the protein that are essential for bioactivity, and that led to the discovery of several novel proteins that fulfill the desired therapeutic characteristics.

SUMMARY

[0011] This disclosure provides for a fusion protein between an IL2αβγ Selective Agonist protein (IL2 Selective Agonist) and a IgG Fc protein. The IL2 Selective Agonist moiety provides a therapeutic activity by selectively activating the IL2αβγ form of the receptor, thus selectively stimulating Tregs. The Fc moiety provides a prolonged circulating half- life compared to the circulating half- life of IL-2 or an IL2 Selective Agonist protein. The Fc moiety increases circulating half-life by increasing the molecular size of the fusion protein to greater than 60,000 daltons, which is the approximate cutoff for glomerular filtration of maeromolecules by the kidney, and by recycling the fusion protein through the Neonatal Fc Receptor (FcRn) protein, the receptor that binds and recycles IgG, thus prolonging its circulating half-life. The Fc moiety will also be deficient in Fc effector functions, such as Complement-Dependent Cytotoxicity (CDC) and Antibody-Dependent Cellular Cytotoxicity (ADCC), enabling the fusion protein to selectively activate Tregs to potentiate Treg function and to expand Treg numbers. The two protein moieties are fused in a manner that maintains robust bioactivity of the IL2 Selective Agonist moiety and enables the Fc moiety to promote a prolonged circulating half-life and thus efficiently potentiate Tregs function and numbers. This potentiation of Tregs will suppress over-exuberant autoimmune or inflammatory responses, and will be of benefit in treating autoimmune and inflammatory diseases. The proteins of this disclosure may be monomeric or dimeric forming dimers through cysteine residues in the Fc moieties or domains.

[0012] More specifically, this disclosure provides for a fusion protein, comprising: a N-terminal human IL-2 variant protein moiety, and a C-terminal IgG Fc protein moiety, wherein said IL-2 fusion protein has the ability to selectively activate the high affinity IL-2 receptor and thus selectively activate human regulatory T cells. The variants of IL-2 include those with substitutions selected from the group consisting of: N88R, N88I, N88G, D20H, Q126L, and Q126F relative to human IL2 protein (SEQ ID NO: 1). In addition the, IL-2 variant protein optionally comprises human IL-2 with the substitution C125S. It is preferred that the proteins of this disclosure are fused wherein both the IL-2 variant protein and the IgG Fc protein have an N-terminus and a C-terminus and said human IL-2 variant protein is fused at its C-terminus to the N-terminus of the IgG Fc protein. It is further preferred that the IL-2

variant domain and the Fc domain are joined or fused through a linker peptide positioned between the IL-2 variant protein and the IgG Fc protein moieties. The IgG Fc protein moiety or domain will preferably be deficient in Fc effector functions or contain one or more amino acid substitutions that reduce the effector functions of the Fc portion of the fusion protein.

[0012a] In one aspect, there is provided a fusion protein, comprising: a. a N-terminal human IL-2 variant protein having at least 95% sequence identity to SEQ ID NO: 1 and comprising the substitution C125S and a substitution selected from the group consisting of: N88R, N88I, N88G, D20H, Q126L and Q126F; b. a C-terminal IgG Fc protein; and c. a linker peptide of between 5 to 20 amino acid residues positioned between the IL-2 variant protein and the IgG Fc protein.

[0013] An example of this disclosure is a protein, comprising: a IL-2 variant protein having amino acid substitutions N88R and C125S relative to human IL-2 (SEQ ID NO: l), a linker peptide as set forth in SEQ ID NO: 15, and a human IgGl Fc protein as set forth in SEQ ID NO:2, wherein said fusion protein has the ability to selectively activate the high affinity IL-2 receptor and thus selectively activate human regulatory T cells. Alternative proteins of this disclosure include: a IL-2 variant protein having amino acid substitutions N88R and C 125S relative to human IL-2 (SEQ ID NO : 1), a linker peptide as set forth in SEQ ID NO : 15 , and a human IgG2 Fc protein as set forth in SEQ ID NO:3.

[0014] A more specific embodiment of this disclosure is a dimeric protein, comprising two identical chains, where each chain comprises a N-terminal human IL-2 variant protein moiety and a C-terminal IgG Fc protein moiety wherein: the N-terminal human IL-2 variant protein moiety has a N-terminus and a C- terminus varies from the human IL-2 wildtype as in SEQ ID NO: 1 by at least one of the substitutions selected from the group consisting of: N88R, N88I, N88G, D20H, Q126L, and Q126F, has at least a 97% sequence identify to Sequence ID No. 1; and, has the ability to activate Treg cells by binding to a IL2Rαβγ on those cells; the N-terminal human IL-2 variant protein is joined at its C-terminal to a N-terminus of an amino acid linker of between 6 to 20 amino acid residues where said linker also has a C-terminus; and, the C-terminus of the amino acid linker is joined to the N-terminus of IgG Fc protein moiety having 97% sequence identify to for example SEQ ID NO:3 (IgG2) or SEQ ID No. 2 IgG1N297A) and containing cysteine residues; and where the two chains are linked to each other through the cysteine residues that form the interchain disulfide bonds of the IgG Fc protein moiety. The dimers of this disclosure may be further substituted at C125S of the IL-2 moiety. The proteins of this disclosure preferably include amino acid linkers consisting a group of glycine residues, serine residues, and a mix of glycine and serine residues. The linkers may comprise a mix of between 12 and 17 serine and glycine residues preferably with a ratio of glycine residues to serine residues in a range of 3:1-5:1, e.g, a 4:1 ratio.

