluding doublets, T<sub>reg</sub>~s were defined as CD3<sup>+</sup>, CD4<sup>+</sup>, Foxp3<sup>+</sup> and subdivided as CD45<sup>+</sup> Foxp3<sup>+</sup> (activated T<sub>reg</sub>~s), CD3<sup>+</sup>, CD4<sup>+</sup>, CD45RA<sup>+</sup>, Foxp3<sup>+</sup> (memory T<sub>reg</sub>~s) and CD3<sup>+</sup>, CD4<sup>+</sup>, Foxp3<sup>+</sup> (naïve T<sub>reg</sub>~s). Conventional CD4<sup>+</sup> T cells were defined as CD3<sup>+</sup>, CD4<sup>+</sup>, CD45RA<sup>+</sup> (naïve) and CD3<sup>+</sup>, CD45RA<sup>+</sup> (memory). CD8<sup>+</sup> T cells were defined as CD3<sup>+</sup>, CD8<sup>+</sup>, CD45RA<sup>+</sup> (memory), CD3<sup>+</sup>, CD8<sup>+</sup>, CD45RA<sup>+</sup> (naïve) and CD3<sup>+</sup>, CD8<sup>+</sup>, CD45RA<sup>+</sup> (memory). NKT cells were defined as CD3<sup>+</sup>, CD56<sup>+</sup> and NK cells were defined as CD3<sup>+</sup>, CD56<sup>+</sup> (activated NK cells) or CD3<sup>+</sup>, CD56<sup>+</sup> (naïve NK cells). Intracellular pSTAT5α levels were quantified in all cell subsets at all doses.

[0161] FIG. 5 shows the dose response of the DP47GS IgG-IL-2 immunoconjugate on T cells, NK cells and NK T cells in human peripheral blood. The hierarchy of responsiveness to DP47GS IgG-IL-2 was the same as that observed when recombiant human IL-2 was used (data not shown): all three T<sub>reg</sub> populations, naïve (CD45RA<sup>+</sup>, CD25<sup>+</sup>), memory (CD45RA<sup>+</sup>, CD25<sup>+</sup>) and activated (CD45RA<sup>+</sup>, CD25<sup>+</sup>) increased pSTAT5α levels at the 0.1 ng/ml concentration of DP47GS IgG-IL-2 whereas other cell populations required 1 (CD56<sup>bright</sup> NK and memory CD4<sup>+</sup> T cells), 10 (memory CD8<sup>+</sup> T cells), or 100 ng/ml (CD56<sup>+</sup> NK cells, naïve CD4<sup>+</sup> T cells, NKT cells and CD45RA<sup>+</sup>CD8<sup>+</sup> T cells) DP47GS IgG-IL-2 to produce detectable increases in pSTAT5α. Also see FIG. 8 for detailed dose responses by the T<sub>reg</sub> populations that display their high sensitivity for DP47GS IgG-IL-2. It is notable that the high expression of IL-2Rβ1 on NK cells with intermediate levels of CD56 (FIG. 3D) as compared to IL-2Rβ1 expression on T cell subsets is not sufficient to allow T<sub>reg</sub>-like IL-2 sensitivity (FIG. 3B). Overall, activated, memory and naïve T<sub>reg</sub> subsets showed the greatest sensitivity to DP47GS IgG-IL-2, while CD56<sup>bright</sup> NK cells and CD4<sup>+</sup> conventional memory T cells were 20-50 fold less sensitive, respectively. Amongst the other subsets analysed, increased pSTAT5α was detected in memory CD8<sup>+</sup> T cells at a 10-fold higher concentration of DP47G IgG-IL-2 than that observed to induce pSTAT5α in CD4<sup>+</sup> conventional memory T cells. Naïve CD4<sup>+</sup> and CD8<sup>+</sup> T cells, NKT cells and “resting” NK cells (positive, not bright staining for CD56) were relatively insensitive to the immunoconjugate (FIG. 3B).

[0162] As observed for DP47GS IgG-IL-2 (FIG. 5), the T<sub>reg</sub> subset were the cells most sensitive to DP47GS IgG-IL-2<sub>2</sub> (activated T<sub>reg</sub>~s) induced pSTAT5α (FIGS. 6 and 8). To more readily compare DP47GS IgG-IL-2 and DP47GS IgG-IL-2<sub>2</sub>, the pSTAT5α values were normalized (FIG. 7). To normalize MFI values, unstimulated pSTAT5α MFI values specific for each gated subset were subtracted from all stimulated MFI values for that cell subset. The resulting values were divided by the highest pSTAT5α MFI value obtained by that subset in the dose response. The EC<sub>50</sub> were estimated based on the amount of IL-2 fusion protein required to reach 50% of the maximal pSTAT5α MFI observed for that subset. As shown in FIG. 7, the DP47GS IgG-IL-2 immunoconjugate produced a more potent and selective induction of pSTAT5α in cells constitutively expressing CD25, potentially as a consequence of increased avidity of the immunoconjugate for the high affinity IL-2 receptor. The EC<sub>50</sub> for pSTAT5α activation was observed to be 20-60 fold lower in T<sub>reg</sub>~s when directly compared to DP47GS IgG-IL-2<sub>2</sub> to DP47GS IgG-IL-2. Table 2 summarizes the EC<sub>50</sub> values and fold differences for pSTAT5α activation by DP47GS IgG-IL-2<sub>2</sub> vs. DP47GS IgG-IL-2 in the different cell subsets.

<table>
<thead>
<tr>
<th>T cell</th>
<th>IgG-IL-2</th>
<th>IgG-(IL-2)&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>activated T&lt;sub&gt;reg&lt;/sub&gt;~</td>
<td>0.20 ng/mL</td>
<td>0.010 ng/mL</td>
<td>20</td>
</tr>
<tr>
<td>memory T&lt;sub&gt;reg&lt;/sub&gt;~</td>
<td>0.60 ng/mL</td>
<td>0.010 ng/mL</td>
<td>60</td>
</tr>
</tbody>
</table>
TABLE 2-continued

<table>
<thead>
<tr>
<th>T cell</th>
<th>IgG-IL-2</th>
<th>IgG-(IL-2)</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>naive T&lt;sub&gt;reg&lt;/sub&gt;</td>
<td>0.60 ng/mL</td>
<td>0.025 ng/mL</td>
<td>24</td>
</tr>
<tr>
<td>CD5&lt;sup&gt;+&lt;/sup&gt; NK</td>
<td>3.5 ng/mL</td>
<td>1.5 ng/mL</td>
<td>2.3</td>
</tr>
<tr>
<td>CD4&lt;sup&gt;+&lt;/sup&gt; Te mem</td>
<td>10 ng/mL</td>
<td>0.4 ng/mL</td>
<td>25</td>
</tr>
<tr>
<td>CD5&lt;sup&gt;+&lt;/sup&gt; NK</td>
<td>100 ng/mL</td>
<td>25 ng/mL</td>
<td>4</td>
</tr>
</tbody>
</table>

Even at extremely limiting concentrations, DP47GS IgG-(IL-2) produced higher levels of pSTAT5a as compared to DP47GS IgG-IL-2 (FIG. 8). For this experiment, blood from three healthy volunteers were tested individually on the same day for responses to a 2-fold titration of DP47 IgG-IL-2 and DP47 IgG-(IL-2) at limiting concentrations. Graphs in FIG. 8 represent the average and SE of the pSTAT5a MF1 for the three donors. In addition to the T<sub>reg</sub> subsets examined individually (FIG. B3-D), a gate was applied to assess pSTAT5a in total CD3<sup>+</sup>, CD4<sup>+</sup>, Foxp3<sup>+</sup> Tregs (FIG. 8A). Polychromatic flow cytometry was performed as described above (see FIG. 5).

CD4<sup>+</sup> conventional memory T cells also responded to lower (25-fold) concentrations of DP47GS IgG-(IL-2) as compared to DP47GS IgG-IL-2. However, although the EC<sub>50</sub> values were reduced for CD5<sup>+</sup> NK cells and NK cells when comparing DP47GS IgG-(IL-2) to DP47 IgG-IL-2, the reductions were only 2.3-fold and 4-fold, respectively. This is likely due to the reliance of these cells on the intermediate IL-2 receptor for IL-2-mediated signalling. This differential shift in the EC<sub>50</sub> for T<sub>reg</sub> vs. NK cells increases the preference for T<sub>reg</sub> activation several fold.

Induction of pSTAT5a in Cynomolgus Peripheral Blood Cell Subsets

As observed in human peripheral blood, there is a preferential induction of pSTAT5a in T<sub>reg</sub> subsets in cynomolgus peripheral blood stimulated with DP47GS IgG-IL-2. In a direct comparison of the ability of DP47GS IgG-(IL-2) and DP47GS IgG-IL-2 to induce pSTAT5a in the three T<sub>reg</sub> subsets, 2.8 fold less DP47GS IgG-(IL-2) was required to reach 50% of the maximal phosphorylation level (Table 3).

Similar to human whole blood, cell surface and intracellular markers were used to identify regulatory T cell subsets and conventional T cells in whole blood from normal healthy cynomolgus monkeys. Blood samples were collected on the same day from three healthy cynomolgus monkeys in sodium heparin tubes and various concentrations of DP47GS IgG-IL-2 or DP47GS IgG-(IL-2) were added to 500 μL of blood and incubated at 37° C. After 10 min at 37° C, samples were lysed and fixed with pre-warmed BD Lyse/Fix buffer (BD Biosciences). After washing, cells were permeabilized with 1 mL methanol for 30 min on ice. Samples were washed 3 times and stained with a panel of Foxp3-Alexa Fluor<sup>®</sup> 647 (clone: 25D, BioLegend), CD4-V500 (clone: L200, BD Biosciences), CD45RA-V450 (clone: 519, BD Biosciences), CD25-PE (clone: 4E3, eBioscience), pSTAT5a-Alexa Fluor<sup>®</sup> 488 (clone: 47, BD Biosciences), and Ki-67-PerCP-Cy5.5 (clone: B56, BD Biosciences) for 1 hour at 4°C. All the samples were acquired by an LSRFortessa cell analyser (Becton Dickinson) and then analysed with FlowJo software (Tree star, Inc., Ashland, USA).

Table 3 summarizes the EC<sub>50</sub> values for pSTAT5a activation by DP47GS IgG-IL-2 vs. DP47GS IgG-(IL-2) in the different cell subsets.

<table>
<thead>
<tr>
<th>T cell</th>
<th>IgG-IL-2</th>
<th>IgG-(IL-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>activated T&lt;sub&gt;reg&lt;/sub&gt;</td>
<td>0.070 ng/mL</td>
<td>0.020 ng/mL</td>
</tr>
<tr>
<td>memory T&lt;sub&gt;reg&lt;/sub&gt;</td>
<td>0.210 ng/mL</td>
<td>0.025 ng/mL</td>
</tr>
<tr>
<td>naive T&lt;sub&gt;reg&lt;/sub&gt;</td>
<td>0.040 ng/mL</td>
<td>0.020 ng/mL</td>
</tr>
<tr>
<td>memory Te mem</td>
<td>&gt;4.00 ng/mL</td>
<td>&gt;1.00 ng/mL</td>
</tr>
</tbody>
</table>

Induction of T<sub>reg</sub> Number in Cynomolgus Monkeys

Cynomolgus animals treated in vivo with DP47GS IgG-IL-2 had dose-dependent increases in the absolute number of T<sub>reg</sub> as well as the fold increase above baseline 7 days post dosing (FIGS. 9A and 9B), respectively. Normal healthy cynomolgus monkeys of both sexes at ages ranging from 3 to 6 years were used in all tests and no animal was used more than once. While under anaesthesia, various doses of DP47GS IgG-IL-2 (n=6) or vehicle (n=3) were injected SC on the lateral dorsum. Individual doses of DP47GS IgG-IL-2 were based on body weight and formulated for injection in a vehicle of sterile PBS pH 7.2 containing 0.5% sterile cynomolgus serum. Blood samples were collected at various times post treatment and tested for haematological changes (CBC and Differential) with an Advia Automated Hematology Analyser as well as cell surface and intracellular markers detailed above (experimental procedures for Table 3). The changes in whole blood CD4<sup>+</sup>, CD25<sup>+</sup>, Foxp3<sup>+</sup>, regulatory T cells on day 7 post treatment are shown in FIG. 9 as the absolute cell number per mm<sup>3</sup> of whole blood (FIG. 9A) and the fold change in T<sub>reg</sub> (FIG. 9B); all data are represented as the mean±SEM. At the higher doses of 25 μg/kg and 36 μg/kg DP47GS IgG-IL-2, average T<sub>reg</sub> increases of nearly 3-fold (range of 111-255%) and 4-fold (range of 110-470%), respectively, were observed. Without wishing to be bound by theory, the ~2-fold increase in T<sub>reg</sub> (ranging from 67-133%) with the 12 DP47GS IgG-IL-2 dose represents the desirable increase in T<sub>reg</sub>s. There is a large range in the numbers of T<sub>reg</sub>s in humans (20-90 T<sub>reg</sub>s per mm<sup>3</sup> of blood; 4 to 10% of CD4<sup>+</sup> T cells) and it is reasonable to assume that an increase of T<sub>reg</sub>s induced by IL-2 within an individual will result in an overall increase in functional suppression. It might be desirable, however, not to increase T<sub>reg</sub> numbers above the normal range for this cell population for a sustained period of time, but primarily enhance the function of these cells.

