

Claims

What is claimed is:

1. A human interleukin-2 (IL-2) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO:1, wherein said IL-2 mutein has one or
5 more mutations of V91K,D20L; D84R,E61Q; V91K,D20A,E61Q,M104T; N88K,M104L; V91H,M104L; V91K,H16E,M104V; V91K,H16R,M104V; V91K,H16R,M104T; V91K,D20A,M104T; V91K,H16E,M104T; V91K,H16E,E61Q,M104T; V91K,H16R,E61Q,M104T; V91K,H16E; V91H,D20A,M104T; H16E,V91H,M104V; V91H,D20A,E61Q,M104T; V91H,H16R,E16Q; V91K,D20A,M104V; H16E,V91H; V91H,D20A,M104V; H16E,V91H,M104T; H16E,V91H,E61Q,M104T; V91K,E61Q,H16E;
10 V91K,H16R,M104L; H16E,V91H,E16Q; V91K,E61Q,H16R; D20W,V91K,E61Q; V91H,H16R; V91K,H16R; D20W,V91K,E61Q,M104T; V91K,D20A; V91H,D20A,E16Q; V91K,D20A,M104L; V91H,D20A; V91K,E61Q,D20A; V91H,M104T; V91H,M104V; V91K,E61Q; V91K,N88K,E61Q,M104T; V91K,N88K,E61Q; V91H,E61Q; V91K,N88K; D20A,H16E,M104T; D20A,M104T; H16E,N88K; D20A,M104V; D20A,M104L; H16E,M104T; H16E,M104V; N88K,M104V; N88K,E61Q; D20A,E61Q;
15 H16R,D20A; D20W,E61Q; H16E,E61Q; H16E,M104L; N88K,M104T; D20A,H16E; D20A,H16E,E16Q; D20A,H16R,E16Q; V91K,D20W; V91A,H16A; V91A,H16D; V91A,H16E; V91A,H16S; V91E,H16A; V91E,H16D; V91E,H16E; V91E,H16S; V91K,H16A; V91K,H16D; V91K,H16S; or V91S,H16E and preferentially stimulates T regulatory cells relative to other T cells.
2. The human IL-2 mutein of claim 1, wherein the IL-2 mutein further preferentially stimulates
20 T regulatory cells relative to NK cells.
3. The human IL-2 mutein of claim 1, wherein said mutein is at least 95% identical to the amino acid sequence set forth in SEQ ID NO:1.
4. The human IL-2 mutein of claim 1, wherein said mutein is at least 99% identical to the amino acid sequence set forth in SEQ ID NO:1.
- 25 5. The human IL-2 mutein of any one of claims 1-4, wherein the amino acid sequence of said mutein differs from the amino acid sequence set forth in SEQ ID NO:1 at C125A and at the mutation V91K,D20L; D84R,E61Q; V91K,D20A,E61Q,M104T; N88K,M104L; V91H,M104L; V91K,H16E,M104V; V91K,H16R,M104V; V91K,H16R,M104T; V91K,D20A,M104T; V91K,H16E,M104T; V91K,H16E,E61Q,M104T; V91K,H16R,E61Q,M104T; V91K,H16E; V91H,D20A,M104T; H16E,V91H,M104V; V91H,D20A,E61Q,M104T; V91H,H16R,E16Q; V91K,D20A,M104V; H16E,V91H;
30 V91H,D20A,M104V; H16E,V91H,M104T; H16E,V91H,E61Q,M104T; V91K,E61Q,H16E; V91K,H16R,M104L; H16E,V91H,E16Q; V91K,E61Q,H16R; D20W,V91K,E61Q; V91H,H16R; V91K,H16R;

