Thus, in one aspect, the present invention refers to a human interleukin-2 (IL-2) mutein comprising a V91K substitution and an amino acid sequence at least 95% identical to the amino acid sequence set forth in SEQ ID NO:1, wherein said IL-2 mutein preferentially stimulates T regulatory cells. In another aspect, the present invention refers to a Fc-fusion protein comprising an Fc and the human IL-2 mutein of any of the present invention. In still another aspect, the present invention refers to an isolated nucleic acid encoding the human IL-2 mutein of any of the present invention. In still another aspect, the present invention refers to an isolated nucleic acid encoding an Fc-fusion protein of any of the present invention. In still another aspect, the present invention refers to an expression vector comprising the isolated nucleic acid of any of the present invention operably linked to a promoter. In another aspect, the present invention refers to a host cell comprising the isolated nucleic acid of any of the present invention. In another aspect, the present invention refers to a method of making a human IL-2 mutein or Fc-fusion protein, comprising culturing a host cell of any of the present invention under conditions in which said promoter is expressed and harvesting the human IL-2 mutein or Fc-fusion protein from said culture. In another aspect, the present invention refers to an *in vitro* method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, comprising contacting the population of T cells with an effective amount of a human IL-2 mutein of the present invention. In still another aspect, the present invention refers to an *in vitro* method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, comprising contacting the population of T cells with an effective amount of an Fc-fusion protein of the present invention. In another aspect, the present invention refers to the use of a human IL-2 mutein of any of the present invention or a Fc-fusion protein of the present invention in the manufacture of a medicament for treating a subject with an autoimmune or inflammatory disorder. In another aspect, the present invention refers tomethod of monitoring the response of a subject to treatment with the human interleukin-2 (IL-2) mutein of the present invention, or the Fc-fusion protein of the present invention, comprising detecting a change in said subject, said change being: a) an increase in body temperature, b) an increase in CRP in said subject's peripheral blood measured in a sample obtained from said subject, c.) a decrease in platelets in said subject's peripheral blood in a sample obtained from said subject, d) a decrease in neutrophils in said subject's peripheral blood in a sample obtained from said subject, or e) a decrease in albumin in said subject's peripheral blood in a sample obtained from said subject, wherein said treatment is to be terminated, suspended, reduced in dosing frequency, or reduced in dosing amount after said change is detected. The IL-2 muteins described herein have a minimal numbers of alterations to IL-2, thereby decreasing the likelihood of creating an immune response against the IL-2 mutein and/or endogenous IL-2, yet maintain Treg preferential expansion and activation. Moreover, in certain ~~embodiment~~examples, the IL-2 mutein is fused to a molecule, e.g. an antibody Fc, that increases the serum half-life when administered to a subject. IL-2 muteins have a short serum half-life (3 to 5 hrs for sub-cutaneous injection). Exemplary IL-2 mutein Fc fusions described herein have a half-life in humans of at least 1 day, at least 3 days, at least 5 days, at least 10 days, at least 15 days, at least 20 days, or at least 25 days. This effect on the pharmacokinetics of the IL-2 muteins allows for decreased or less frequent dosing of the IL-2 mutein therapeutic.

Moreover, when creating a pharmaceutical large molecule, consideration must be made for the ability to produce the large molecule in large quantities, while minimizing aggregation and maximizing the stability of the molecule. The IL-2 mutein Fc-fusion molecules demonstrate such attributes.

Additionally, in certain ~~embodiment~~examples, the IL-2 mutein Fc-fusion protein contains an IgG1 Fc region. When it is desirable to abolish the effector functions of IgG1 (e.g., ADCC activity), it was found that mutation of the asparagine at position 297 to glycine (N297G; EU numbering scheme) provided greatly improved purification efficiency and biophysical properties over other mutations that lead to an aglycosylation IgG1 Fc. In preferred ~~embodiment~~examples, cysteines are engineered into the Fc to allow disulfide bonds, which increased stability of the aglycosylated Fc-containing molecule. The usefulness of the aglycosylated Fc goes beyond the IL-2 mutein Fc-fusion context. Thus, provided herein are Fc-containing molecules, Fc-fusions and antibodies, comprising a N297G substitution and optionally substitution of one or more additional residues to cysteine.

Another ~~aspect of the invention~~example described herein includes glycosylated peptide linkers. Preferred linker peptides that are amenable to N-glycosylation include GGNGT (SEQ ID NO:6) or YGNGT (SEQ ID NO:7).

~~In another aspect, the present invention provides~~Also described herein is a human interleukin-2 (IL-2) mutein comprising a V91K substitution and an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:1, wherein said IL-2 mutein preferentially stimulates T regulatory cells. In one ~~embodiment~~example, the human IL-2 mutein comprises an amino acid sequence at least 95% identical to the amino acid sequence set forth in SEQ ID NO:1. In another ~~embodiment~~example, said mutein comprises the amino acid sequence set forth in SEQ ID NO:1. In another ~~embodiment~~example, position 125 is an alanine. In another ~~embodiment~~example, position 125 is a cysteine.