In the DP47GS IgG-IL-2 dose response test outlined above (FIG. 9) cynomolgus Nature Killer cells (NK) were also examined for treatment related changes. Cynomolgus NK cells are not CD56<sup>+</sup> so an alternative staining strategy was used to define NK cells in cynomolgus blood; a panel of CD3-Alexa Fluor<sup>®</sup> 488 (clone: SP34-2, BD Biosciences), CD16-APC (clone: 3G8, BD Biosciences), and CD8-V500 (clone: SK1, BD Biosciences) was used to stain another set of blood samples with NK cells identified as CD5<sup>+</sup>, CD8<sup>+</sup> CD16<sup>+</sup> as shown. Vehicle treated monkeys (n=3) and those treated with DP47GS IgG-IL-2 (n=6) are shown in FIG. 9 as total blood NK cells (x10<sup>5</sup> per ml). Importantly, at the 12 μg/kg dose, no increase in NK cells was observed. At the
higher doses of 25 μg/kg and 36 μg/kg DP47GS IgG-IL-2, average NK increases of approximately 2-fold and 3-fold (range of 110-470%), respectively, were observed (FIG. 10). These data strongly support the hypothesis that low dose IL-2 can preferentially stimulate increases in Treg numbers in vivo.

[0170] A comparison of the ability of DP47GS IgG-IL-2 to induce an increase in Tregs in vivo in cynomolgus monkeys with that of Proleukin is shown in FIG. 11. Normal healthy cynomolgus monkeys (groups of n=5) were treated with low doses of DP47GS IgG-IL-2 or high doses of Proleukin and the change in regulatory T cells tested at day 10. On days 0 and 7, DP47 IgG-IL-2 was given SC at a dose of 16,800 IU/kg. Proleukin treatment was given SC 3 times per week (MWF) for a total of 5 doses at 200,000 IU/kg. The results are shown in FIG. 11 as means±SEM for the change in total Treg cells per mm³ blood (FIG. 11A), the fold increase in Treg cells (FIG. 11B), and the change in the ratio of Treg cells to conventional CD4⁺FoxP³ cells (FIG. 11C).

[0171] Because of the short half-life of Proleukin (see FIG. 14), it is normally dosed 5 days per week in humans. Therefore, during a 10 day study of Treg induction comparing DP47GS IgG-IL-2 and Proleukin, two doses of 16,800 IU/kg of DP47GS IgG-IL-2 (12 μg/kg) were administered (days 0 and 7) whereas five doses (days 0, 2, 4, 7, 9) of Proleukin at 200,000 μU/kg were given. The dose of Proleukin was based on an extrapolation from human studies where Treg numbers had been shown to be increased following the administration of Proleukin. Although nearly 30-fold less units of DP47GS IgG-IL-2 activity were administered over the 10 day period, DP47GS IgG-IL-2 induced a larger increase in the number of Treg cells than Proleukin (FIG. 11A, p=0.06). The increase in Treg cells above baseline (FIG. 11B) and the increase in Treg cells relative to conventional CD4⁺ T cells (FIG. 11C) were also larger (p=0.0011 and p=0.016, respectively) in animals dosed with DP47GS IgG-IL-2 as compared to Proleukin. In humans the ratio of regulatory CD4 T cells (usually defined as Foxp3⁺ or by a combination of surface markers) to non-regulatory CD4 T cells (referred to as conventional or effector cells) is often used to define the functional levels of Treg cells in patients through time.

In Vivo Response of Cynomolgus Peripheral Blood Cell Subsets to Low Dose DP47GS IgG-IL-2 Treatment

[0172] The in vivo cellular specificity of low dose IL-2 treatment is a critical parameter. We have determined that in vivo cell activation induced by DP47GS IgG-IL-2 or Proleukin can be sensitively monitored by measuring pSTAT5a levels ex vivo at various times after dosing cynomolgus monkeys or mice. The in vivo response of all cell populations that can be monitored in vitro (FIGS. 5-7) can be examined ex vivo.

[0173] One and three days after in vivo administration of a single low dose of DP47GS IgG-IL-2 (12 μg/kg) to healthy cynomolgus monkeys (n=5), whole blood was collected and tested for STAT5 phosphorylation as described above (experimental procedures to Table 3). Each monkey was bled on day 0 before treatment and the amount of STAT5 phosphorylation was measured and used individually to assess fold-changes post treatment. The fold change in pSTAT5 in Treg cells on days 1 and 3 is shown in FIG. 12A, the fold change in pSTAT5 in conventional CD4⁺CD45⁻ memory T cells in FIG. 12B, and the fold change in pSTAT5 in naive T cells is in FIG. 12C.

[0174] Cynomolgus blood cells obtained one and three days after a single low dose of DP47GS IgG-IL-2 (12 μg/kg) showed preferential pSTAT5a increases in Treg cells as compared to naive and memory conventional CD4⁺ T cells (FIG. 12).

[0175] The increase in Treg cells in the peripheral blood after low dose IL-2 treatment could reflect a change in the distribution of the cells in the body rather than an actual increase of the cells. To substantiate that Treg increases in vivo are at least in part due to the induction of cell division by IL-2 treatment, the intracellular marker of proliferation Ki-67 was assessed. Ki-67 is a protein that can be detected in the nucleus during G1, S, G2, and mitosis but is absent from resting cells that are in the G0 phase of the cell cycle. The cynomolgus monkeys treated with DP47GS IgG-IL-2 as described above (FIG. 12) were also monitored for ex vivo changes in the intracellular marker Ki-67 as described above (experimental procedures to Table 3) to assess the extent of proliferation in vivo. The percentage of cells that were in cell cycle (Ki-67⁺) on day 0 was compared to the percentage of cells Ki-67⁺ at 2 and 7 days post treatment. Ki-67⁺ Treg cells are shown in FIG. 13A, conventional CD4⁺CD45⁺ memory T cells are in FIG. 13B, and naive CD4⁺CD45RA⁺ T cells are in FIG. 13C. Cynomolgus blood cells obtained two and seven days after a single low dose of DP47GS IgG-IL-2 (12 μg/kg) showed preferential Ki-67 increases in Treg cells as compared to naive and memory conventional CD4⁺ T cells (FIG. 13).

Pharmacokinetic Properties of DP47GS IgG-IL-2

[0176] Prior to beginning functional studies in mice, the pharmacokinetic properties of the immunoconjugate DP47 GS IgG-IL-2 were compared to those of Proleukin (Novartis) (FIG. 14).

[0177] NOD mice were injected IP or SC with the indicated doses of DP47GS IgG-IL-2 or Proleukin in PBS containing 0.5% mouse serum and bled at various times after the injections. Doses of DP47GS IgG-IL-2 are summarized in Table 4. Human IL-2 was assessed in serum samples using mouse anti-human IL-2 mAb, BD Pharmingen, Cat #555051, clone 5344.111 to coat 96-well plates to capture the IL-2. IL-2 was detected using biotinylated mouse anti-human IL-2 mAb, BD Pharmingen, Cat #555040, clone H33-2. Binding was visualized using Europium-conjugated streptavidin.

[0178] As described previously, Proleukin is cleared rapidly. In contrast, DP47GS IgG-IL-2 is cleared about 10-100 times more slowly. Results from comparing the PK of DP47GS IgG-IL-2 in normal mice and CD25KO scid mice support the hypothesis that a major component driving the in vivo clearance of DP47GS IgG-IL-2 is the high affinity IL-2 receptor (data not shown).

| TABLE 4 |
|-----------------|-----------------|
| Doses of DP47GS IgG-IL-2 for PK study shown in FIG. 14. |
| IgG-IL-2 | IU/25g | IgG-IL-2 | mg/kg |
| 105,000 | 105 | 3.0 |
| 10,500 | 105 | 0.0 |
| 4,000 | 105 | 0.114 |
| 1,000 | 105 | 0.0286 |
| 200 | 105 | 0.0086 |
Foxp3 and CD25 MEL Increase in T<sub>reg</sub> after Treatment with IgG-IL-2

[0179] To compare the abilities of the immunoconjugate DP47GS IgG-IL-2 and recombinant human IL-2 to stimulate Foxp<sup>3</sup><sup>+</sup> Treg cells in vivo, mice were injected subcutaneously with either Proleukin (Novartis, 4,000 or 40,000 IU) or DP47 GS IgG-IL-2 (4,000 IU) and Tregs were monitored for changes in the expression of CD25 and Foxp3 one and three days later (FIG. 15).

[0180] NOD mice (3 mice/treatment group, including control cohorts at 24 and 72 h) were injected subcutaneously with either Proleukin (Novartis, 4,000 or 40,000 IU) or DP47 GS IgG-IL-2 (4,000 IU). Doses were delivered in 100 μl sterile PBS pH 7.2 containing 0.5% sterile-filtered mouse serum. After 24 and 72 h mice were euthanized by cervical dislocation and spleens excised. A single cell suspension of splenocytes was generated in 1 nl 1L-15 media and stored on ice, until further processing. A filtered aliquot of the single cell suspension, 40 μl, was transferred to FACS tubes and washed with 2 ml FACs buffer (600g, 5 min). Samples were then incubated with fluorochrome-conjugated antibodies directed against cell surface antigens: CD4 (clone RM4-5, fluorochrome A700), CD25 (eBio7134, A488), CD44 (IM7, e605), CD62L (HEL-14, PE), ICOS (C9384A, PE/Cy7), CD103 (2C7, APC). Staining was performed for 30 min, at 4 °C in 100 μl FACs buffer (PBS pH 7.2, 0.2% BSA). Following cell surface staining, samples were washed with 4 ml FACs buffer (600g, 5 min) before intracellular staining (according to the eBioscience intracellular staining protocol). Briefly, samples were resuspended in 200 μl fixation/permeabilisation buffer (eBioscience 00-5521) and incubated for 1 h, 4 °C. 1 ml of 1x permeabilisation buffer (eBioscience 00-8333) was added to samples before 3 ml FACs buffer and washing (600g, 5 min). Intracellular antigens, Ki67 (B56, PerCP Cy5.5) and Foxp3 (FJK-16S, e450), were stained in 100 μl 1x permeabilisation buffer for 1 h, 4 °C. Samples were washed with 4 ml FACs buffer (600g, 5 min—twice) and data acquired on a BD Fortessa Analyzer and analysed using FlowJo software (Tree Star Inc.). T<sub>reg</sub>s were defined as CD4<sup>+</sup>, Foxp3<sup>+</sup> from singlets within the lymphocyte gate; from this population, CD25 and Foxp3 mean fluorescence intensity (MFI) were calculated for all samples.

[0181] As shown in FIG. 15, 4,000 IU DP47 IgG-IL-2 induced greater up-regulation of Foxp3 and CD25 compared to 40,000 Proleukin. There was no significant increase in Foxp3 or CD25 expression when mice were treated with 4,000 IU Proleukin. In all treatment groups, Foxp3 and CD25 levels returned to baseline 72 h after IL-2 treatment.

In Vivo Treatment with DP47GS IgG-IL-2 Suppresses Immune Responses in Mice

[0182] Since we observed that a 4,000 IU DP47GS IgG-IL-2 dose activated mouse Foxp3<sup>+</sup> regulatory T cells in vivo, this dose was used to assess its ability to suppress immune responses in mice (FIGS. 16 and 17).

[0183] NOD mice and C57BL/6 mice (n=7) were immunized IV with sheep red blood cells (srbc) and challenged 3 days later with a bolus of srbc in a single hind foot to induce a delayed type hypersensitivity (DTH) response. One day after challenge, mice were euthanized with CO<sub>2</sub> and the paws excised and weighed. The magnitude of the DTH response is shown as the change in paw weight compared to non-immunized mice (A paw weight). DP47GS IgG-IL-2 was given SC at 4,000 IU per mouse 3 days before and on the day of srbc immunization and the vehicle was sterile PBS pH 7.2. Statistical significance was derived from the Mann Whitney test in GraphPad Prism.

[0184] Dosing DP47GS IgG-IL-2 three days before and on the day of sheep red blood cell immunization suppressed the subsequent delayed type hypersensitivity response to a sheep blood cell challenge by 51% in NOD mice (FIG. 16A; p=0.0023) and 38% in C57BL/6 mice (FIG. 16B; p=0.002).

[0185] DP47GS IgG-IL-2 was also able to suppress KLH-specific IgG responses in C57BL/6 (78% inhibition, p=0.0007, FIG. 17A) and NOD mice (67% inhibition, p=0.004, FIG. 17B). For this experiment, healthy young C57BL/6 mice (n=7-10) and NOD mice (n=13-14) were immunized IP with 100 μg of human vaccine grade KLH without adjuvant as recommended by the manufacturer (Stellar). DP47GS IgG-IL-2 treatment consisted of 1 (NOD) or 2 (C57BL/6) weekly treatments with 4,000 IU per mouse SC initiated on the day of immunization. Seven days (NOD) and 21 days (C57BL/6) after immunization, blood was collected and serum KLH-specific IgG responses were measured by ELISA.

[0186] The ability of DP47GS IgG-IL-2 to suppress immune responses in vivo supports the hypothesis that the regulatory T cell activation induced by low dose IL-2 produces functional regulatory T cells that mediate a reduction in the immune response.