[0014a] In another aspect, there is provided a dimeric protein, comprising two identical chains, wherein each chain comprises a fusion protein described herein, and wherein the two chains are linked to each other through cysteine residues in the IgG Fc protein.

[0015] This disclosure further provides for the compositions above in a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

[0015a] In another aspect, there is provided a pharmaceutical composition comprising the fusion protein described herein, or the dimeric protein described herein, and a pharmaceutically acceptable carrier.

[0016] This disclosure further provides for nucleic acids encoding the proteins described herein. The nucleic acids or preferably operably linked to expression cassettes that can be either designed for recombination with a host cell genome or introduced on an independently replicating plasmid or extrachromosomal nucleic acid.

[0016a] In another aspect, there is provided a nucleic acid encoding the fusion protein described herein.

[0017] This disclosure further provides for methods of selectively activating human regulatory T cells in a patient in need thereof, the method comprising administering a pharmaceutical composition comprising the compositions described administered at therapeutically effective doses until human regulatory T cell concentrations reach desired levels.

[0017a] In another aspect, there is provided the fusion protein described herein, the dimeric protein described herein, or the pharmaceutical composition described herein, for use in a method of treating an autoimmune disease. In another aspect, there is provided use of the fusion protein described herein, the dimeric protein described herein, or the pharmaceutical composition described herein, in the manufacture of a medicament for treating an autoimmune disease.

[0018] A method of measuring the numbers of Treg cells in a human blood sample by contacting human blood cells with the fusion protein of claim 1 at a concentration of between 1 nM and 0.01 nM, and then detecting cells that bind to the protein by flow cytometry.

[0018a] In another aspect, there is provided an *in vitro* method of measuring the numbers of regulatory T cells in a human blood sample by contacting human blood cells with the fusion protein described herein at a concentration of between 1 nM and 0.01 nM, and then detecting cells that bind to the protein by flow cytometry.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] FIGURE 1 is a diagrammatic illustration of the relationship between circulating half-life, peak drug level, the biological effective concentration, and the duration necessary to stimulate Treg cell proliferation after a single dose of IL-2 or an IL2-Fc fusion protein with increased half-life. The dashed line represents the blood level over time of IL-2 following a subcutaneous injection, and the solid line represents the blood level over time of an IL2-Fc fusion protein. The horizontal dotted lines indicate the concentrations (EC50 values) necessary to activate cells expressing IL2Rαβγ and IL2Rβγ, respectively) are indicated. The double-headed arrow indicates the duration of exposure (5-6 hr) to IL-2 at the EC50 necessary to stimulate cell proliferation.

[0020] FIGURE 2 shows the design configurations for Fc fusion proteins. The fusion partner (X), can be fused at the N terminus (X-Fc) or the C-terminus (Fc-X) of the Fc protein. Linker peptides can be inserted between X and the Fc.

[0021] FIGURE 3 shows a dose-response of IL-2 and N88RL9AG1 stimulated STAT5 phosphorylation in CD4+ T cells as measured by flow cytometry. Cells were treated with the IL-2 or N88RFc at the concentrations indicated on the top for 10 minutes at 37 C, fixed,

WHAT IS CLAIMED IS:

1. A fusion protein, comprising:
a. a N-terminal human IL-2 variant protein having at least 95% sequence identity to SEQ ID NO: 1 and comprising the substitution C125S and a substitution selected from the group consisting of: N88R, N88I, N88G, D20H, Q126L and Q126F;
b. a C-terminal IgG Fc protein; and
c. a linker peptide of between 5 to 20 amino acid residues positioned between the IL-2 variant protein and the IgG Fc protein.

2. The fusion protein of claim 1, wherein the IgG Fc protein contains one or more amino acid substitutions that reduce the effector functions of the IgG Fc protein.

3. The fusion protein of claim 1 or claim 2, wherein the linker peptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19.

4. The fusion protein of claim 1 or claim 2, comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10.

5. A dimeric protein, comprising two identical chains, wherein each chain comprises a fusion protein according to any one of the preceding claims, and wherein the two chains are linked to each other through cysteine residues in the IgG Fc protein.

6. The dimeric protein of claim 5, wherein the IL-2 variant protein comprises the N88R substitution.

7. A pharmaceutical composition comprising the fusion protein of any one of claims 1-4, or the dimeric protein of claim 5 or claim 6, and a pharmaceutically acceptable carrier.

8. A nucleic acid encoding the fusion protein of any one of claims 1-4.

9. The fusion protein according to any one of claims 1-4, the dimeric protein according to claim 5 or claim 6, or the pharmaceutical composition according to claim 7, for use in a method of treating an autoimmune disease.

10. An *in vitro* method of measuring the numbers of regulatory T cells in a human blood sample by contacting human blood cells with the fusion protein of claim 1 at a concentration of between 1 nM and 0.01 nM, and then detecting cells that bind to the protein by flow cytometry.

11. Use of the fusion protein according to any one of claims 1-4, the dimeric protein according to claim 5 or claim 6, or the pharmaceutical composition according to claim 7, in the manufacture of a medicament for treating an autoimmune disease.