- D20W,V91K,E61Q,M104T; V91K,D20A; V91H,D20A,E16Q; V91K,D20A,M104L; V91H,D20A;
V91K,E61Q,D20A; V91H,M104T; V91H,M104V; V91K,E61Q; V91K,N88K,E61Q,M104T;
V91K,N88K,E61Q; V91H,E61Q; V91K,N88K; D20A,H16E,M104T; D20A,M104T; H16E,N88K;
D20A,M104V; D20A,M104L; H16E,M104T; H16E,M104V; N88K,M104V; N88K,E61Q; D20A,E61Q;
5 H16R,D20A; D20W,E61Q; H16E,E61Q; H16E,M104L; N88K,M104T; D20A,H16E; D20A,H16E,E16Q;
D20A,H16R,E16Q; V91K,D20W; V91A,H16A; V91A,H16D; V91A,H16E; V91A,H16S; V91E,H16A;
V91E,H16D; V91E,H16E; V91E,H16S; V91K,H16A; V91K,H16D; V91K,H16S; or V91S,H16E.
6. The human IL-2 mutein of any one of claims 1-5, wherein the IL-2 mutein has less than 30% pSTAT activation of T effector cells at 1nM IL-2 treatment, using the protocol of Example 1.
- 10 7. The human IL-2 mutein of any one of claims 1-5, wherein the IL-2 mutein has less than 30% pSTAT activation of T effector cells and greater than 40% pSTAT activation of T regulatory cells at 1nM IL-2 treatment, using the protocol of Example 1.
8. The human IL-2 mutein of any one of claims 1-5, wherein the IL-2 mutein has greater than 40% pSTAT activation of T regulatory cells at 1nM IL-2 treatment, using the protocol of Example 1.
- 15 9. The human IL-2 mutein of any one of claims 1-8, further wherein the IL-2 mutein has greater than 30% stability, using the protocol of Example 2.
10. An Fc-fusion protein comprising an Fc and the human IL-2 mutein of any of claims 1-9.
11. The Fc-fusion protein of claim 10, wherein the Fc is a human IgG1 Fc.
12. The Fc-fusion protein of claim 11, wherein the human IgG1 Fc comprises one or more
20 mutations altering effector function of said Fc.
13. The Fc-fusion protein of claim 12, wherein the human IgG1 comprises a substitution at N297.
14. The Fc-fusion protein of claim 13, wherein the substitution at N297 is N297G.
15. The Fc-fusion protein of any of claims 11-14, comprising a substitution or deletion of the C-terminal lysine of said human IgG Fc.
- 25 16. The Fc-fusion protein of claim 15, wherein the C-terminal lysine of said human IgG Fc is deleted.
17. The Fc-fusion protein of any of claims 11-16, wherein a linker connects the Fc and human IL-2 mutein portions of said protein.

18. The Fc-fusion protein of claim 17, wherein the linker is GGGGS (SEQ ID NO: 5), GGNGT, or (SEQ ID NO: 6), and YGNGT (SEQ ID NO: 7).
19. The Fc-fusion protein of claim 18, wherein the linker is GGGGS (SEQ ID NO: 5).
20. The Fc-fusion protein of any of claims 11-19, 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.
21. The Fc-fusion protein of claim 20, wherein the IL-2 mutein comprises a T3 substitution.
22. The Fc-fusion protein of claim 21, wherein the IL-2 mutein comprises a T3N or T3A substitution.
23. The Fc-fusion protein of claim 22, wherein the IL-2 mutein comprises a T3N substitution.
24. The Fc-fusion protein of claim 23, wherein the IL-2 mutein further comprises an S5 mutation.
25. The Fc-fusion protein of claim 24, wherein the IL-2 mutein further comprises an S5T mutation.
26. The Fc-fusion protein of any of claim 11-25, wherein said Fc-fusion protein comprises an Fc dimer.
27. The Fc-fusion protein of claim 26, wherein said Fc-fusion protein comprises two IL-2 muteins.
28. The Fc-fusion protein of claim 26, wherein said Fc-fusion protein comprises a single IL-2 mutein.
29. An isolated nucleic acid encoding the human IL-2 mutein of any of claims 1-9.
30. An isolated nucleic acid encoding an Fc portion of an antibody and the human IL-2 muteins of any of claims 1-9.
31. The isolated nucleic acid of claim 30, wherein said Fc portion of an antibody and the human IL-2 mutein are encoded within a single open-reading frame.
32. The isolated nucleic acid of claim 30 or 31, wherein the Fc is a human IgG1 Fc.
33. The isolated nucleic acid of claim 32, wherein the human IgG1 Fc comprises one or more mutations altering effector function of said Fc.