~~In another aspect, the present invention provides~~Also described herein is an Fc-fusion protein comprising an Fc and a human IL-2 mutein. In one ~~embodiment~~example, the Fc is a human IgG1 Fc. In another ~~embodiment~~example, the human IgG1 Fc comprises one or more mutations altering effector function of said Fc. In another ~~embodiment~~example, the human IgG1 comprises a substitution at N297. In another ~~embodiment~~example, the substitution at N297 is N297G. In another ~~embodiment~~example, the Fc-fusion protein comprises a substitution or deletion of the C-terminal lysine of said human IgG Fc. In another ~~embodiment~~example, the C-terminal lysine of said human IgG Fc is deleted. In another ~~embodiment~~example, a linker connects the Fc and human IL-2 mutein portions of said protein. In another ~~embodiment~~example, the linker is GGGGS, GGNGT, or YGNGT. In

another ~~embodiment~~example, the linker is GGGGS. In another ~~embodiment~~example, the IL-2 mutein further comprises an amino acid addition, substitution, or deletion altering glycosylation of said Fc-fusion protein when expressed in mammalian cells. In another ~~embodiment~~example, the IL-2 mutein comprises a T3 substitution. In another ~~embodiment~~example, the IL-2 mutein comprises a T3N or T3A substitution. In another ~~embodiment~~example, the IL-2 mutein comprises a T3N substitution. In another ~~embodiment~~example, the IL-2 mutein further comprises an S5 mutation. In another ~~embodiment~~example, the IL-2 mutein further comprises an S5T mutation. In another ~~embodiment~~example, the Fc-fusion protein comprises an Fc dimer. In another ~~embodiment~~example, said Fc-fusion protein comprises two IL-2 muteins. In another ~~embodiment~~example, said Fc-fusion protein comprises a single IL-2 mutein.

~~In another aspect, the present invention provides~~Also described herein is an isolated nucleic acid encoding a human IL-2 mutein or an Fc portion of an antibody and a human IL-2 muteins. In one ~~embodiment~~example, said Fc portion of an antibody and the human IL-2 mutein are encoded within a single open-reading frame. In another ~~embodiment~~example, the Fc is a human IgG1 Fc. In another ~~embodiment~~example, the human IgG1 Fc comprises one or more mutations altering effector function of said Fc. In another ~~embodiment~~example, the human IgG1 comprises a substitution at N297. In another ~~embodiment~~example, the substitution at N297 is N297G. In another ~~embodiment~~example, the human IgG1 Fc comprises a substitution or deletion of the C-terminal lysine. In another ~~embodiment~~example, the C-terminal lysine of said human IgG Fc is deleted. In another ~~embodiment~~example, the nucleic acid further encodes a linker connecting the Fc portion of an antibody and the human IL-2 mutein. In another ~~embodiment~~example, the linker is GGGGS, GGNGT, or YGNGT. In another ~~embodiment~~example, the linker is GGGGS. In another ~~embodiment~~example, the IL-2 mutein further comprises an amino acid addition, substitution, or deletion altering glycosylation of a protein comprising said IL-2 mutein when expressed in mammalian cells. In another ~~embodiment~~example, the IL-2 mutein comprises a T3 substitution. In another ~~embodiment~~example, the IL-2 mutein comprises a T3N or T3A substitution. In another ~~embodiment~~example, the IL-2 mutein comprises a T3N substitution. In another ~~embodiment~~example, the IL-2 mutein further comprises an S5 mutation. In another ~~embodiment~~example, the IL-2 mutein further comprises an S5T mutation.

~~In another aspect, the present invention provides~~Also described herein is an expression vector comprising an isolated nucleic acid as described above operably linked to a promoter.

~~In another aspect, the present invention provides~~Also described herein is a host cell comprising an isolated nucleic acid as described above. In one ~~embodiment~~example, the isolated nucleic acid is operably linked to a promoter. In another ~~embodiment~~example, said host cell is a prokaryotic cell. In another ~~embodiment~~example, the host cell is *E. coli*. In another ~~embodiment~~example, said host cell is a eukaryotic cell. In another ~~embodiment~~example, the host cell is a mammalian cell. In another ~~embodiment~~example, the host cell is a Chinese hamster ovary (CHO) cell line.

~~In another aspect, the present invention provides~~Also described herein is a method of making a human IL-2 mutein, comprising culturing a host cell as described above under conditions in which said promoter is expressed and harvesting the human IL-2 mutein from said culture. In one ~~embodiment~~example, the method comprises culturing a host cell as described above under conditions in which said promoter is expressed and harvesting the Fc-fusion protein from said culture.