[0187] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.
<table>
<thead>
<tr>
<th>20</th>
<th>25</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Ala Thr Glu Leu Lys His Leu Glu Cys Leu Glu Glu Glu Leu Lys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile Ser Thr Leu Thr</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

-continued

<table>
<thead>
<tr>
<th>35</th>
<th>40</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Ala Thr Glu Leu Lys His Leu Glu Cys Leu Glu Glu Glu Leu Lys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>50</th>
<th>55</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Ala Thr Glu Leu Lys His Leu Glu Cys Leu Glu Glu Glu Leu Lys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>65</th>
<th>70</th>
<th>75</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Ala Thr Glu Leu Lys His Leu Glu Cys Leu Glu Glu Glu Leu Lys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>90</th>
<th>95</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Ala Thr Glu Leu Lys His Leu Glu Cys Leu Glu Glu Glu Leu Lys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

210: SEQ ID NO 2
211: LENGTH: 399
212: TYPE: DNA
213: ORGANISM: Artificial Sequence
220: FEATURE:
222: OTHER INFORMATION: Wild-type human IL-2
400: SEQUENCE: 2

```plaintext
acactcactt cagcttctc aacgaaacaa cagcttacaac tggagcattt actgtggtgat 60
ttcagcagat tttgaatgg aataataat tacaaacat ccaaacac caggatgctc 120
acattaaga tttaagaatc cacaagcatc cagacattg caacatcctc gtagctgagaa 180
gacagactc taacctgcga ggaagtgc aattagctc aacgaaacaa ctttcaccta 240
agccacgcg acattcagc caaatcaca gtaatgctc tggagcataac gggatcagaa 300
acaacacca tgtctgga aacgagc aacgaaacaa tctgagattt tctgacacaga 360
tggacttcttt tttcagcattt cagcttacac tggagcattt actgtggtgat 399
```

210: SEQ ID NO 3
211: LENGTH: 133
212: TYPE: PRT
213: ORGANISM: Artificial Sequence
220: FEATURE:
222: OTHER INFORMATION: Wild-type human IL-2 (C125A)
400: SEQUENCE: 3

```plaintext
Ala Pro Thr Ser Ser Ser Thr Lys Thr Gln Leu Gln Leu Glu His 1 5 10 15
Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys 20 25 30
Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys 35 40 45
Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys 50 55 60
Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu 65 70 75 80
Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu 85 90 95
Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala 100 105 110
```
<210> SEQ ID NO 4
<211> LENGTH: 399
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Wild-type human IL-2 (C125A) (1)

<400> SEQUENCE: 4

gtcctctacat cctccagcac caagaaacc cagctcagc tggaacatct cctgctggtat
  60
ctgggagatga tcgtgaaagg catcaacaac tacagaacc ccagactgc cggagacttg
  120
acccgtaagt tttcatgccc caagaggtcc acggagctga aacatctgca tggctcggaa
  180
ggccactgta aggctcctgga aagagtcttgac aacgtgagccc agtgagcaag ccctcagcttg
  240
agggctccaggg acctctgactc caacatcaac gtgagctggtc tggaactgaa gcggctcggg
  300
acacatctca tgctgatgta agcgcagcac acagtcacca tcgtgagctt tctgacggg
  360
tggatccatctc ggcgcacagc cattctgcc acctgagcc
  399

<210> SEQ ID NO 5
<211> LENGTH: 399
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Wild-type human IL-2 (C125A) (2)

<400> SEQUENCE: 5

gcactctcttg caagttctac caagaaaca caagttctac ccagtcacac ccagtcactt acctgctggtat
  60
tttgagtttt gtttatttatt attttatttt tatttttttt ttgctggtagc ccagactgc cggagacttg
  120
accaaaaggg ttctctgccc caagaggtcc acggagctga aacatctgca tggctcggaa
  180
ggagagcactgta ggaagctcagta aatattgagttt ccagagcaaa ccccctgttta
  240
acccagctgagc agctctgaag caaatctcactgta gtaatagctg tggagcatgtg gggagctgga
  300
acacatctca tgctgagtttt gttgctgagc acagcagcag ctgctgagtttt tctgacggg
  360
tggatccatctc ggcgcacagc cattctgcc acctgagcc
  399

<210> SEQ ID NO 6
<211> LENGTH: 399
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Wild-type human IL-2 (C125A) (3)

<400> SEQUENCE: 6

gtcctctacat cagctcagc caagaaacc cagctcagc tggaacatct gctgctggtat
  60
tggggagatga tcgtgaaagg catcaacaac tacagaacc ccagactgc cggagacttg
  120
acccgtaagt tttcatgccc caagaggtcc acggagctga aacatctgca tggctcggaa
  180
ggccactgta aggctcctgga aagagtcttgac aacgtgagccc agtgagcaag ccctcagcttg
  240
agggctccaggg acctctgactc caacatcaac gtgagctggtc tggaactgaa gcggctcggg
  300
acacatctca tgctgatgta agcgcagcac acagtcacca tcgtgagctt tctgacggg
  360
<210> SEQ ID NO: 7
<211> LENGTH: 399
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<222> FEATURE: OTHER INFORMATION: Wild-type IL-2 (C125A) (4)
<400> SEQUENCE: 7

tggatcacct tgccccagag catatcgac accctgaca 399

tacatgta tttgaaatgg aattataaat tacaagaact ccaacttcac caggatgc 120

<210> SEQ ID NO: 8
<211> LENGTH: 227
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 8

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1  5  10  15
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
20  25  30
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
35  40  45
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Gly Val
50  55  60
His Asn Ala lys Thr Lys Pro Arg Glu Glu Gin Tyr Asn Ser Thr Tyr
65  70  75  80
Arg Val Val Ser Val Leu Thr Val Leu His Gin Asp Trp Leu Asn Gly
85  90  95
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
100 105 110
Glu Lys Thr Ile Ser Lys Ala Lys Gin Gin Pro Arg Glu Pro Gin Val
115 120 125
Tyr Thr Leu Pro Pro Ser Asp Glu Leu Thr Lys Asn Gin Val Ser
130 135 140
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145 150 155 160
Trp Glu Ser Asn Gin Pro Glu Asn Gin Tyr Lys Thr Thr Pro Pro
165 170 175
Val Leu Asp Ser Asp Gin Ser Asp Phe Leu Tyr Ser Lys Leu Thr Val
180 185 190
Asp Lys Ser Arg Trp Gin Gin Gly Asn Val Phe Ser Cys Ser Val Met
195 200 205
His Glu Ala Leu His Asn His Tyr Thr Gin Lys Ser Leu Ser Leu Ser
210 215 220
Pro Gly Lys
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser 50 55 60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu 65 70 75 80
Pro Glu Asp Phe Ala Val Tyr Cys Gln Gln Tyr Gly Ser Ser Pro 85 90 95
Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys 100 105

<210> SEQ ID NO 12
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DP47GS VL

<400> SEQUENCE: 12

gaaatcgcgt taaacgcgtc tccaggcacc ctagtctttgt tctcagggga aagagccacc 60
cctctttgca ggcgcgctca gactgttagc agcagcact ttagcctgta cccagcagaa 120
cctgagcagctcaggcagtctctatccagcgcctcatt gctgcgtccga gctgcgtccga 180
gaaggtagc gtaggagcctagcttagcctactccttgcta cagcttcag cagcttcag 240
cctgagagcttcctgctctcgcttagcttggctagcttggctagcttggctagcttggctag 300
caggtgacca aagtggaat caga 324

<210> SEQ ID NO 13
<211> LENGTH: 592
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DP47GS HC(Fc Knob, P329G LALA)-IL2

<400> SEQUENCE: 13

Glu Val Gln Leu Leu 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
Ala Met Ser Thr Val Arg Gln Ala Pro Gly Lys Gly Leu Gln Trp Val 30 35 40
Ser Ala Ile Ser Gly Ser Gly Ser Thr Tyr Ala Asp Ser Val 45 50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Aaa Ser Lys Thr Leu Tyr 65 70 75 80
Leu Gln Met Aaa Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Cys 95 90 95
Ala Lys Gly Ser Gly Phe Asp Tyr Trp Gly Gin Gly Thr Leu Val Thr 100 105 110
Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro 115 120 125
Ser Ser Lys Ser Thr Ser Gly Thr Ala Ala Leu Gly Cys Leu Val 130 135 140
Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Thr Asp Ser Gly Ala 145 150 155 160
Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gin Ser Ser Gly 165 170 175
<table>
<thead>
<tr>
<th>Leu</th>
<th>Tyr</th>
<th>Ser</th>
<th>Leu</th>
<th>Ser</th>
<th>Ser</th>
<th>Val</th>
<th>Val</th>
<th>Thr</th>
<th>Val</th>
<th>Pro</th>
<th>Ser</th>
<th>Ser</th>
<th>Ser</th>
<th>Leu</th>
<th>Gly</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>185</td>
<td>190</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td>Gln</td>
<td>Thr</td>
<td>Tyr</td>
<td>Ile</td>
<td>Cys</td>
<td>Asn</td>
<td>Val</td>
<td>Asn</td>
<td>His</td>
<td>Lys</td>
<td>Pro</td>
<td>Ser</td>
<td>Asn</td>
<td>Thr</td>
<td>Lys</td>
</tr>
<tr>
<td>195</td>
<td>200</td>
<td>205</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>Asp</td>
<td>Lys</td>
<td>Val</td>
<td>Glu</td>
<td>Pro</td>
<td>Lys</td>
<td>Ser</td>
<td>Cys</td>
<td>Asp</td>
<td>Lys</td>
<td>Thr</td>
<td>His</td>
<td>Thr</td>
<td>Cys</td>
<td></td>
</tr>
<tr>
<td>210</td>
<td>215</td>
<td>220</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>Pro</td>
<td>Cys</td>
<td>Pro</td>
<td>Ala</td>
<td>Pro</td>
<td>Glu</td>
<td>Ala</td>
<td>Ala</td>
<td>Gly</td>
<td>Gly</td>
<td>Pro</td>
<td>Ser</td>
<td>Val</td>
<td>Phe</td>
<td>Leu</td>
</tr>
<tr>
<td>225</td>
<td>230</td>
<td>235</td>
<td>240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>Pro</td>
<td>Pro</td>
<td>Lys</td>
<td>Pro</td>
<td>Lys</td>
<td>Asp</td>
<td>Thr</td>
<td>Leu</td>
<td>Met</td>
<td>Ile</td>
<td>Ser</td>
<td>Arg</td>
<td>Thr</td>
<td>Pro</td>
<td>Glu</td>
</tr>
<tr>
<td>245</td>
<td>255</td>
<td>255</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>Thr</td>
<td>Cys</td>
<td>Val</td>
<td>Val</td>
<td>Val</td>
<td>Val</td>
<td>Ser</td>
<td>His</td>
<td>Glu</td>
<td>Asp</td>
<td>Pro</td>
<td>Glu</td>
<td>Val</td>
<td>Lys</td>
<td></td>
</tr>
<tr>
<td>260</td>
<td>265</td>
<td>270</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>Asn</td>
<td>Trp</td>
<td>Tyr</td>
<td>Val</td>
<td>Asp</td>
<td>Gly</td>
<td>Val</td>
<td>Glu</td>
<td>Val</td>
<td>His</td>
<td>Asn</td>
<td>Ala</td>
<td>Lys</td>
<td>Thr</td>
<td>Lys</td>
</tr>
<tr>
<td>275</td>
<td>280</td>
<td>285</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>Arg</td>
<td>Glu</td>
<td>Glu</td>
<td>Gln</td>
<td>Tyr</td>
<td>Asn</td>
<td>Ser</td>
<td>Thr</td>
<td>Tyr</td>
<td>Arg</td>
<td>Val</td>
<td>Ser</td>
<td>Val</td>
<td>Leu</td>
<td></td>
</tr>
<tr>
<td>290</td>
<td>295</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td>Val</td>
<td>Leu</td>
<td>His</td>
<td>Gln</td>
<td>Asp</td>
<td>Trp</td>
<td>Leu</td>
<td>Asn</td>
<td>Gly</td>
<td>Lys</td>
<td>Glu</td>
<td>Tyr</td>
<td>Lys</td>
<td>Cys</td>
<td>Lys</td>
</tr>
<tr>
<td>305</td>
<td>310</td>
<td>315</td>
<td>320</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>Ser</td>
<td>Asn</td>
<td>Lys</td>
<td>Ala</td>
<td>Leu</td>
<td>Gly</td>
<td>Ala</td>
<td>Pro</td>
<td>Ile</td>
<td>Glu</td>
<td>Lys</td>
<td>Thr</td>
<td>Ile</td>
<td>Ser</td>
<td>Lys</td>
</tr>
<tr>
<td>325</td>
<td>330</td>
<td>335</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>Lys</td>
<td>Gly</td>
<td>Gln</td>
<td>Pro</td>
<td>Arg</td>
<td>Glu</td>
<td>Pro</td>
<td>Gln</td>
<td>Val</td>
<td>Tyr</td>
<td>Thr</td>
<td>Leu</td>
<td>Pro</td>
<td>Pro</td>
<td>Cys</td>
</tr>
<tr>
<td>340</td>
<td>345</td>
<td>350</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>Asp</td>
<td>Glu</td>
<td>Leu</td>
<td>Thr</td>
<td>Lys</td>
<td>Asn</td>
<td>Val</td>
<td>Ser</td>
<td>Leu</td>
<td>Thr</td>
<td>Trp</td>
<td>Cys</td>
<td>Leu</td>
<td>Val</td>
<td>Lys</td>
</tr>
<tr>
<td>355</td>
<td>360</td>
<td>365</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>Phe</td>
<td>Tyr</td>
<td>Pro</td>
<td>Ser</td>
<td>Asp</td>
<td>Ile</td>
<td>Ala</td>
<td>Val</td>
<td>Glu</td>
<td>Trp</td>
<td>Glu</td>
<td>Ser</td>
<td>Asn</td>
<td>Gly</td>
<td>Gln</td>
</tr>
<tr>
<td>370</td>
<td>375</td>
<td>380</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>Glu</td>
<td>Asn</td>
<td>Asn</td>
<td>Tyr</td>
<td>Lys</td>
<td>Thr</td>
<td>Thr</td>
<td>Pro</td>
<td>Val</td>
<td>Leu</td>
<td>Asp</td>
<td>Ser</td>
<td>Asp</td>
<td>Gly</td>
<td></td>
</tr>
<tr>
<td>385</td>
<td>390</td>
<td>395</td>
<td>400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>Phe</td>
<td>Phe</td>
<td>Leu</td>
<td>Tyr</td>
<td>Ser</td>
<td>Lys</td>
<td>Leu</td>
<td>Thr</td>
<td>Val</td>
<td>Asp</td>
<td>Ser</td>
<td>Arg</td>
<td>Trp</td>
<td>Gln</td>
<td></td>
</tr>
<tr>
<td>405</td>
<td>410</td>
<td>415</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln</td>
<td>Gly</td>
<td>Asn</td>
<td>Val</td>
<td>Phe</td>
<td>Ser</td>
<td>Cys</td>
<td>Ser</td>
<td>Val</td>
<td>Met</td>
<td>His</td>
<td>Glu</td>
<td>Ala</td>
<td>Leu</td>
<td>His</td>
<td>Asn</td>
</tr>
<tr>
<td>420</td>
<td>425</td>
<td>430</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>His</td>
<td>Tyr</td>
<td>Thr</td>
<td>Gln</td>
<td>Lys</td>
<td>Ser</td>
<td>Leu</td>
<td>Ser</td>
<td>Leu</td>
<td>Ser</td>
<td>Pro</td>
<td>Gly</td>
<td>Gly</td>
<td>Gly</td>
<td>Gly</td>
<td>Gly</td>
</tr>
<tr>
<td>435</td>
<td>440</td>
<td>445</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>Gly</td>
<td>Gly</td>
<td>Gly</td>
<td>Ser</td>
<td>Gly</td>
<td>Gly</td>
<td>Gly</td>
<td>Gly</td>
<td>Ser</td>
<td>Ala</td>
<td>Pro</td>
<td>Thr</td>
<td>Ser</td>
<td>Ser</td>
<td></td>
</tr>
<tr>
<td>450</td>
<td>455</td>
<td>460</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>Thr</td>
<td>Lys</td>
<td>Thr</td>
<td>Gln</td>
<td>Leu</td>
<td>Gln</td>
<td>Leu</td>
<td>Glu</td>
<td>His</td>
<td>Leu</td>
<td>Leu</td>
<td>Asp</td>
<td>Leu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>465</td>
<td>470</td>
<td>475</td>
<td>480</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln</td>
<td>Met</td>
<td>Ile</td>
<td>Leu</td>
<td>Asn</td>
<td>Gly</td>
<td>Ile</td>
<td>Asn</td>
<td>Asn</td>
<td>Tyr</td>
<td>Lys</td>
<td>Asn</td>
<td>Pro</td>
<td>Lys</td>
<td>Leu</td>
<td>Thr</td>
</tr>
<tr>
<td>485</td>
<td>490</td>
<td>495</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>Met</td>
<td>Leu</td>
<td>Thr</td>
<td>Phe</td>
<td>Lys</td>
<td>Phe</td>
<td>Tyr</td>
<td>Met</td>
<td>Pro</td>
<td>Lys</td>
<td>Ala</td>
<td>Thr</td>
<td>Glu</td>
<td>Leu</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>505</td>
<td>510</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>His</td>
<td>Leu</td>
<td>Gln</td>
<td>Cys</td>
<td>Leu</td>
<td>Glu</td>
<td>Glu</td>
<td>Leu</td>
<td>Lys</td>
<td>Pro</td>
<td>Leu</td>
<td>Glu</td>
<td>Glu</td>
<td>Val</td>
<td></td>
</tr>
<tr>
<td>515</td>
<td>520</td>
<td>525</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Asn</td>
<td>Leu</td>
<td>Ala</td>
<td>Gln</td>
<td>Ser</td>
<td>Lys</td>
<td>Asn</td>
<td>Phe</td>
<td>His</td>
<td>Leu</td>
<td>Arg</td>
<td>Pro</td>
<td>Arg</td>
<td>Asp</td>
<td>Leu</td>
</tr>
<tr>
<td>530</td>
<td>535</td>
<td>540</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile</td>
<td>Ser</td>
<td>Asn</td>
<td>Ile</td>
<td>Asn</td>
<td>Val</td>
<td>Ile</td>
<td>Val</td>
<td>Leu</td>
<td>Glu</td>
<td>Leu</td>
<td>Lys</td>
<td>Gly</td>
<td>Ser</td>
<td>Glu</td>
<td>Thr</td>
</tr>
<tr>
<td>545</td>
<td>550</td>
<td>555</td>
<td>560</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td>Phe</td>
<td>Met</td>
<td>Cys</td>
<td>Glu</td>
<td>Tyr</td>
<td>Ala</td>
<td>Asp</td>
<td>Glu</td>
<td>Thr</td>
<td>Ala</td>
<td>Thr</td>
<td>Ile</td>
<td>Val</td>
<td>Glu</td>
<td>Phe</td>
</tr>
<tr>
<td>565</td>
<td>570</td>
<td>575</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Asn</td>
<td>Arg</td>
<td>Trp</td>
<td>Ile</td>
<td>Thr</td>
<td>Phe</td>
<td>Ala</td>
<td>Gln</td>
<td>Ser</td>
<td>Ile</td>
<td>Ile</td>
<td>Ser</td>
<td>Thr</td>
<td>Leu</td>
<td>Thr</td>
</tr>
<tr>
<td>580</td>
<td>585</td>
<td>590</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
<210> SEQ ID NO 15
<211> LENGTH: 445
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DP47GS HC (Pc knob, P329G LALA) - IL2