34. The isolated nucleic acid of claim 33, wherein the human IgG1 comprises a substitution at N297.
35. The isolated nucleic acid of claim 32, wherein the substitution at N297 is N297G.
36. The isolated nucleic acid of any of claims 32-35 encoding a substitution or deletion of the C-terminal lysine of said human IgG Fc.
37. The isolated nucleic acid of claim 36, wherein the C-terminal lysine of said human IgG Fc is deleted.
38. The isolated nucleic acid of any of claims 30-37, further encoding a linker connecting the Fc portion of an antibody and the human IL-2 mutein.
39. The isolated nucleic acid of claim 38, wherein the linker is GGGGS (SEQ ID NO: 5), GGNGT, or (SEQ ID NO: 6), and YGNGT (SEQ ID NO: 7).
40. The isolated nucleic acid of claim 39, wherein the linker is GGGGS (SEQ ID NO: 5).
41. The isolated nucleic acid of any of claims 30-40, 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.
42. The isolated nucleic acid of claim 41, wherein the IL-2 mutein comprises a T3 substitution.
43. The isolated nucleic acid of claim 42, wherein the IL-2 mutein comprises a T3N or T3A substitution.
44. The isolated nucleic acid of claim 43, wherein the IL-2 mutein comprises a T3N substitution.
45. The isolated nucleic acid of claim 44, wherein the IL-2 mutein further comprises an S5 mutation.
46. The isolated nucleic acid of claim 45, wherein the IL-2 mutein further comprises an S5T mutation.
47. An expression vector comprising the isolated nucleic acid of any of claims 29-46 operably linked to a promoter.
48. A host cell comprising the isolated nucleic acid of any of claims 29-46.
49. The host cell of claim 48, wherein the isolated nucleic acid is operably linked to a promoter.

50. The host cell of claim 48 or 49, wherein said host cell is a prokaryotic cell.
51. The host cell of claim 50, wherein the host cell is *E. coli*.
52. The host cell of claim 48 or 49, wherein said host cell is a eukaryotic cell.
53. The host cell of claim 52, wherein the host cell is a mammalian cell.
- 5 54. The host cell of claim 53, wherein the host cell is a Chinese hamster ovary (CHO) cell line.
55. A method of making a human IL-2 mutein, comprising culturing a host cell of any of claims 49 to 54 under conditions in which said promoter is expressed and harvesting the human IL-2 mutein from said culture.
56. A method of making a Fc-fusion protein, comprising culturing a host cell of any of claims 49
10 to 54 under conditions in which said promoter is expressed and harvesting the Fc-fusion protein from said culture.
57. 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 of any of claims 1-9.
- 15 58. The method of claim 57, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.
59. The method of claim 58, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.
60. 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
20 an Fc-fusion protein of any of claims 10-28.
61. The method of claim 60, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.
62. The method of claim 61, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.
63. A method of increasing the ratio of regulatory T cells (Tregs) to non-regulatory T cells within
25 peripheral blood of a subject, comprising administering an effective amount of a human IL-2 mutein of any of claims 1-9.
64. The method of claim 63, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.

65. The method of claim 64, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.
66. 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 of any of claims 10-28.
67. The method of claim 66, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases.
68. The method of claim 67, wherein the ratio of CD3+FoxP3+ cells to CD3+FoxP3- increases at least 50%.
69. 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 of any of claims 1-9.
70. The method of claim 69, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases.
71. The method of claim 70, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases at least 50%.
72. 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 of any of claims 10-28.
73. The method of claim 72, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases.
74. The method of claim 73, wherein the ratio of CD3+FoxP3+ cells to CD3-CD19- lymphocytes expressing CD56 and/or CD16 increases at least 50%.
75. 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 of any of claims 1-9.
76. 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 of any of claims 10-25.

77. The method of treating a subject with an inflammatory or autoimmune disease of claim 75 or 76, wherein administration causes reduction of at least one symptom of the disease.
78. The method of claim 77, wherein the ratio of regulatory T cells (Tregs) to non-regulatory T cells within the peripheral blood of a subject increases after the administration.
- 5 79. The method of claim 77, 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.
80. The method of any of claims 75-79, wherein the inflammatory or autoimmune disease is lupus, graft-versus-host disease, hepatitis C-induced vasculitis, type I diabetes, type II diabetes, multiple sclerosis, rheumatoid arthritis, alopecia areata, atherosclerosis, psoriasis, organ transplant
- 10 rejection, Sjögren's Syndrome, Behcet's disease, spontaneous loss of pregnancy, atopic diseases, asthma, or inflammatory bowel diseases.