~~In another aspect, the present invention provides~~Also described herein is a method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, comprising contacting the population of T cells with an effective amount of a human IL-2 mutein described above. In one ~~embodiment~~example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases. In another ~~embodiment~~example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

~~In another aspect, the present invention provides~~Also described herein is a method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, comprising contacting the population of T cells with an effective amount of an Fc-fusion protein described above. In one ~~embodiment~~example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases. In another ~~embodiment~~example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

~~In another aspect, the present invention provides~~Also described herein is a method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within peripheral blood of a subject, comprising administering an effective amount of a human IL-2 mutein described above. In one ~~embodiment~~example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases. In another ~~embodiment~~example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

~~In another aspect, the present invention provides~~Also described herein is a method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject, comprising administering an effective amount of an Fc-fusion protein described above. In one ~~embodiment~~example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases. In another ~~embodiment~~example, the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

~~In another aspect, the present invention provides~~Also described herein is a method of increasing the ratio of regulatory T cells (Tregs) to natural killer (NK) cells within the peripheral blood of a subject, comprising administering an effective amount of a human IL-2 mutein described above. In one ~~embodiment~~example, the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases. In another ~~embodiment~~example, the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases at least 50%.

~~In another aspect, the present invention provides~~Also described herein is a method of increasing the ratio of regulatory T cells (Tregs) to natural killer (NK) cells within the peripheral blood of a subject, comprising administering an effective amount of an Fc-fusion protein described above. In one ~~embodiment~~example, the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases. In another ~~embodiment~~example, the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases at least 50%.

~~In another aspect, the present invention provides~~Also described herein is a method of treating a subject with an inflammatory or autoimmune disease, said method comprising administering to said subject a therapeutically effective amount of an IL-2 mutein described above.

~~In another aspect, the present invention provides~~Also described herein is a method of treating a subject with an inflammatory or autoimmune disease, said method comprising administering to said subject a

therapeutically effective amount of an Fc-fusion protein described above. In one ~~embodiment~~example, the administration causes reduction of at least one symptom of the disease. In another ~~embodiment~~example, the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject increases after the administration. In another ~~embodiment~~example, the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject remains essentially the same after the administration. In another ~~embodiment~~example, the inflammatory or autoimmune disease is lupus, graft-versus-host disease, hepatitis C-induced vasculitis, Type I diabetes, multiple sclerosis, spontaneous loss of pregnancy, atopic diseases, or inflammatory bowel diseases.

~~In another aspect, the present invention provides~~Also described herein is an Fc region of a human IgG1 antibody wherein said Fc region comprises an N297G mutation and said Fc region of a human IgG1 comprises at least 90% identity to the amino acid sequence set forth in SEQ ID NO:3. In one ~~embodiment~~example, the Fc region of a human IgG1 comprises at least 95% identity to the amino acid sequence set forth in SEQ ID NO:3. In another ~~embodiment~~example, the Fc region of a human IgG1 comprises the amino acid sequence set forth in SEQ ID NO:3. In another ~~embodiment~~example, the Fc region of a human IgG1 further comprises one or more mutations to stabilize the polypeptide. In another ~~embodiment~~example, one or more amino acids set forth in SEQ ID NO:3 are substituted with cysteine. In another ~~embodiment~~example, V259, A287, R292, V302, L306, V323, or I332 of the amino acid sequence set forth in SEQ ID NO:3 is substituted with cysteine. In another ~~embodiment~~example, the Fc region comprises an A287C and L306C substitution within the amino acid sequence set forth in SEQ ID NO:3. In another ~~embodiment~~example, the Fc region comprises an V259C and L306C substitution within the amino acid sequence set forth in SEQ ID NO:3. In another ~~embodiment~~example, the Fc region comprises an R292C and V302C substitution within the amino acid sequence set forth in SEQ ID NO:3. In another ~~embodiment~~example, the Fc region comprises an V323C and I332C substitution within the amino acid sequence set forth in SEQ ID NO:3.

~~In another aspect, the present invention provides~~Also described herein is an antibody comprising an Fc region described above.

~~In another aspect, the present invention provides~~Also described herein is an Fc-fusion protein comprising the Fc region described above.

~~In another aspect, the present invention provides~~Also described herein is a polypeptide comprising a linker, wherein the linker is GGNGT or YGNGT. In one ~~embodiment~~example the linker comprises N-glycosylation. In another ~~embodiment~~example, the linker is inserted into or replaces a loop in the polypeptide structure.

~~In another aspect, the present invention provides~~Also described herein is a method of making an aglycosylated IgG1 Fc-containing molecule, said method comprising expressing a nucleic acid encoding a polypeptide described above in a mammalian cell culture and harvesting the aglycosylated IgG1 Fc-containing molecule from said culture.

~~In another aspect, the present invention provides~~Also described herein is a method of making an IgG1 Fc-containing molecule aglycosylated when expressed in mammalian cells, said method comprising the step of mutating a codon for N297 in the Fc region to a glycine codon.