<400> SEQUENCE: 15

gaggytcgaat tgtggagct tgtggagagc tgtggagagc tgtggagagc tgtggagagc 60
tctgtgcag ccttcggatt cactttagc agattgcoca tgtgcgggggt gcgcctagct 120
cagggaag ggtgcggagt gtgtcgtact attagtctgt tgtgcgtttag ccacatctac 180
gcaagtccog tgaaggggag gttacacttc cccagacca attccaaagac caagtcgtat 240
cctgaagttga gacgctgtag agcgag gagacgctgtata ctactgtgg aaagcagcag 300
gatatgtcc atctgggccc agggacctctg tgcgtgcct cgagtcgctg caccaagcgc 360
cattggtct tctcctgtgg aacccctccc aagagacact cttcgggccc agggggcctg 420
ggcggcgcc tctacagca cctcaggtact ctcacagct ctacagcctc 480
cctgacagcc gcgtacagcc cttcagctgc tgcgtgcct ctacagcctc 540
ggcggcacgc tgcgtgcct ctacagcctgc tgtggcagccc agagccatac ctgcaagcgt 600
aatcacaacg cccaccaacac caggtggagc agccaaagtt agccaaatact tgtgcaacaa 660
acctcaacgt gcccagcactg cccagacact gaaggtgccag gggacactgt gcggtccttc 720
tttcctccac ccacccagga caccctctag atctccggga cctcctgagtc cacatgcgtg 780
ggtgcggcg tgcagcagca agacccttag gttacagttc cactgttaagt gggagcctg 840
gagctgctata atgcaccacg acagcgccgg gcggacgctg acacacgac gcacgtcgtg 900
gtcacagtc ccagcgtcct gcacaccggc tgcgtaagag gcagaggtga caagtgaaag 960
gtcgacacca cagctgctgg gcggccctac gacccacac ctcacccaaac cagggcag 1020
cccgagacac caacgctgta cacttgccoe ccagtgcgggg atgagcgcac caaagcag 1080
gtcacagcgtg ggcgctgctag caagcttctc tctccagcagc acatcgcgtg gggttgagag 1140
agcaatgggc agcgggagaa caatcacaag acacgcgctc cgtgcctgga ctcagacgcc 1200
tctctctcc tctacacca gtcacccgtg gcacaacgca ggcgtgccag cggggacgctc 1260
tttcagctg cctgctgact cggagctcag gcacacctc acacgcgaaag ggcctctgcc 1320
cggcgccag gcggcggagg aggcgcgagc ggggagagtt tgtggagagc ggcggcgcag 1380
cctctcaca gttcctcaac caaagcagag ctcaagctct gcgacgtctg cgtggatatta 1440
cagctcgatt ttaaatagc aacagtcaca caaagcagag atgctcaca 1500
}
Glu Val Gln Leu Leu 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
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Glu Trp Val
35   40   45
Ser Ala Ile Ser Gly Ser Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50   55   60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
45   70   75   80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85   90
95
Ala Lys Gly Ser Gly Phe Asp Tyr Trp Gly Gin Gin Gly Thr Leu Val Thr
100 105   110
Val Ser Ser Ala Thr Lys Pro Ser Val Phe Pro Leu Ala Pro
115 120  125
Ser Ser Lys Ser Thr Ser Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135  140
Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150  155  160
Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gin Ser Ser Gly
165 170  175
Leu Tyr Ser Leu Ser Val Val Thr Val Ser Ser Ser Leu Gly
180 185  190
Thr Glu Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200  205
Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215  220
Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu
225 230  235  240
Phe Pro Pro Lys Pro Lys Phe Pro Thr Met Ile Ser Arg Thr Pro Glu
245 250  255
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
260 265  270
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280  285
Pro Arg Glu Glu Gin Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
290 295  300
Thr Val Leu His Gin Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310  315  320
Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330  335
Ala Lys Gly Gin Pro Arg Glu Pro Gin Val Cys Thr Leu Pro Pro Ser
340 345  350
Arg Asp Glu Leu Thr Lys Asn Gin Val Ser Leu Ser Cys Ala Val Lys
355 360  365
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gin
370 375  380
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
-continued

385 390 395 400
Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Ann
420 425 430
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> SEQ ID NO: 16
<211> LENGTH: 1335
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DP470S HC (Fc wt, P329G LALA)

<400>SEQUENCE: 16

gaggtgcaat tgggtggtgc tggggagggc tgggtacagc ctaggcccct cctgagactc 60
tcgtggtcct ccacgctgat cacccttctg cgtattgtaa cggaagtgggt ctacactacc 120
ccaggaagag gcctttggtg cggcgtctag atcatagctc ttgggaagct ctcactacc 180
gcagcgctt gcggccctcg cgggctacag ctcacccaga ttttagcagc ttcaggtgta 240
cctcagagct aacgctttgc aacgaggagc aacggcctgt aagtaagctg gaaagcggcc 300
ggagttgact actgtgagcg cagagaaactct gtcagcagtg caggaagtgct cagcaggtga 360
cacccggttg ctcacccgcag ccccgcagcg ggcgggagc aagcggctgt cgttccgcct 420
ggagttgctgg ttcagccgct gccggccgcc cctctgtgcc gcgtgtgctg ctacactacc 480
cggctgcctgc ggtggtcctgc cactgggtcc tgtcagcctg ccacagggct ctcgcagcag 540
agcgcggttc gcacggccgc tcctgagcag ccataagcct cgtcagcag 600
aacaggaagc caagcgagcag cccgggctag ctcacccgcag ctcctgctgc gcggccgctg 660
aadcccgctg ccggacagat ggccgctgct cgtgcgagcg cccggagggc 720
ttcaccccaag cccggggtgc ccccgccttc atggccgggt ctgtgggact ctacactacc 780
gggctggctgg cagccgagc cagcgtctgg cttccggctgc gcgtgtgctg gcgtgtgctg 840
ggaggtgcaat atgcaggcgc cgaggaggtc gcggcagcc gcggcagccgc gcggcagccgc 900
gtgcagcgct ctcacccgct gcacggcggc gcggccgctg gcggccgctg gcggccgctg 960
gtcaggggagt gcccgggagt gcccgggagt gcccgggagt gcccgggagt gcccgggagt 1020
cagaggggagc cgggctccgc cccccggctcc gcggcagccgc gcggcagccgc gcggcagccgc 1080
gtgcagcctc gcggcagccgc gcggcagccgc gcggcagccgc gcggcagccgc gcggcagccgc 1140
agcaggggagt gcccgggagt gcccgggagt gcccgggagt gcccgggagt gcccgggagt 1200
ttcacccgcag gcggcagccgc gcggcagccgc gcggcagccgc gcggcagccgc gcggcagccgc 1260
ttcacccgcag gcggcagccgc gcggcagccgc gcggcagccgc gcggcagccgc gcggcagccgc 1320
cagaggggagc cgggctccgc cccccggctcc gcggcagccgc gcggcagccgc gcggcagccgc 1385

<210> SEQ ID NO: 17
<211> LENGTH: 592
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DP470S HC (Fc wt, P329G LALA)-IL2

<400>SEQUENCE: 17
Glu Val Gln Leu Leu Leu 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
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 35 40 45
Ser Ala Ile Ser Gly Ser Gly Ser Thr Tyr Tyr Ala Asp Ser Val 50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr 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 Lys Gly Ser Gly Phe Asp Tyr Trp Gly Gin Gly Thr Leu Val Thr 100 105 110
Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro 115 120 125
Ser Ser Lys Ser Thr Ser Gly Thr Ala Ala Leu Gly Cys Leu Val 130 135 140
Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala 145 150 155 160
Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly 165 170 175
Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly 180 185 190
Thr Gln Thr Tyr Ile Cys Asn Val Val Asn His Lys Pro Ser Asn Thr Lys 195 200 205
Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys 210 215 220
Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu 225 230 235 240
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu 245 250 255
Val Thr Cys Val Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys 260 265 270
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys 275 280 285
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu 290 295 300
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys 305 310 315 320
Val Ser Asn Asp Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys 325 330 335
Ala Lys Gly Gln Pro Arg Glu Pro Glu Val Tyr Thr Leu Pro Pro Ser 340 345 350
Arg Asp Glu Leu Thr Lys Asn Glu Val Ser Leu Thr Cys Leu Val Lys 355 360 365
Gly Phe Tyr Pro Ser Asp Ile Ala Ala Glu Trp Glu Ser Asn Gly Gln 370 375 380
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly 385 390 395 400
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln 405 410 415
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn 420
425 430
His Tyr Thr Gin Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly Gly
435 440 445
Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Ser Pro Thr Ser Ser
450 455 460
Ser Thr Lys Thr Gin Leu Gin Leu Glu His Leu Leu Leu Asp Leu
465 470 475 480
Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr
485 490 495
Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys Ala Thr Glu Leu
500 505 510
Lys His Leu Gin Cys Leu Gin Glu Gin Leu Gin Leu Lys Pro Leu Glu Glu Val
515 520 525
Leu Asn Leu Ala Gin Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu
530 535 540
Ile Ser Asn Ile Val Ile Val Leu Gin Leu Lys Gly Ser Glu Thr
545 550 555 560
Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Val Gin Phe
565 570 575
Leu Asn Arg Trp Ile Thr Phe Ala Gin Ser Ile Ser Ile Ser Thr Leu Thr
580 585 590

