

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 more mutations of V91K,H16E; 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; 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; 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; H16R,D20A; D20W,E61Q; H16E,E61Q; H16E,M104L; N88K,M104T; D20A,H16E; D20A,H16E,E16Q; D20A,H16R,E16Q; or V91K,D20W; 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 T regulatory cells relative to NK cells.

3. The human IL-2 mutein of any one of claims 1-2, 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,H16E; 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; 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; 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; H16R,D20A; D20W,E61Q; H16E,E61Q; H16E,M104L; N88K,M104T; D20A,H16E; D20A,H16E,E16Q; D20A,H16R,E16Q; or V91K,D20W.

4. The human IL-2 mutein of any one of claims 1-3, wherein the IL-2 mutein has: (a) less than 30% pSTAT activation of T effector cells at 1nM IL-2 treatment; (b) less than 30% pSTAT activation of T effector cells and greater than 40% pSTAT activation of T regulatory cells at 1nM IL - 2 treatment; or (c) greater than 40% pSTAT activation of T regulatory cells at 1nM IL - 2 treatment; using the protocol of Example 1.

5. The human IL-2 mutein of any one of claims 1-4, further wherein the IL-2 mutein has greater than 30% stability, using the protocol of Example 2.

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: (a) the Fc is a human IgG1 Fc, wherein the human IgG1 Fc comprises one or more mutations altering effector function of said Fc, wherein the human IgG1 comprises a substitution N297G; (b) the Fc-fusion protein comprises a substitution or deletion of the C-terminal lysine of said human IgG Fc; (c) a linker connects the Fc and human IL- 2 mutein portions of said protein, wherein the linker is GGGGS (SEQ ID NO: 5), GGNGT, (SEQ ID NO: 6), or YGNGT (SEQ ID NO: 7); (d) 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; (e) the IL-2 mutein comprises a T3 substitution, wherein the IL-2 mutein comprises a T3N or T3A substitution (f) the IL-2 mutein further comprises an S5 mutation, wherein the IL-2 mutein further comprises an S5T mutation; (g) said Fc-fusion protein comprises an Fc dimer; (h) said Fc-fusion protein comprises two IL-2 muteins; or (i) said Fc-fusion protein comprises a single IL-2 mutein.

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

9. An isolated nucleic acid encoding an Fc portion of an antibody and the human IL-2 muteins of claim 6 or 7.

10. The isolated nucleic acid of claim 30, wherein: (a) said Fc portion of an antibody and the human IL-2 mutein are encoded within a single open-reading frame; (b) the Fc is a human IgG1 Fc; (c) the human IgG1 Fc comprises one or more mutations altering effector function of said Fc, wherein the human IgG1 comprises an N297G substitution; (d) the isolated nucleic acid encodes a substitution or

deletion of the C-terminal lysine of said human IgG Fc; (e) , further encoding a linker connecting the Fc portion of an antibody and the human IL-2 mutein, wherein the linker is GGGGS (SEQ ID NO: 5), GGNGT, (SEQ ID NO: 6), or YGNGT (SEQ ID NO: 7); or (f) the nucleic acid further encodes an amino acid addition, substitution, or deletion altering glycosylation of said Fc-fusion protein when expressed in mammalian cells, wherein the substitution is a T3N, T3A, or S5T mutation.

11. An expression vector comprising the isolated nucleic acid of any of claims 7-10 operably linked to a promoter.

12. A host cell comprising the isolated nucleic acid of any of claims 7-10, wherein: (a) the isolated nucleic acid is operably linked to a promoter; (b) said host cell is a prokaryotic cell; (c) the host cell is *E. coli*; (d) said host cell is a eukaryotic cell; (e) the host cell is a mammalian cell; or (f) the host cell is a Chinese hamster ovary (CHO) cell line.

13. A method of making a human IL-2 mutein, comprising culturing a host cell of claim 12 under conditions in which said promoter is expressed and harvesting the human IL-2 mutein from said culture.

14. A method of making a Fc-fusion protein, comprising culturing a host cell of claim 12 under conditions in which said promoter is expressed and harvesting the Fc-fusion protein from said culture.

15. Use 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.

16. Use of an Fc-fusion protein of claim 6 or 7 in the manufacture of a medicament for treating a subject with an inflammatory or autoimmune disease

17. The use of claim 15 or 16, wherein administration causes reduction of at least one symptom of the disease.

18. The use of any of claims 15-17, 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 rejection, Sjögren's Syndrome, Behcet's disease, spontaneous loss of pregnancy, atopic diseases, asthma, or inflammatory bowel diseases.