~~In another aspect, the present invention provides~~Also described herein is an Fc-fusion protein consisting of the sequence of SEQ ID NO:18 or SEQ ID NO:20. Also described herein is~~In one embodiment, the present invention provides~~ a nucleic acid encoding the Fc-fusion. Also described herein is~~In another embodiment, the present invention provides~~ a cell comprising the nucleic acid. Also described herein is~~In another embodiment, the present invention provides~~ a method of making the Fc-fusion protein comprising incubating the cell under conditions allowing it to express said Fc-fusion protein. Also described herein is~~In another embodiment, the present invention provides~~ a method of treating an inflammatory or auto-immune condition in a subject comprising administering an effective amount of the Fc-fusion protein to said subject. In another ~~embodiment~~example, the inflammatory or auto-immune condition is graft versus host disease.

~~In another aspect, the present invention provides~~Also described herein is a method of monitoring the response of a subject to treatment with a human interleukin-2 (IL-2) mutein described above or an Fc-fusion protein described above, comprising detecting a change in said subject, said change being an increase in body temperature, an increase in CRP in said subject's peripheral blood, a decrease in platelets in said subject's peripheral blood, a decrease in neutrophils in said subject's peripheral blood, or a decrease in albumin in said subject's peripheral blood, wherein said treatment is terminated, suspended, reduced in dosing frequency, or reduced in dosing amount after said change is detected. In one ~~embodiment~~example, said change comprises an increase in body temperature of at least 0.5° C, an increase in CRP in said subject's peripheral blood of at least 0.2 mg/mL, a decrease in platelets in said subject's peripheral blood of at least 0.8-fold, a decrease in neutrophils in said subject's peripheral blood of at least 0.8-fold, or a decrease in albumin in said subject's peripheral blood of at least 0.4-fold.

Brief Description of the Figures

FIG. 1 In a short term stimulation assay, homodimerization by fusion to the C-terminus of IgG-Fc does not alter the activity of IL-2 muteins with reduced potency and with high affinity for CD25.

FIG. 2A and FIG. 2B IL-2 muteins with the indicated mutations and fused to the C-terminus of one side of an Fc-heterodimer were tested for their ability to stimulate STAT5 phosphorylation in T cells. These muteins also contained three mutations conferring high affinity for CD25 (V69A, N71R, Q74P). Their activity was compared to three forms of IL-2 without Fc fusion (open symbols): WT IL-2, HaWT (high affinity for CD25) (N29S, Y31H, K35R, T37A, K48E, V69A, N71R, Q74P), and HaD (high affinity for CD25 and reduced signaling activity) (N29S, Y31H, K35R, T37A, K48E, V69A, N71R, Q74P, N88D). Phospho-STAT5 responses are shown for gated FOXP3+CD4+ and FOXP3-CD4+ T cells.

FIG. 3 Proliferation of T cell subsets in response to titrations of IL-2 muteins fused to Fc-heterodimer. Activity of fusion proteins was compared to three forms of IL-2 without Fc fusion (open symbols): WT IL-2, HaWT (high affinity for CD25) (N29S, Y31H, K35R, T37A, K48E, V69A, N71R, Q74P), and HaD (high affinity for CD25 and reduced signaling activity) (N29S, Y31H, K35R, T37A, K48E, V69A, N71R, Q74P, N88D)

Claims

What is claimed is:

1. A human interleukin-2 (IL-2) mutein comprising a V91K substitution and an amino acid sequence at least 95% identical to the amino acid sequence set forth in SEQ ID NO:1, wherein said IL-2 mutein preferentially stimulates T regulatory cells.

2. The human IL-2 mutein of claim 1, wherein said mutein comprises the amino acid sequence set forth in SEQ ID NO:1.

3. The human IL-2 mutein of claim 1 or 2, wherein position 125 is an alanine.

4. The human IL-2 mutein of claim 1 or 2, wherein position 125 is a cysteine.

5. An Fc-fusion protein comprising an Fc and the human IL-2 mutein of any of claims 1-4.

6. The Fc-fusion protein of claim 5, wherein the Fc is a human IgG1 Fc.

7. The Fc-fusion protein of claim 6, wherein the human IgG1 Fc comprises one or more mutations altering effector function of said Fc.

8. The Fc-fusion protein of claim 7, wherein the human IgG1 comprises a substitution at N297.

9. The Fc-fusion protein of claim 8, wherein the substitution at N297 is N297G.

10. The Fc-fusion protein of any of claims 6-9, comprising a substitution or deletion of the C-terminal lysine of said human IgG Fc.

11. The Fc-fusion protein of claim 10, wherein the C-terminal lysine of said human IgG Fc is deleted.

12. The Fc-fusion protein of any of claims 5-11, wherein a linker connects the Fc and human IL-2 mutein portions of said protein.