<210> SEQ ID NO 18
<211> LENGTH: 1776
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DP47GS HC (Fc wt, P329G LALA)-IL2
<400> SEQUENCE: 18

...
D.M. Wilson et al., "Comparison of the antioxidant properties of..." (Manuscript not shown)
<210> SEQ ID NO 20  
<211> LENGTH: 645  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: DP470S LC  

<400> SEQUENCE: 20  

```
gaactgcgtg taaacgagtgc tccaggccac cttgtctttgt ctoctcagggga aagagccacc  
gctcttgcga ggcggcagtc gatgttgtac acgcacgtact tagcttggta ccaacgaga  
cctgcagcag cctccagctt cctcactatat ggaagatcca gcaagggccac tggcatcoca  
gcaggtgctc gttgcagtgct atcggagacag acttcacaactc tcacactcag cagacttgag  
cctgagatt tgtgctgtca cagataggtta gctcaccgct gaagttgagc  
caggggaaca aagttgaaac caacagctacg gttgcttgac cactctgtctt catotctccg  
ccatctgtgy agactgtgaa attcggagcc gcctctgttg bgtgcctgtc gataaactcc  
tatccgagc agcggcaagtc acagttgaa gttgataaag cccctcaac gcggtaaactcc  
cagaggtgt ccacagacga gcaagacagg cagacagctc acagcacttc cagacactcctg  
aegctgagaco aagagacga caagagacac aaggtcaac cttcgaaagt caccatccag  
ggcctcgagt gcgcctctac aacagggag aagtgt  
```  

<210> SEQ ID NO 21  
<211> LENGTH: 466  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Human IL-2R-beta-Fc(hole) fusion protein  

<400> SEQUENCE: 21  

```
Met Asp Met Arg Val Pro Ala Leu Leu Gly Leu Leu Leu Leu Leu Leu Trp  
Phe Pro Gly Ala Arg Cys Ala Val Asn Gly Thr Ser Gln Phe Thr Cys  
Phe Tyr Asn Ser Arg Ala Asn Ile Ser Cys Val Trp Ser Gln Asp Gly  
Ala Leu Gln Asp Thr Ser Cys Gin Val His Ala Trp Pro Asp Arg Arg  
Arg Trp Asn Gin Thr Cys Glu Leu Leu Pro Val Ser Gin Ala Ser Trp  
Ala Cys Asn Leu Ile Leu Gly Ala Pro Asp Ser Gin Lys Leu Thr Thr  
Val Asp Ile Val Thr Leu Arg Val Leu Cys Arg Gin Gly Val Arg Trp  
Arg Val Met Ala Ile Gin Asp Phe Lys Pro Phe Gln Asn Leu Arg Leu  
Met Ala Pro Ile Ser Leu Gin Val Val His Val Gly Thr His Arg Cys  
Asn Ile Ser Thr Glu Ile Ser Gin Ala Ser His Tyr Phe Glu Arg His  
Leu Glu Phe Glu Ala Arg Thr Leu Ser Pro Gin His Thr Trp Glu  
```
-continued

<table>
<thead>
<tr>
<th>Ala</th>
<th>Pro</th>
<th>Leu</th>
<th>Leu</th>
<th>Thr</th>
<th>Leu</th>
<th>Lys</th>
<th>Glu</th>
<th>Gln</th>
<th>Glu</th>
<th>Trp</th>
<th>Ile</th>
<th>Cys</th>
<th>Leu</th>
<th>Glu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td>Leu</td>
<td>Thr</td>
<td>Pro</td>
<td>Asp</td>
<td>Thr</td>
<td>Gln</td>
<td>Tyr</td>
<td>Glu</td>
<td>Phe</td>
<td>Glu</td>
<td>Val</td>
<td>Arg</td>
<td>Val</td>
<td>Lys</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Gln</td>
<td>Gln</td>
<td>Glu</td>
<td>Phe</td>
<td>Thr</td>
<td>Glu</td>
<td>Phe</td>
<td>Thr</td>
<td>Trp</td>
<td>Ser</td>
<td>Pro</td>
<td>Trp</td>
<td>Ser</td>
<td>Glu</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>Arg</td>
<td>Thr</td>
<td>Lys</td>
<td>Ala</td>
<td>Ala</td>
<td>Leu</td>
<td>Gln</td>
<td>Gly</td>
<td>Asp</td>
<td>Thr</td>
<td>Gly</td>
<td>Ala</td>
<td>Gln</td>
<td>Asp</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>Thr</td>
<td>His</td>
<td>Thr</td>
<td>Cys</td>
<td>Pro</td>
<td>Pro</td>
<td>Cys</td>
<td>Pro</td>
<td>Ala</td>
<td>Pro</td>
<td>Glu</td>
<td>Leu</td>
<td>Leu</td>
<td>Gly</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>Ser</td>
<td>Val</td>
<td>Phe</td>
<td>Leu</td>
<td>Phe</td>
<td>Pro</td>
<td>Pro</td>
<td>Lys</td>
<td>Pro</td>
<td>Lys</td>
<td>Thr</td>
<td>Leu</td>
<td>Met</td>
<td>Ile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>Arg</td>
<td>Thr</td>
<td>Pro</td>
<td>Glu</td>
<td>Val</td>
<td>Thr</td>
<td>Cys</td>
<td>Val</td>
<td>Val</td>
<td>Val</td>
<td>Asp</td>
<td>Val</td>
<td>Ser</td>
<td>His</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp</td>
<td>Pro</td>
<td>Glu</td>
<td>Val</td>
<td>Lys</td>
<td>Phe</td>
<td>Asn</td>
<td>Trp</td>
<td>Tyr</td>
<td>Val</td>
<td>Asp</td>
<td>Gly</td>
<td>Val</td>
<td>Glu</td>
<td>Val</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asn</td>
<td>Ala</td>
<td>Lys</td>
<td>Thr</td>
<td>Lys</td>
<td>Pro</td>
<td>Arg</td>
<td>Glu</td>
<td>Glu</td>
<td>Tyr</td>
<td>Asn</td>
<td>Ser</td>
<td>Thr</td>
<td>Tyr</td>
<td>Arg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>Val</td>
<td>Ser</td>
<td>Val</td>
<td>Leu</td>
<td>Thr</td>
<td>Val</td>
<td>Leu</td>
<td>His</td>
<td>Gln</td>
<td>Asp</td>
<td>Trp</td>
<td>Leu</td>
<td>Asn</td>
<td>Gly</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>Tyr</td>
<td>Lys</td>
<td>Cys</td>
<td>Lys</td>
<td>Val</td>
<td>Ser</td>
<td>Asn</td>
<td>Lys</td>
<td>Ala</td>
<td>Leu</td>
<td>Pro</td>
<td>Ala</td>
<td>Pro</td>
<td>Ile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>Thr</td>
<td>Ile</td>
<td>Ser</td>
<td>Lys</td>
<td>Ala</td>
<td>Lys</td>
<td>Glu</td>
<td>Pro</td>
<td>Arg</td>
<td>Glu</td>
<td>Pro</td>
<td>Glu</td>
<td>Pro</td>
<td>Glu</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td>Leu</td>
<td>Pro</td>
<td>Pro</td>
<td>Ser</td>
<td>Arg</td>
<td>Asp</td>
<td>Glu</td>
<td>Leu</td>
<td>Thr</td>
<td>Lys</td>
<td>Asn</td>
<td>Glu</td>
<td>Val</td>
<td>Ser</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>Cys</td>
<td>Ala</td>
<td>Val</td>
<td>Lys</td>
<td>Gly</td>
<td>Phe</td>
<td>Tyr</td>
<td>Pro</td>
<td>Ser</td>
<td>Asp</td>
<td>Ile</td>
<td>Ala</td>
<td>Val</td>
<td>Glu</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>Ser</td>
<td>Asn</td>
<td>Gly</td>
<td>Glu</td>
<td>Pro</td>
<td>Glu</td>
<td>Asn</td>
<td>Tyr</td>
<td>Lys</td>
<td>Thr</td>
<td>Thr</td>
<td>Pro</td>
<td>Pro</td>
<td>Val</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Asp</td>
<td>Ser</td>
<td>Asp</td>
<td>Gly</td>
<td>Ser</td>
<td>Phe</td>
<td>Leu</td>
<td>Val</td>
<td>Ser</td>
<td>Lys</td>
<td>Leu</td>
<td>Thr</td>
<td>Val</td>
<td>Asp</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>Ser</td>
<td>Arg</td>
<td>Trp</td>
<td>Gln</td>
<td>Gln</td>
<td>Gly</td>
<td>Asn</td>
<td>Val</td>
<td>Phe</td>
<td>Ser</td>
<td>Cys</td>
<td>Ser</td>
<td>Val</td>
<td>Met</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>Ala</td>
<td>Leu</td>
<td>His</td>
<td>Asn</td>
<td>His</td>
<td>Tyr</td>
<td>Thr</td>
<td>Glu</td>
<td>Lys</td>
<td>Ser</td>
<td>Leu</td>
<td>Ser</td>
<td>Leu</td>
<td>Ser</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>Lys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<210> SEQ ID NO 22
<211> LENGTH: 1401
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Human IL-2R-βc[hole] fusion protein
<400> SEQUENCE: 22
atggaacatga   ggctccocgc   tcagctcctg   ggccctcgctg   tgccctggtt   ccacagctgccc   60
aggtgtggcg   tgaatggcag   ttcacccagtct   acatgctctt   acacactcgag   gcacacatcc   120
tccgtcctg   ggagcccatg   ttggggtcctg   caggaacattg   cctgcacaagct   ccaagcctg   180
tcgggacagcc   ggctgctgaa   ccaacacttg   gacgctgctcc   cgctgtacag   agcactctgg   240
gctgcacacc   tgcacctcgag   ggcccccgat   ttcgacaacgt   tgaccagctg   300
-continued

accttgagg ccgtgtgccg tggaggggtg cagttgaggg gtagtggcct cgaggacttc
  360
aagccctttg agaacacctt gctgatggcc ccacactcct ttagcagttg cccagttgag
  420
accelacagat gcacataaag otgggaaatct cccacagctt ccaccagctt tggagacac
  480
cggaggtgct agggcggcgc gctctccccc gcacacacct gggagggagg cccgcctgct
  540
actctcaagc agaagagagga atggatctgc otggagacgc tccaccca gagacagat
  600
gagtttcagg tggagggctca gctcttgcaaa ggcaggtctca cgaccttgagg ccccttgagc
  660
cagcccctgg ccttcagaaac aagcetctgca gcctttgaggg gaggacaccgg aggtccaggac
  720
aatactcaca cagctccacac gttgccgacca ctgtaacctc cggggagacc actagcttcc
  780
cctccctccc cccacaccct ggacacacct caggctctcgg gcggccctaa gcggccctag
  840
gtgggttggg aggtagacgc c gagacacggct taggtcaggt cggcagcagc aggccctcggc
  900
gtaggggtgc ataatggcacc gagaagagcgg cggagggcgc aggccacagg cagctatggc
  960
gtgctcgagc tctctcagct cggcagcagc gagtcggctga atggcagggg atcagacagt
 1020
aaggctccta acaacagcttc cccaccccc ctcagcagcct ctcagcagc cagcagcagc
1080
cagcccgagc aacgagaggg ggttcacact cccgcctccc cggatgagct gaccaagaac
1140
caggtccagc tctcgctccc agtaacaggg ttcctatccc ggacacattc gctggaggtgg
1200
gagcacagt gggccgaggg gacaactacct cgcagctcggc ggtacggtgg gcacgagac
1260
ggtctcctct tgtctctgag caggtctaccc gtggcagcag gcaggtgaggg gcagggagac
1320
gtctctcact gcgcgcagct gcagttggtc gctgcaagcag ccgggtggag gcagctcagc
1380
tctctgcct cggtgaaaaag a
1401

<210> SEQ ID NO 23
<211> LENGTH: 492
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Human IL-2R-gamma-Pc(knob) fusion protein
<400> SEQUENCE: 23

Met Leu Lys Pro Ser Leu Pro Phe Thr Ser Leu Leu Phe Leu Gln Leu
  1    5  10  15
Pro Leu Leu Gly Val Gly Leu Asn Thr Thr Thr Ile Leu Thr Pro Asn Gly
  20  25  30
Asn Glu Asp Thr Thr Ala Asp Phe Leu Thr Thr Met Pro Thr Asp
  35  40  45
Ser Leu Ser Val Ser Thr Leu Pro Leu Pro Glu Val Glu Cys Phe Val
  50  55  60
Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro
  65  70  75  80
Gln Pro Thr Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn
  85  90  95
Asp Lys Val Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Ile Thr
 100 105 110
Ser Gly Cys Gln Leu Gln Lys Glu Ile His Leu Tyr Gln Thr Phe
 115 120 125
Val Val Gln Leu Gln Asp Pro Arg Glu Pro Arg Glu Ala Thr Gln
 130 135 140
Met Leu Lys Leu Glu Asn Leu Val Ile Pro Thr Ala Pro Glu Asn Leu
 145 150 155 160
Thr Leu His Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn 165 170 175
Arg Phe Leu Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp 180 185 190
Trp Asp His Ser Trp Thr Gln Ser Val Asp Tyr Arg His Lys Phe 195 200 205
Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg 210 215 220
Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp 225 230 235 240
Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Pro Phe 245 250 255
Leu Phe Ala Leu Glu Ala Gly Ala Gln Asp Lys Thr His Thr Cys Pro 260 265 270
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe 275 280 285
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val 290 295 300
Thr Cys Val Val Val Asp Ser His Gln Asp Pro Gln Val Lys Phe 305 310 315 320
Asn Trp Tyr Val Asp Gly Val Val His Asn Ala Lys Thr Lys Pro 325 330 335
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Ser Val Leu Thr 340 345 350
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val 355 360 365
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala 370 375 380
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg 385 390 395 400
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly 405 410 415
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro 420 425 430
Glu Asn Asn Tyr Lys Thr Thr Pro Val Leu Asp Ser Asp Gly Ser 435 440 445
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln 450 455 460
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His 465 470 475 480
Tyr Thr Glu Lys Ser Leu Ser Leu Ser Pro Gly Lys 485 490

<210> SEQ ID NO 24
<211> LENGTH: 1479
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Human IL-2R-gamma-Fc (knob) fusion protein
<400> SEQUENCE: 24
atgtgaaagc catcattacc attoacatcc ctcttatcct tgcagctgcc cctgctgga 60
gtggggtcag acaogacaat tctgaogcccc atatggaagt aagacaccac agtcgtatttc 120
<table>
<thead>
<tr>
<th>Amino Acid Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly</td>
</tr>
<tr>
<td>Val His Ser Glu Leu Cys Asp Asp Asp Pro Pro Pro Glu Ile Pro His Ala</td>
</tr>
<tr>
<td>Thr Phe Lys Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Aam Cys Glu</td>
</tr>
<tr>
<td>Cys Lys Arg Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu</td>
</tr>
<tr>
<td>Cys Thr Gly Asn Ser Ser His Ser Ser Thr Arg Asp Asn Gln Cys Gln Cys</td>
</tr>
<tr>
<td>Thr Ser Ser Ala Thr Arg Ann Thr Lys Glu Val Thr Pro Glu Pro</td>
</tr>
</tbody>
</table>

**Other Information:** Human IL-2R alpha subunit + Avi-tag + His-tag
Pro Val Asp Gln Ala Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro 115
120 125
Trp Glu Asn Gln Ala Thr Glu Arg Ile Tyr His Phe Val Val Gly Gln 130
135 140
Met Val Tyr Tyr Gln Cys Val Gln Gln Gly Tyr Arg Ala Leu His Arg Gly 145
150 155 160
Pro Ala Glu Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr 165
170 175
Gln Pro Glu Leu Ile Cys Thr Gly Val Asp Glu Gln Leu Tyr Phe Gln 180
185 190
Gly Gly Ser Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp 195
200 205
His Glu Ala Arg Ala His His His His His His 210
215

<210> SEQ ID NO 26
<211> LENGTH: 660
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Human IL-2R alpha subunit + Avi-tag + His-tag

<400> SEQUENCE: 26
atgggatggg gctgtatcat ccttctcttg gtagcaacag ctacggtgct gcattccgag 60
cctgtgagc atgacccgag gagatccctg aacgccacat aaaaagcccag 120
gagaagcgct ttgtagcttg tgaatgcaag agaggtttcc gcagataaaa aagcggttca 180
cctatattg tgtgacagc aacgcattgc cactgctct gggccaccac atgtcaatcg 240
cccttgctcc ccacgtggaa ccaacgaaa caaagcagac ctcaacattga agaacaagaa 300
ggaagaaaa cccacagat gcaagttcca atcgagccag tggacaacgc gacgcttccca 360
ggatcactggc ggagaccttc acacagggaa aatgaacgca cagagagat tttcattttc 420
gtggcgcc ggatgttta ttcatcgcc gctccaggat acaagctttt acacacagtt 480
cctgtggaga ggtgtcggaa aatgacccac gggaagacaa gttggacaaca gcccaccgct 540
atgtccagc tgtgcgggc acaattatat ttccagggcg gttcgaggct gaacagacac 600
ttgagggcc agacagagta gttggcaggg gttggagcgt accacactca ccaagactga 660

<210> SEQ ID NO 27
<211> LENGTH: 473
<212> TYPE: PRO
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Murine IL-2R beta-Pr(hole) fusion protein

<400> SEQUENCE: 27
Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp 1
5 10 15
Phe Pro Leu Leu Leu Leu Leu Trp Phe Pro Gly Ala Arg Cys Ala Val Lye 20
25 30
Asn Cys Ser His Leu Glu Cys Phe Tyr Asn Ser Arg Ala Asn Val Ser 35
40 45
Cys Met Trp Ser His Glu Glu Ala Leu Asn Val Thr Thr Cys His Val 50
55 60
His Ala Lys Ser Asn Leu Arg His Trp Asn Lys Thr Cys Glu Leu Thr
Lieu Val Arg Gln Ala Ser Trp Ala Cys Asn Leu Ile Leu Gly Ser Phe
85  90  95
Pro Glu Ser Gln Ser Leu Thr Ser Val Asp Leu Leu Asp Ile Asn Val
100 105 110
Val Cys Trp Glu Glu Lys Gly Trp Arg Arg Val Lys Thr Cys Asp Phe
115 120 125
His Pro Phe Asp Asn Leu Arg Leu Val Ala Pro His Ser Leu Gln Val
130 135 140
Leu His Ile Asp Thr Gln Arg Cys Asn Ile Ser Trp Lys Val Ser Gln
145 150 155 160
Val Ser His Tyr Ile Glu Pro Tyr Leu Glu Phe Glu Ala Arg Arg
165 170 175
Leu Leu Gly His Ser Trp Glu Asp Ala Ser Val Leu Ser Leu Lys Gln
180 185 190
Arg Gln Gln Trp Leu Phe Leu Glu Met Leu Ile Pro Ser Thr Ser Tyr
195 200 205
Glu Val Gln Val Arg Val Lys Ala Gln Arg Asn Asn Thr Gly Thr Trp
210 215 220
Ser Pro Trp Ser Gln Pro Leu Thr Phe Arg Thr Arg Pro Ala Asp Pro
225 230 235 240
Met Lys Glu Gln Gln Asp Asn Thr His Thr Cys Pro Pro Cys Pro
245 250 255
 Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
260 265 270
 Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Gln Val Thr Cys Val
275 280 285
Val Val Asp Val Ser His Gln Asp Pro Glu Val Lys Phe Asn Trp Tyr
290 295 300
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
305 310 315 320
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
325 330 335
Gln Asp Trp Leu Asn Gly Lys Tyr Lys Cys Lys Val Ser Asn Lys
340 345 350
 Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
355 360 365
 Pro Arg Glu Pro Glu Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu
370 375 380
Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro
385 390 395 400
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Glu Pro Glu Asn Asn
405 410 415
 Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
420 425 430
Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
435 440 445
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
450 455 460
Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470
-continued-

<210> SEQ ID NO 28
<211> LENGTH: 1422
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Murine IL-2R-gamma-Fc(knob) fusion protein

<400> SEQUENCE: 28

atggacatga ggtcgcgcgc tcagcctctg tgctctcttt tcacccctctg 60
cgctctcttg tccagacgct cagctgacag gtagaaact agtccacact tgaagtcctc 120
tacaacctag ggaagccagt ccctgtcacc gtagagcttt gatgtcaca 180
aagttgagcc aactcctgct ggctgtaac ccagctgtgta gatagttaact 240
tttgaggAGc aggctagcgg tcagcctctg gctctctctg aagttgagcc 300
tcactacGct tcagcctctg ccctgtcacc gtagaaact agtccacact 360
cgtagagcc aagccctgcgc ccacccctctg tggacacgcc gtggtgctgt tctgggcac 420
tcctcccaag tctgccact ttgatacccg agatgtaaca taagctgctta gtggtgctgt 480
gttctcctag aactgagggc ataatgctcc cttaagagag gaagctgctc ctctcctgag 540
agttgagcc aagccctgcgc ccacccctctg tggacacgcc gtggtgctgt tctgggcac 600
tcctcccaag tctgccact ttgatacccg agatgtaaca taagctgctta gtggtgctgt 660
tcctcccaag tctgccact ttgatacccg agatgtaaca taagctgctta gtggtgctgt 720
tcctcccaag tctgccact ttgatacccg agatgtaaca taagctgctta gtggtgctgt 780
ctgggggagc gctgagctct accttccctc ccacacaccc aacacacactgt atatcct 840
cgagctcctag aagctgctc cttaagagag gaagctgctc ctctcctgag 900
tcaacctgtct aagctgagggc gctgagctct accttccctc ccacacaccc aacacacactgt atatcct 960
cgagctcctag aagctgctc cttaagagag gaagctgctc ctctcctgag 1020

<210> SEQ ID NO 29
<211> LENGTH: 500
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Murine IL-2R-gamma-Fc(knob) fusion protein

<400>SEQUENCE: 29

Met Aep Met Arg Val Pro Ala Gln Leu Leu Gln Leu Leu Leu Leu Trp 1 5 10 15
Phe Pro Leu Leu Leu Leu Trp Phe Pro Gln Ala Arg Cye Trp Ser Ser 20 25 30
Lys Val Leu Met Ser Ser Ala Asn Glu Asp Ile Lys Ala Amp Leu Ile 35 40 45
-continued

Leu Thr Ser Thr Ala Pro Glu His Leu Ser Ala Pro Thr Leu Pro Leu
50 55 60
Pro Glu Val Gln Cys Phe Val Phe Asn Ile Glu Tyr Met Asn Cys Thr
45 70 75 80
Trp Asn Ser Ser Ser Glu Pro Gln Ala Thr Asn Leu Thr Leu His Tyr
85 90 95
Arg Tyr Lys Val Ser Asp Asn Thr Phe Glu Gln Cys Ser His Tyr
100 105 110
Leu Phe Ser Lys Glu Ile Thr Ser Gly Cys Gln Ile Gln Lys Glu Asp
115 120 125
Ile Gln Leu Tyr Gln Thr Phe Val Glu Leu Glu Gln Asp Pro Gln Lys
130 135 140
Pro Gln Arg Arg Ala Val Gln Lys Leu Asn Leu Gln Asn Leu Val Ile
145 150 155 160
Pro Arg Ala Pro Glu Asn Leu Thr Leu Ser Asn Leu Ser Glu Ser Gln
165 170 175
Leu Glu Leu Arg Trp Lys Ser Arg His Ile Lys Glu Arg Cys Leu Gln
180 185 190
Tyr Leu Val Gln Tyr Arg Ser Asn Arg Asp Arg Ser Thr Thr Glu Leu
195 200 205
Ile Val Asn His Glu Pro Arg Phe Ser Leu Pro Ser Val Asp Glu Leu
210 215 220
Lys Arg Tyr Thr Phe Arg Val Arg Ser Arg Tyr Asn Pro Ile Cys Gly
225 230 235 240
Ser Ser Gln Glu Trp Ser Lys Trp Ser Gln Pro Val His Trp Gln Ser
245 250 255
His Thr Val Glu Glu Asn Pro Ser Leu Phe Ala Leu Glu Ala Gly Ala
260 265 270
Gln Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Glu Leu Leu
275 280 285
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Asp Thr Leu
290 295 300
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Asp Val Ser
305 310 315 320
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
325 330 335
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
340 345 350
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
355 360 365
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
370 375 380
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gin Pro Arg Glu Pro Gin
385 390 395 400
Val Tyr Thr Leu Pro Pro Cys Arg Asp Leu Thr Lys Asn Gin Val
405 410 415
Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
420 425 430
Glu Trp Glu Ser Asn Gly Gin Pro Glu Asn Asn Tyr Lys Thr Thr Pro
435 440 445
Pro Val Leu Asp Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr
450 455 460
Val Asp Lys Ser Arg Thr Gln Gln Gly Asn Val Phe Ser Cys Ser Val
465 470 475 480
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
485 490 495 499
Ser Pro Gly Lys
500

<210> SEQ ID NO 30
<211> LENGTH: 503
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Murine IL-2R-gamma-Fc(chink) fusion protein
<400> SEQUENCE: 30