13. The Fc-fusion protein of claim 12, wherein the linker is GGGGS (SEQ ID NO: 5), GGNGT, or (SEQ ID NO: 6), and YGNGT (SEQ ID NO: 7).

14. The Fc-fusion protein of claim 13, wherein the linker is GGGGS (SEQ ID NO: 5).

15. The Fc-fusion protein of any of claims 5-14, wherein the IL-2 mutein further comprises an amino acid addition, substitution, or deletion altering glycosylation of said Fc-fusion protein when expressed in mammalian cells.

16. The Fc-fusion protein of claim 15, wherein the IL-2 mutein comprises a T3 substitution.

17. The Fc-fusion protein of claim 16, wherein the IL-2 mutein comprises a T3N or T3A substitution.

18. The Fc-fusion protein of claim 17, wherein the IL-2 mutein comprises a T3N substitution.

19. The Fc-fusion protein of claim 18, wherein the IL-2 mutein further comprises an S5 mutation.

20. The Fc-fusion protein of claim 19, wherein the IL-2 mutein further comprises an S5T mutation.

21. The Fc-fusion protein of any of claim 5-20, wherein said Fc-fusion protein comprises an Fc dimer.

22. The Fc-fusion protein of claim 21, wherein said Fc-fusion protein comprises two IL-2 muteins.

23. The Fc-fusion protein of claim 21, wherein said Fc-fusion protein comprises a single IL-2 mutein.

24. An isolated nucleic acid encoding the human IL-2 mutein of any of claims 1-4.

25. An isolated nucleic acid encoding an Fc-fusion protein of any of claims 5-23.

26. The isolated nucleic acid of claim 25, wherein said Fc portion of an antibody and the human IL-2 mutein are encoded within a single open-reading frame.

27. An expression vector comprising the isolated nucleic acid of any of claims 24-26 operably linked to a promoter.

28. A host cell comprising the isolated nucleic acid of any of claims 24-26.

29. The host cell of claim 28, wherein the isolated nucleic acid is operably linked to a promoter.

30. The host cell of claim 28 or 29, wherein said host cell is a prokaryotic cell.

31. The host cell of claim 30, wherein the host cell is *E. coli*.

32. The host cell of claim 28 or 29, wherein said host cell is a eukaryotic cell.

33. The host cell of claim 32, wherein the host cell is a mammalian cell.

34. The host cell of claim 33, wherein the host cell is a Chinese hamster ovary (CHO) cell line.

35. A method of making a human IL-2 mutein or Fc-fusion protein, comprising culturing a host cell of any of claims 28 to 34 under conditions in which said promoter is expressed and harvesting the human IL-2 mutein or Fc-fusion protein from said culture.

36. An *in vitro* method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, comprising contacting the population of T cells with an effective amount of a human IL-2 mutein of any of claims 1-4.

37. The method of claim 36, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases, or wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

38. An *in vitro* method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, comprising contacting the population of T cells with an effective amount of an Fc-fusion protein of any of claims 5-23.

39. The method of claim 38, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases, or wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

40. Use of a human IL-2 mutein of any of claims 1-4 or a Fc-fusion protein of any of claims 5-23 in the manufacture of a medicament for treating a subject with an autoimmune or inflammatory disorder.

41. The use of claim 40, wherein the disorder is selected from the group consisting of inflammation, atopic diseases, paraneoplastic autoimmune diseases, cartilage inflammation, arthritis, rheumatoid arthritis, juvenile arthritis, juvenile rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, juvenile ankylosing spondylitis, juvenile enteropathic arthritis, juvenile reactive arthritis, juvenile Reiter's Syndrome, SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome), juvenile dermatomyositis, juvenile psoriatic arthritis, juvenile scleroderma, juvenile systemic lupus erythematosus, juvenile vasculitis, pauciarticular rheumatoid arthritis, polyarticular rheumatoid arthritis, systemic onset rheumatoid arthritis, ankylosing spondylitis, enteropathic arthritis, reactive arthritis, Reiter's Syndrome, dermatomyositis, psoriatic arthritis, scleroderma, vasculitis, myolitis, polymyolitis, dermatomyolitis, polyarteritis nodossa, Wegener's granulomatosis, arteritis, ploymyalgia rheumatica, sarcoidosis, sclerosis, primary biliary sclerosis, sclerosing cholangitis, Sjogren's syndrome, psoriasis, plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, erythrodermic psoriasis, dermatitis, atopic dermatitis, atherosclerosis, lupus, Still's disease, Systemic Lupus Erythematosus (SLE), myasthenia gravis, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, celiac disease, multiple sclerosis (MS), asthma, COPD, , rhinosinusitis, rhinosinusitis with polyps, eosinophilic esophogitis, eosinophilic bronchitis, Guillain-Barre disease, Type I diabetes mellitus, thyroiditis, Addison's disease, Raynaud's phenomenon, autoimmune hepatitis, graft-versus-host disease (GVHD), transplantation rejection, kidney damage, hepatitis C-induced vasculitis, and spontaneous loss of pregnancy.

42. The use of claim 41, wherein the disorder is selected from the group consisting of lupus, graft-versus-host disease (GVHD), hepatitis C-induced vasculititis, Systemic Lupus Erythematosus (SLE), rheumatoid arthritis, psoriasis, Crohn's disease, ulcerative colitis, celiac disease, multiple sclerosis (MS), Type I diabetes mellitus, spontaneous loss of pregnancy, atopic diseases, and inflammatory bowel diseases.

43. The use of any of claims 40-42, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases, or wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases at least 50%.

44. A method of monitoring the response of a subject to treatment with the human interleukin-2 (IL-2) mutein of any of claims 1-4, or the Fc-fusion protein of any of claims 5-23, comprising detecting a change in said subject, said change being:

a) an increase in body temperature,

b) an increase in CRP in said subject's peripheral blood measured in a sample obtained from said subject,

c.) a decrease in platelets in said subject's peripheral blood in a sample obtained from said subject,

d) a decrease in neutrophils in said subject's peripheral blood in a sample obtained from said subject, or

e) a decrease in albumin in said subject's peripheral blood in a sample obtained from said subject,

wherein said treatment is to be terminated, suspended, reduced in dosing frequency, or reduced in dosing amount after said change is detected.

45. The method of claim 44, wherein said change comprises:

a) an increase in body temperature of at least 0.5° C,

b) an increase in CRP in said subject's peripheral blood of at least 0.2 mg/mL,

c) a decrease in platelets in said subject's peripheral blood of at least 0.8-fold,

d) a decrease in neutrophils in said subject's peripheral blood of at least 0.8-fold, or

e) a decrease in albumin in said subject's peripheral blood of at least 0.4-fold.

Claims

What is claimed is:

1. A human interleukin-2 (IL-2) mutein comprising a V91K substitution and an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:1, wherein said IL-2 mutein preferentially stimulates T regulatory cells.

2. The human IL-2 mutein of claim 1, wherein said mutein comprises an amino acid sequence at least 95% identical to the amino acid sequence set forth in SEQ ID NO:1

3. The human IL-2 mutein of claim 1 or claim 2, wherein said mutein comprises the amino acid sequence set forth in SEQ ID NO:1

4. The human IL-2 mutein of claim 1, 2, or 3, wherein position 125 is an alanine.

5. The human IL-2 mutein of claim 1, 2, or 3, wherein position 125 is a cysteine.

6. An Fc-fusion protein comprising an Fc and the human IL-2 mutein of any of claims 1-5.

7. The Fc-fusion protein of claim 6, wherein the Fc is a human IgG1 Fc.

8. The Fc-fusion protein of claim 7, wherein the human IgG1 Fc comprises one or more mutations altering effector function of said Fc.

9. The Fc-fusion protein of claim 8, wherein the human IgG1 comprises a substitution at N297.

10. The Fc-fusion protein of claim 9, wherein the substitution at N297 is N297G.

11. The Fc-fusion protein of any of claims 7-10, comprising a substitution or deletion of the C-terminal lysine of said human IgG Fc.

12. The Fc-fusion protein of claim 11, wherein the C-terminal lysine of said human IgG Fc is deleted.

13. The Fc-fusion protein of any of claims 6-12, wherein a linker connects the Fc and human IL-2 mutein portions of said protein.

14. The Fc-fusion protein of claim 13, wherein the linker is GGGGS (SEQ ID NO: 5), GGNGT, or (SEQ ID NO: 6), and YGNGT (SEQ ID NO: 7).

15. The Fc-fusion protein of claim 14, wherein the linker is GGGGS (SEQ ID NO: 5).

16. The Fc-fusion protein of any of claims 6-15, wherein the IL-2 mutein further comprises an amino acid addition, substitution, or deletion altering glycosylation of said Fc-fusion protein when expressed in mammalian cells.

17. The Fc-fusion protein of claim 16, wherein the IL-2 mutein comprises a T3 substitution.

18. The Fc-fusion protein of claim 17, wherein the IL-2 mutein comprises a T3N or T3A substitution.

19. The Fc-fusion protein of claim 18, wherein the IL-2 mutein comprises a T3N substitution.

20. The Fc-fusion protein of claim 19, wherein the IL-2 mutein further comprises an S5 mutation.

21. The Fc-fusion protein of claim 20, wherein the IL-2 mutein further comprises an S5T mutation.

22. The Fc-fusion protein of any of claim 6-21, wherein said Fc-fusion protein comprises an Fc dimer.

23. The Fc-fusion protein of claim 22, wherein said Fc-fusion protein comprises two IL-2 muteins.

24. The Fc-fusion protein of claim 22, wherein said Fc-fusion protein comprises a single IL-2 mutein.

25. An isolated nucleic acid encoding the human IL-2 mutein of any of claims 1-5.

26. An isolated nucleic acid encoding an Fc portion of an antibody and the human IL-2 muteins of any of claims 1-5.

27. The isolated nucleic acid of claim 26, wherein said Fc portion of an antibody and the human IL-2 mutein are encoded within a single open-reading frame.