atgagacatga ggctccgccg tcagctcctg ggcctctcgg tgcctctgtt cccctctcctg 60
cgctcgtgtgg tcccaagttc cgggtttgag aagctcaagtg tcctctaagctg 120
gagacatgac aagtgactgtt gatctcagct tctactagcccc tgaacacccct caggtctctc 180
acgccgcccc ttccagagtt gcagtctgggt gttgtcaacca ttaggtaccat gaaattcgact 240
tgaggtgaga cctcgaaacc cacaatctac cggtcaccag tgaagttcctag ccagctcagag 300
tctctctactc atacatctcag ggagttgcaact caagcatcagac aaactcaagag tgtcttgtac 360
ggcagcagta tccaaaaaga agatattcact catctaccatg gattgtgctg tctcaagctcagc 420
gagcagcagc cagacacagc gatgagagta ccaagattagt gcaatagcact cagattcactg 480
tgagagctga gcagcagcagc gatgagagta ccaagattagt gcaatagcact cagattcactg 540
tcggagcagc gctgtggagga gatttcacctc tattagtcaggt tctgcacagc cggcagcagc 600
gagcagcagc cagacacagc gatgagagta ccaagattagt gcaatagcact cagattcactg 660
tcggagcagc gctgtggagga gatttcacctc tattagtcaggt tctgcacagc cggcagcagc 720
gagcagcagc cagacacagc gatgagagta ccaagattagt gcaatagcact cagattcactg 780
tcggagcagc gctgtggagga gatttcacctc tattagtcaggt tctgcacagc cggcagcagc 840
gagcagcagc cagacacagc gatgagagta ccaagattagt gcaatagcact cagattcactg 900
tcggagcagc gctgtggagga gatttcacctc tattagtcaggt tctgcacagc cggcagcagc 960
gagcagcagc cagacacagc gatgagagta ccaagattagt gcaatagcact cagattcactg 1020
tcggagcagc gctgtggagga gatttcacctc tattagtcaggt tctgcacagc cggcagcagc 1080
gagcagcagc cagacacagc gatgagagta ccaagattagt gcaatagcact cagattcactg 1140
tcggagcagc gctgtggagga gatttcacctc tattagtcaggt tctgcacagc cggcagcagc 1200
gagcagcagc cagacacagc gatgagagta ccaagattagt gcaatagcact cagattcactg 1260
tcggagcagc gctgtggagga gatttcacctc tattagtcaggt tctgcacagc cggcagcagc 1320
gagcagcagc cagacacagc gatgagagta ccaagattagt gcaatagcact cagattcactg 1380
tcggagcagc gctgtggagga gatttcacctc tattagtcaggt tctgcacagc cggcagcagc 1440
gagcagcagc cagacacagc gatgagagta ccaagattagt gcaatagcact cagattcactg 1500
tgacattgga cctgtccaccct cacaatctac gcgtctctcct cgcgtggtgag 1503
-continued

```
FEATURE: OTHER INFORMATION: Murine IL-2R alpha subunit + Avi-tag + His-tag

<400> SEQUENCE: 31
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
  1   5   10   15
Val His Ser Glu Leu Cys Leu Tyr Asp Pro Pro Glu Val Pro Asn Ala
  20  25  30
Thr Phe Lys Ala Leu Ser Tyr Lys Asn Gly Thr Ile Leu Asn Cys Glu
  35  40  45
Cys Lys Arg Gly Phe Arg Leu Glu Val Tyr Met Arg Cys
  50  55  60
Leu Gly Asn Ser Thr Ser Ser Asn Cys Gin Cys Thr Ser Asn Ser His
  65  70  75  80
Asp Lys Ser Arg Lys Gin Val Thr Ala Gin Leu Glu His Gin Lys Glu
  85  90  95
Gln Gin Thr Thr Thr Asp Met Gin Lys Pro Thr Gin Ser Met Gin
 100 105 110
Glu Asn Leu Thr Gly His Cys Arg Glu Pro Pro Pro Thr Lys His Glu
 115 120 125
Asp Ser Lys Arg Ile Tyr His Phe Val Glu Gly Gin Ser Val His Tyr
 130 135 140
Glu Cys Ile Pro Gly Tyr Lys Ala Leu Gin Arg Gly Pro Ala Ile Ser
 145 150 155 160
Ile Cys Lys Met Lys Cys Gly Lys Thr Gly Trp Thr Gin Pro Gin Leu
 165 170 175
Thr Cys Val Asp Glu Gin Leu Tyr Phe Gin Gly Ser Gly Leu Asn
 180 185 190
Asp Ile Phe Glu Ala Gin Lys Ile Glu Thr Gin Ala Arg Ala His
 195 200 205
His His His His
 210

<210> SEQ ID NO 32
<211> LENGTH: 642
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE: OTHER INFORMATION: Murine IL-2R alpha subunit + Avi-tag + His-tag
<400> SEQUENCE: 32
atgggatgga gcgtgatacat cctcttcttg gtgcacacag ctacggttgt gcattcggas 60
c tgtgtcgtgt atgaccocacc ggaagtcccc aatggcacat tcaagcosct cttotacaag 120
aaccgcacca tcotaaactc tgaatcgcag agaggttccc gaagactaaagaaggatggtc 180
tatagcgtga gcgttagaaa cttctggagc agcaactgccc agtgccacag caacctcccat 240
gaaatcagca gaagcagact tacaacctgaa cttaaacgcc agaaagagc caaaccaca 300
agaagacatg agaagacaacc acagtctcat ccacagca gactacagc actctgcaag 360
gagccacccc tctggtaacca tgaagattcc aagagactc atcacttgtg ggaaggaccg 420
agttgtcaact acaggtgtat tcggggatat aaggtacac agagaggtcc tgttatagtcc 480
atctggaga tgaagtctgg gaaaaaaggg tggactcagc ccacgtcagc atctgtcgac 540
gacagtttat atttttcaggg cagcgtcaggg ctgaacgaca tcttgaggg ccagaagatc 600
```
<210> SEQ ID NO: 33
<211> LENGTH: 480
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cynomolgous IL-2R-beta-Pc(knob) fusion protein + Avi-tag

<400> SEQUENCE: 33

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 Val His Ser Ala Val Asn Gly Thr Ser Arg Phe Thr Cys Phe Tyr Asn
 Ser Arg Ala Asn Ile Ser Cys Val Trp Ser Gin Asp Gly Ala Leu Gln
 Asp Thr Ser Cys Gin Val His Ala Trp Pro Asp Arg Arg Arg Trp Asn
 Gin Thr Cys Glu Leu Leu Pro Val Ser Gin Ala Asr Ser Trp Ala Cys Asn
 Leu Ile Leu Gly Thr Pro Asp Ser Gin Lys Leu Thr Ala Val Asp Ile
 Val Thr Leu Arg Val Met Cys Arg Glu Gly Val Arg Trp Arg Met Met
 Ala Ile Gin Asp Phe Lys Pro Phe Glu Asn Leu Arg Leu Met Ala Pro
 Ile Ser Leu Gin Val His Val Glu Thr His Arg Cys Asn Ile Ser
 Trp Lys Ile Ser Gin Ala Ser His Tyr Phe Glu Arg His Leu Glu Phe
 Glu Ala Arg Thr Leu Ser Pro Gly His Thr Trp Glu Glu Ala Pro Leu
 Met Thr Leu Leu Gly Lys Glu Glu Trp Ile Cys Leu Glu Thr Leu Thr
 Pro Asp Thr Gin Tyr Glu Phe Gin Val Arg Val Lys Pro Leu Gin Gly
 Glu Phe Thr Thr Trp Ser Pro Ser Gin Pro Leu Ala Phe Arg Thr
 Lys Pro Ala Ala Leu Gly Asp Thr Gly Ala Gin Asp Lys Thr His
 Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
 Phe Leu Phe Pro Pro Lys Pro Asp Thr Leu Met Ile Ser Arg Thr
 Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 Thr Lys Pro Arg Glu Glu Gin Tyr Asn Ser Thr Tyr Arg Val Val Ser
 Val Leu Thr Val Leu His Gin Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
-continued

<table>
<thead>
<tr>
<th></th>
<th>340</th>
<th>345</th>
<th>350</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Glu Val Tyr Thr Leu Pro</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>355</td>
<td>360</td>
<td>365</td>
</tr>
<tr>
<td>Pro Cys Arg Amp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>375</td>
<td>380</td>
</tr>
<tr>
<td>Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>385</td>
<td>390</td>
<td>395</td>
</tr>
<tr>
<td>Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Val Leu Asp Ser</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>405</td>
<td>410</td>
<td>415</td>
</tr>
<tr>
<td>Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>420</td>
<td>425</td>
<td>430</td>
</tr>
<tr>
<td>Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Gln Ala Leu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>435</td>
<td>440</td>
<td>445</td>
</tr>
<tr>
<td>His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ser</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>455</td>
<td>460</td>
</tr>
<tr>
<td>Gly Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Gly Trp His Glu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>465</td>
<td>470</td>
<td>475</td>
</tr>
</tbody>
</table>

<210> SEQ ID NO 34
<211> LENGTH: 1443
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cynomolgous IL-2R-beta-Pc(knob) fusion protein + Avrl-tag

<400> SEQUENCE: 34

atggaatgaa gctgtatcat cctcttcttg tgtggaacac atcaggttgt gcattcgcag 60

tgcaacctgc cttcccggtt cacatgttct tcaaaactga gcacccacat cttctgtgct 120
tggagccag atggggtctt gcagagaaact tctggcaag tccagctcggt gcggcagca 180
cgcaggtgaa acacaactct ttctggcttc ctgcgtgagt gcaagcagcc 240
cgctgtctcg gaacccacag tttctcagaa cttgaccgac tagtatgctg cagctgtgag 300
gttctgctgg cttgaaaggt gcctgtgagg atgatgccca cccagggcttt 360
gcacatcct gctgtatgac ccccagttcc tctcaagtgc tcaagtggga gacccacag 420
tgcacaatgg cctggaatg cttccagcagc tttccactct tggaaagaca cgcggggttt 480
gaucagaag cagcttgctg cggaggggag ccocctgtg catctgcaag 540
cggaagcag tcgggatgct gccagcagtc ccaaaacctg acacacagtg cttcgttccag 600

gtcgggggcc aagccattggt acagcattgg ggacacatg cggtaggtc ggaagctggc 660
gccctttgca cggaggtctg gggcagccgt ccagcagcag 720
acactgcaaac cggagccgag cctggaggggc gctgtccttc ctctgccagcag 780
ccacaaaccgg aacgacaacct cccgagccctt ggctgtgagtc cgtgtcctg 840
gactgggaac cagagcaacc ttgagcctag tccacctgtg cgtggaggtgc 900
cataatggca caaagcaagc gcggaggagc ctcagctcag cggatgctct 960
gtctctacacg tctggccaga gcaagctgagt ctgggagca cggagctgagc 1020
gaggggggcag cccagcagct ggtgggagct gagctgctcttc ctctgccagcag 1180
ctgtgggtgc tgtgtaacgg cttcttaccc ggagacatacg cctgtgagtt ggagagcaat 1240
-continued-

```
GGGCAGCCGG AGAACAAC GTAAGACGCG CTTCTCAGCTC CTCAGCGGC
CTCTTCGGCAGCAGGCGAAGCAGGCCTTTAGGCG

SEQ ID NO 35
LENGTH: 489
TYPE: PRT
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Cynomolgous IL-2R-gamma-Fc(hole) fusion protein

SEQUENCE: 35

MET GLY TRP SER CYE ILE ILE LEU PHE LEU VAL ALA THR ALA THR GLY

VAL HIS SER LEU AMH THR ILE LEU THR PRO ASN GLY ASN GLU ASP

ALA THR THR ASP PHE PHE LEU THR SER MET PRO THR ASP SER LEU SER

VAL SER THR LEU PRO LEU PRO GLU VAL GLN CYE PHE VAL PHE ASN VAL

GLU TYR MET ASN CYE THR TRP ASN SER SER GLU PRO GLN PRO THR

ASN LEU THR LEU HIS TYR TRP TYR LYNS ASN SER ASP ASP LYS VAL

GLN LYS CYE SER HIS TYR LEU PHE SER GLU GLU ILE THR SER GLY CYE

GLN LEU GLN LYS GLU ILE HIS LEU TYR GLN THR PHE VAL VAL GLN

LEU GLN ASP PRO ARG GLU PRO ARG GLNALA THR GLN MET LEU LYS

LEU GLN ASN LEU VAL ILE PRO TRP ALA PRO GLU ASN LEU THR LEU ARG

LYN LEU SER GLU SER GLN LEU GLU LEU ASN TRP ASP ARG PHE LEU

ASN HIS CYE LEU GLU HIS LEU VAL GLN TYR ARG THR ASP TRP ASP HIS

SER TRP THR GLU GLN SER VAL ASP TYR ARG HIS LYE PHE SER LEU PRO

SER VAL ASP GLY GLN LYS ARG TYR THR PHE ARG VAL ARG SER ARG PHE

ANN PRO LEU CYE GLY SER ALA GLN HIS TRP SER GLU TRP SER HIS PRO

ILE HIS TRP GLY SER ASN SER LYS GLU ASN PRO PHE LEU PHE ALA

LEU GLU ALA GLY ALA GLN ASP LYS THR HIS THR CYE PRO PRO CYE PRO

ALA PRO GLU LEU LEU GLY GLY PRO SER VAL PHE LEU PHE PRO PRO LYE

PRO LYS ASP THR LEU MET ILE SER ARG THR PRO GLU VAL THR CYE VAL
```