28. The isolated nucleic acid of claim 26 or 27, wherein the Fc is a human IgG1 Fc.

29. The isolated nucleic acid of claim 28, wherein the human IgG1 Fc comprises one or more mutations altering effector function of said Fc.

30. The isolated nucleic acid of claim 29, wherein the human IgG1 comprises a substitution at N297.

31. The isolated nucleic acid of claim 28, wherein the substitution at N297 is N297G.

32. The isolated nucleic acid of any of claims 28-31 encoding a substitution or deletion of the C-terminal lysine of said human IgG Fc.

33. The isolated nucleic acid of claim 32, wherein the C-terminal lysine of said human IgG Fc is deleted.

34. The isolated nucleic acid of any of claims 26-33, further encoding a linker connecting the Fc portion of an antibody and the human IL-2 mutein.

35. The isolated nucleic acid of claim 34, wherein the linker is GGGGS (SEQ ID NO: 5), GGNGT, or (SEQ ID NO: 6), and YGNGT (SEQ ID NO: 7).

36. The isolated nucleic acid of claim 35, wherein the linker is GGGGS (SEQ ID NO: 5).

37. The isolated nucleic acid of any of claims 26-36, wherein the IL-2 mutein further comprises an amino acid addition, substitution, or deletion altering glycosylation of a protein comprising said IL-2 mutein when expressed in mammalian cells.

38. The isolated nucleic acid of claim 37, wherein the IL-2 mutein comprises a T3 substitution.

39. The isolated nucleic acid of claim 38, wherein the IL-2 mutein comprises a T3N or T3A substitution.

40. The isolated nucleic acid of claim 39, wherein the IL-2 mutein comprises a T3N substitution.

41. The isolated nucleic acid of claim 40, wherein the IL-2 mutein further comprises an S5 mutation.

42. The isolated nucleic acid of claim 41, wherein the IL-2 mutein further comprises an S5T mutation.

43. An expression vector comprising the isolated nucleic acid of any of claims 25-42 operably linked to a promoter.

44. A host cell comprising the isolated nucleic acid of any of claims 25-42.

45. The host cell of claim 44, wherein the isolated nucleic acid is operably linked to a promoter.

46. The host cell of claim 44 or 45, wherein said host cell is a prokaryotic cell.

47. The host cell of claim 46, wherein the host cell is *E. coli*.

48. The host cell of claim 44 or 45, wherein said host cell is a eukaryotic cell.

49. The host cell of claim 48, wherein the host cell is a mammalian cell.

50. The host cell of claim 49, wherein the host cell is a Chinese hamster ovary (CHO) cell line.

51. A method of making a human IL-2 mutein, comprising culturing a host cell of any of claims 45 to 50 under conditions in which said promoter is expressed and harvesting the human IL-2 mutein from said culture.

52. A method of making a Fc-fusion protein, comprising culturing a host cell of any of claims 45 to 50 under conditions in which said promoter is expressed and harvesting the Fc-fusion protein from said culture.

53. An *in vitro* method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, comprising contacting the population of T cells with an effective amount of a human IL-2 mutein of any of claims 1-5.

54. The method of claim 53, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.

55. The method of claim 54, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

56. An *in vitro* method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within a population of T cells, comprising contacting the population of T cells with an effective amount of an Fc-fusion protein of any of claims 6-24.

57. The method of claim 56, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.

58. The method of claim 57, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

59. Use of a human IL-2 mutein of any of claims 1-5 in the manufacture of a medicament for increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within peripheral blood of a subject.

60. The use of claim 59, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.

61. The use of claim 60, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

62. Use of an Fc-fusion protein of any of claims 6-24 in the manufacture of a medicament for increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject.

63. The use of claim 62, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.

64. The use of claim 63, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.

65. Use of a human IL-2 mutein of any of claims 1-5 in the manufacture of a medicament for increasing the ratio of regulatory T cells (Tregs) to natural killer (NK) cells within the peripheral blood of a subject.

66. The use of claim 65, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases.

67. The use of claim 66, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases at least 50%.

68. Use of an Fc-fusion protein of any of claims 6-24 in the manufacture of a medicament for increasing the ratio of regulatory T cells (Tregs) to natural killer (NK) cells within the peripheral blood of a subject.

69. The use of claim 68, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases.

70. The use of claim 63, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases at least 50%.

71. Use of a therapeutically effective amount of an IL-2 mutein of any of claims 1-5 in the manufacture of a medicament for treating a subject with an inflammatory or autoimmune disease.

72. Use of an Fc-fusion protein of any of claims 6-21 in the manufacture of a medicament for treating a subject with an inflammatory or autoimmune disease.

73. The use of claim 71 or 72, wherein administration causes reduction of at least one symptom of the disease.

74. The use of claim 73, wherein the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject increases after the administration.

75. The use of claim 73, wherein the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject remains essentially the same after the administration.

76. The use of any of claims 71-75, wherein the inflammatory or autoimmune disease is lupus, graft-versus-host disease, hepatitis C-induced vasculitis, Type I diabetes, multiple sclerosis, spontaneous loss of pregnancy, atopic diseases, or inflammatory bowel diseases.

77. A polypeptide comprising an Fc region of a human IgG1 antibody wherein said Fc region comprises an N297G mutation and said Fc region of a human IgG1 comprises at least 90% identity to the amino acid sequence set forth in SEQ ID NO:3.

78. The polypeptide of claim 77, wherein said Fc region of a human IgG1 comprises at least 95% identity to the amino acid sequence set forth in SEQ ID NO:3.

79. The polypeptide of claim 77, wherein said Fc region of a human IgG1 comprises the amino acid sequence set forth in SEQ ID NO:3.

80. The polypeptide of claim 77, wherein said Fc region of a human IgG1 further comprises one or more mutations to stabilize the polypeptide.

81. The polypeptide of claim 80, wherein one or more amino acids set forth in SEQ ID NO:3 are substituted with cysteine.

82. The polypeptide of claim 81, wherein V259, A287, R292, V302, L306, V323, or I332 of the amino acid sequence set forth in SEQ ID NO:3 is substituted with cysteine.

83. The polypeptide of claim 82, wherein said Fc region comprises an A287C and L306C substitution within the amino acid sequence set forth in SEQ ID NO:3.

84. The polypeptide of claim 82, wherein said Fc region comprises an V259C and L306C substitution within the amino acid sequence set forth in SEQ ID NO:3.

85. The polypeptide of claim 82, wherein said Fc region comprises an R292C and V302C substitution within the amino acid sequence set forth in SEQ ID NO:3.

86. The polypeptide of claim 82, wherein said Fc region comprises an V323C and I332C substitution within the amino acid sequence set forth in SEQ ID NO:3.

87. An antibody comprising an Fc region of any of claims 77-86.

88. An Fc-fusion protein comprising the Fc region of any of claims 77-86.

89. A polypeptide comprising a linker, wherein the linker is GGNGT (SEQ ID NO: 6) or YGNGT (SEQ ID NO: 7).

90. The polypeptide of claim 89, wherein the linker comprises N-glycosylation.

91. The polypeptide of claim 89, wherein the linker is inserted into or replaces a loop in the polypeptide structure.

92. A method of making an aglycosylated IgG1 Fc-containing molecule, said method comprising:

a) expressing a nucleic acid encoding a polypeptide of any of claims 77-86 in a mammalian cell culture; and

b) harvesting the aglycosylated IgG1 Fc-containing molecule from said culture.

93. A method of making an IgG1 Fc-containing molecule aglycosylated when expressed in mammalian cells, said method comprising the step of mutating a codon for N297 in the Fc region to a glycine codon.

94. An Fc-fusion protein consisting of the sequence of SEQ ID NO:18 or SEQ ID NO:20.

95. A nucleic acid encoding the Fc-fusion of claim 94.

96. A cell comprising the nucleic acid of claim 95.

97. A method of making an Fc-fusion protein comprising incubating the cell of claim 96 under conditions allowing it to express said Fc-fusion protein.

98. Use of a Fc-fusion protein of claim 94 in the manufacture of a medicament for treating an inflammatory or auto-immune condition in a subject.

99. The use of claim 98 wherein said inflammatory or auto-immune condition is graft versus host disease.

100. An *in vitro* method of monitoring the response of a subject to treatment with the human interleukin-2 (IL-2) mutein of claim 1, the Fc-fusion protein of claim 6, or the Fc-fusion protein of claim 94, comprising detecting a change in said subject, said change being:

a) an increase in body temperature,

b) an increase in CRP in said subject's peripheral blood,

c.) a decrease in platelets in said subject's peripheral blood,

d) a decrease in neutrophils in said subject's peripheral blood, or

e) a decrease in albumin in said subject's peripheral blood

wherein said treatment is terminated, suspended, reduced in dosing frequency, or reduced in dosing amount after said change is detected.

101. The method of claim 100, wherein said change comprises:

a) an increase in body temperature of at least 0.5° C,

b) an increase in CRP in said subject's peripheral blood of at least 0.2 mg/mL,

c) a decrease in platelets in said subject's peripheral blood of at least 0.8-fold,

d) a decrease in neutrophils in said subject's peripheral blood of at least 0.8-fold, or

e) a decrease in albumin in said subject's peripheral blood of at least 0.4-fold.