1260

1320

1380

1440

1443
<table>
<thead>
<tr>
<th>Val</th>
<th>Val</th>
<th>Asp</th>
<th>Val</th>
<th>Ser</th>
<th>His</th>
<th>Glu</th>
<th>Asp</th>
<th>Pro</th>
<th>Glu</th>
<th>Val</th>
<th>Lys</th>
<th>Phe</th>
<th>Asn</th>
<th>Trp</th>
<th>Tyr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>Asp</td>
<td>Gly</td>
<td>Val</td>
<td>Glu</td>
<td>Val</td>
<td>His</td>
<td>Asn</td>
<td>Ala</td>
<td>Lys</td>
<td>Thr</td>
<td>Lys</td>
<td>Pro</td>
<td>Arg</td>
<td>Glu</td>
<td>Glu</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln</td>
<td>Tyr</td>
<td>Asn</td>
<td>Ser</td>
<td>Thr</td>
<td>Tyr</td>
<td>Arg</td>
<td>Val</td>
<td>Val</td>
<td>Ser</td>
<td>Val</td>
<td>Thr</td>
<td>Val</td>
<td>Leu</td>
<td>Thr</td>
<td>Val</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln</td>
<td>Asp</td>
<td>Trp</td>
<td>Leu</td>
<td>Arg</td>
<td>Gly</td>
<td>Lys</td>
<td>Trp</td>
<td>Lys</td>
<td>Lys</td>
<td>Cys</td>
<td>Val</td>
<td>Ser</td>
<td>Asn</td>
<td>Lys</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>Leu</td>
<td>Gly</td>
<td>Ala</td>
<td>Pro</td>
<td>Ile</td>
<td>Glu</td>
<td>Val</td>
<td>Thr</td>
<td>Ile</td>
<td>Ser</td>
<td>Ala</td>
<td>Lys</td>
<td>Gly</td>
<td>Gln</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>Arg</td>
<td>Glu</td>
<td>Pro</td>
<td>Glu</td>
<td>Val</td>
<td>Cys</td>
<td>Thr</td>
<td>Leu</td>
<td>Pro</td>
<td>Pro</td>
<td>Ser</td>
<td>Arg</td>
<td>Asp</td>
<td>Glu</td>
<td>Leu</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td>Lys</td>
<td>Asn</td>
<td>Gln</td>
<td>Val</td>
<td>Ser</td>
<td>Leu</td>
<td>Ser</td>
<td>Cys</td>
<td>Ala</td>
<td>Val</td>
<td>Lys</td>
<td>Gly</td>
<td>Phe</td>
<td>Tyr</td>
<td>Pro</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>Asp</td>
<td>Ile</td>
<td>Ala</td>
<td>Val</td>
<td>Glu</td>
<td>Trp</td>
<td>Gln</td>
<td>Ser</td>
<td>Arg</td>
<td>Val</td>
<td>Gly</td>
<td>Gln</td>
<td>Pro</td>
<td>Glu</td>
<td>Asn</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyr</td>
<td>Lys</td>
<td>Thr</td>
<td>Thr</td>
<td>Pro</td>
<td>Pro</td>
<td>Val</td>
<td>Leu</td>
<td>Asp</td>
<td>Ser</td>
<td>Asp</td>
<td>Gly</td>
<td>Ser</td>
<td>Phe</td>
<td>Phe</td>
<td>Leu</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>Ser</td>
<td>Lys</td>
<td>Leu</td>
<td>Thr</td>
<td>Val</td>
<td>Lys</td>
<td>Ser</td>
<td>Arg</td>
<td>Trp</td>
<td>Gln</td>
<td>Gln</td>
<td>Gly</td>
<td>Asn</td>
<td>Val</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>Ser</td>
<td>Cys</td>
<td>Ser</td>
<td>Val</td>
<td>Met</td>
<td>His</td>
<td>Ala</td>
<td>Leu</td>
<td>His</td>
<td>Asn</td>
<td>His</td>
<td>Tyr</td>
<td>Thr</td>
<td>Gln</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>Ser</td>
<td>Leu</td>
<td>Ser</td>
<td>Leu</td>
<td>Ser</td>
<td>Pro</td>
<td>Gly</td>
<td>Lys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SEQ ID NO 36
LENGTH: 1470
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE: OTHER INFORMATION: Cynomolgous IL-2R-gamma-Fc(hole) fusion protein
SEQUENCE: 36

```
ATG GAT CTT CTG TAC CTA TCT CTT CTT GCT CAC CCA AA CAA GAG CCG GAA TAA
AA AAG CAG CCG CTC GGC GGC GGT GGT GCT CTC CTC CTC CTC CTC CTC
GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT
GCT CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG
```

51
-continued

gtggacggtg tggagtggtc taattgccaag acaaacgcgc gggaggagca gtacagcagc O2O acgt accgtg tdgtcagcgt cct caccgtc...

Gly Lieu. Asn Asp Ile Phe Glu Ala Glin Lys Ile Glu Trp His Glu 195 200 205

<210> SEQ ID NO 37
<211> LENGTH: 217
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE: OTHER INFORMATION: Cytoplasmic IL-2R alpha subunit + Avi-tag + His-tag

<400> SEQUENCE: 37

Met Gly Trp Ser Cys lle Ile Leu Phe Leu Val Ala Thr Ala Thr Gly 1 5 10 15
Glu Leu Cys Asp Asp Asp Pro Pro Lyv lle Thr His Ala Thr Phe Lys 20 25 30
Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg 35 40 45
Gly Phe Arg Arg lle Lys Ser Gly Ser Pro Tyr Met Leu Cys Thr Gly 50 55 60
Asn Ser Ser His Ser Ser Thr Asp Asn Gln Cys Glu Cys Thr Ser Ser 65 70 75 80
Ala Ala Arg Asn Thr Thr Lys Glu Val Thr Pro Gln Pro Glu Glu Gln 85 90 95
Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Met Gln Leu Ala Asp 100 105 110
Gln Val Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro Thr Glu Ann 115 120 125
Glu Ala Thr Glu Arg lle Tyr His Phe Val Val Gly Glu Thr Val Tyr 130 135 140
Tyr Glu Cys Val Glu Gly Tyr Arg Ala Leu His Arg Arg Gly Pro Ala Glu 145 150 155 160
Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Thr Glu Pro Glu 165 170 175
Leu lle Cys Thr Gly Glu Val Arg Glu Leu Tyr Phe Glu Gly Gly 180 185 190
Ser Gly Leu Asn Asp lle Phe Glu Ala Gln Lys lle Glu Trp His Glu 195 200 205
Ala Arg Ala His His His His His His His 210 215

<210> SEQ ID NO 38
<211> LENGTH: 654
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220>  FEATURE:  Cynomolgous IL-2R alpha subunit + Avi-tag + His-tag
<223>  OTHER INFORMATION:  Cynomolgous IL-2R alpha subunit + Avi-tag + His-tag
<400>  SEQUENCE:  38
atggatgag acctctctctt gtagcacaac actacggtgta gctctgtgac  60
gctgacccga caaaaactca acagtccac cagcgttacag cagacagacc 120
atggtgact gtagcaca gagaagtttc gccaagttaa aagaggggtc accctatag 180
cctgtacag gaaactctag ccaactgtgc tggacaaac aatgtcaatg cacaagctct 240
gctgtcgac gaaaccaaca acaagtggaca ccatcacaag gagaacagaa aagagaaa 300
accacagaa tgcacagctca aatgcatctg gcaacacag tcagcctcttc aggtcatctgc 360
agggacccct cccgggtgga aatgacagcc acagaaaaaa ttatatcattt cggtgtgagg 420
cagacggtt ttactacagtt cgttccaggga tacaggtctc tacaacaggg tctggtgag 480
agcgtctgca aatgacccca cgggacacca atagtggacc acccagagt catatgcaca 540
ggtggaagct acaagacggct tttttttcag ggggtcctag gcctgaacga catctctgag 600
gcocaaga gctggactgtg cggagctctga gtcaccaccc atcaccacca ctaga 654

<210>  SEQ ID NO  39
<211>  LENGTH:  19
<212>  TYPE:  PRT
<213>  ORGANISM:  Artificial Sequence
<220>  FEATURE:  leader sequence
<223>  OTHER INFORMATION:  leader sequence
<400>  SEQUENCE:  39
Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Gly
1     5     10      15
Ala His Ser

<210>  SEQ ID NO  40
<211>  LENGTH:  57
<212>  TYPE:  DNA
<213>  ORGANISM:  Artificial Sequence
<220>  FEATURE:  leader sequence
<223>  OTHER INFORMATION:  leader sequence
<400>  SEQUENCE:  40
atggatgagacctctctcttgtagcacaacactacggtgta gctctgtgac  57

<210>  SEQ ID NO  41
<211>  LENGTH:  57
<212>  TYPE:  DNA
<213>  ORGANISM:  Artificial Sequence
<220>  FEATURE:  leader sequence
<223>  OTHER INFORMATION:  leader sequence
<400>  SEQUENCE:  41
atggatgagacctctctcttgtagcacaacactacggtgta gctctgtgac  57

<210>  SEQ ID NO  42
<211>  LENGTH:  22
<212>  TYPE:  PRT
<213>  ORGANISM:  Artificial Sequence
<220>  FEATURE:  leader sequence
<223>  OTHER INFORMATION:  leader sequence
<400>  SEQUENCE:  42
Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Leu Trp
1  5  10  15

Phe Pro Gly Ala Arg Cys
20

<210> SEQ ID NO 43
<211> LENGTH: 66
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: leader sequence

<400> SEQUENCE: 43
atggacatga ggctccgcgc tcagctctcg ggctctctgc tgctctgtgt cccaggtgcc 60
aggtgt

<210> SEQ ID NO 44
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: leader sequence

<400> SEQUENCE: 44
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1  5  10  15

Val His Ser

<210> SEQ ID NO 45
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: leader sequence

<400> SEQUENCE: 45
atgggatgga gctgtatcat cctttctctg gtgacacag ctacoggtgt gcaaactcc 57

<210> SEQ ID NO 46
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: leader sequence

<400> SEQUENCE: 46
atgggtctgt gctgcatcat cctttctctg gtgacacag ccacggtgt gcaaactcc 57

<210> SEQ ID NO 47
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: leader sequence

<400> SEQUENCE: 47
atgggtctgt gctgcatcat cctttctctg gtgacacag ccacggtgt gcaaactcc
1. A fusion protein comprising (i) an immunoglobulin molecule comprising a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification, and (ii) two interleukin-2 (IL-2) molecules.

2. The fusion protein of claim 1, wherein said immunoglobulin molecule is an IgG-class immunoglobulin molecule.

3. The fusion protein of claim 2, wherein said IgG-class immunoglobulin molecule is IgG1.

4. The fusion protein of claim 2, wherein said immunoglobulin molecule is a human immunoglobulin molecule.

5. The fusion protein of claim 1, wherein said immunoglobulin molecule is not capable of specific binding to an antigen.

6. The fusion protein of claim 1, wherein said immunoglobulin molecule comprises a heavy chain variable region sequence based on the human V/H3-23 germline sequence.

7. The fusion protein of claim 1, wherein said immunoglobulin molecule comprises the heavy chain variable region sequence comprising SEQ ID NO: 9.

8. The fusion protein of claim 1, wherein said immunoglobulin molecule comprises a light chain variable region sequence based on the human V/K3-20 germline sequence.

9. The fusion protein of claim 1, wherein said immunoglobulin molecule comprises the light chain variable region sequence comprising SEQ ID NO: 11.

10. The fusion protein of claim 1, wherein said Fc receptor is an Fcy receptor.

11. The fusion protein of claim 1, wherein said Fcy receptor is a human Fcy receptor.

12. The fusion protein of claim 1, wherein said Fcy receptor is an activating Fc receptor.

13. The fusion protein of claim 1, wherein said Fc receptor is an activating Fc receptor.

14. The fusion protein of claim 1, wherein said Fc receptor is a human FcyRIIIa.

15. The fusion protein of claim 1, wherein said Fc receptor is selected from the group of FcyRIIa (CD16a), FcyRI (CD64), FcyRIla (CD32) and FceRI (CD23).

16. The fusion protein of claim 1, wherein said Fc receptor is human FcyRIIIa.

17. The fusion protein of claim 16, wherein said Fc receptor is human FcyRIIIa.

18. The fusion protein of claim 1, wherein said immunoglobulin molecule comprises an amino acid substitution at position 329 of the immunoglobulin heavy chains.

19. The fusion protein of claim 18, wherein said amino acid substitution is P329G.

20. The fusion protein of claim 1, wherein said immunoglobulin molecule comprises amino acid substitutions at positions 234 and 235 of the immunoglobulin heavy chains.

21. The fusion protein of claim 20, wherein said amino acid substitutions are L234A and L235A (LALA).

22. The fusion protein of claim 1, wherein said immunoglobulin molecule comprises the amino acid substitutions L234A, L235A and P329G in the immunoglobulin heavy chains.

23. The fusion protein of claim 1, wherein said IL-2 molecules are wild-type IL-2 molecules.

24. The fusion protein of claim 1, wherein said IL-2 molecules are human IL-2 molecules.

25. The fusion protein of claim 1, wherein said IL-2 molecules comprise the sequence of SEQ ID NO: 1 or SEQ ID NO: 3, particularly the sequence of SEQ ID NO: 3.

26. The fusion protein of claim 1, wherein said IL-2 molecules are each fused at their N-terminal amino acid to the C-terminal amino acid of one of the immunoglobulin heavy chains of said immunoglobulin molecule, optionally through a peptide linker.

27. The fusion protein of claim 1, wherein the fusion protein comprises the polypeptide sequences of SEQ ID NO: 17 and SEQ ID NO: 19.

28. A polynucleotide encoding the fusion protein of claim 1.

29. An expression vector, comprising the polynucleotide of claim 28.

30. A host cell comprising the expression vector of claim 29.

31. A method for producing a fusion protein, said method comprising the steps of: (i) culturing a host cell containing a polynucleotide encoding the fusion protein of claim 1, under conditions suitable for expression of the fusion protein; and (ii) recovering the fusion protein.

32. A fusion protein comprising: (i) an immunoglobulin molecule comprising: a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification, and (ii) two interleukin-2 (IL-2) molecules produced by the method of claim 31.

33. A pharmaceutical composition comprising the fusion protein of claim 1 and a pharmaceutically acceptable carrier.

34. A pharmaceutical composition comprising the fusion protein of claim 32 and a pharmaceutically acceptable carrier.

35. A method for treating or preventing an autoimmune disease, said method comprising: administering the pharmaceutical composition of claim 33 to a patient.

36. The method of claim 35, wherein said autoimmune disease is selected from the group of type 1 diabetes, systemic lupus erythematosus, Crohn’s disease and multiple sclerosis.

37. A method for treating or preventing transplant rejection or graft-versus-host disease said method comprising: administering the pharmaceutical composition of claim 33 to a patient.

38. A method of treating a disease in an individual, said method comprising: administering to said individual a therapeutically effective amount of a composition comprising the fusion protein of claim 1 in a pharmaceutically acceptable form.

39. The method of claim 38, wherein said disease is an autoimmune disease.

40. The method of claim 39, wherein said autoimmune disease is selected from the group of type 1 diabetes, systemic lupus erythematosus, Crohn’s disease and multiple sclerosis.

41. The method of claim 39, wherein said disease is transplant rejection or graft-versus-host disease.

42. A method for selectively activating regulatory T cells in vitro or in vivo, said method comprising: administering a fusion protein of claim 1.

43. The method of claim 42, wherein said activation comprises induction of proliferation and/or induction of IL-2 receptor signaling.

44. The method of claim 42, wherein said method is in vitro and said fusion protein is used at a concentration of about 1 ng/mL or less, particularly about 0.1 ng/mL or less.
45. The method of claim 42, wherein said method is in vivo and said fusion protein is used at a dose of about 20 μg/kg body weight or less.

46. The method of claim 45, wherein said dose is less than or equal to about 12 μg/kg body weight.