WO 2010/85495 describes IL-2 variants that selectively promote activity in Treg cells over non-regulatory T cells, for the treatment of inflammatory disorders. The IL-2 variants described comprise a combination of eight or more amino acid substitutions affecting binding to the different subunits of the IL-2 receptor.

The IL-2 fusion proteins of the present invention preferentially activate human and non-human Tregs with little or no effect on human $CD4^+$ memory T effector cells, 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

The disclosure provides a fusion protein comprising (i) an immunoglobulin molecule, and (ii) two mutant interleukin-2 (IL-2) molecules comprising an amino acid mutation that reduces affinity of the mutant IL-2 molecule to the intermediate affinity IL-2 receptor, as compared to a wild-type IL-2 molecule. In one aspect, the disclosure provides a fusion protein comprising (i) an immunoglobulin molecule that is not capable of specific binding to an antigen and (ii) two mutant interleukin-2 (IL-2) molecules comprising an amino acid mutation that reduces affinity of the mutant IL-2 molecule to the intermediate affinity IL-2 receptor, as compared to a wild-type IL-2 molecule, wherein said mutant IL-2 molecules comprise the sequence of SEQ ID NO: 58.

In one embodiment, said immunoglobulin molecule is an IgG-class immunoglobulin molecule, particularly an IgG_1-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 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. In one embodiment, said immunoglobulin molecule is not capable of specific binding to an antigen, and comprises a heavy chain variable region sequence based on the human Vh3-23 germline

activation is determined by measurement of intracellular STAT, particularly STAT5, phosphorylation levels. In one embodiment, said measurement of intracellular STAT phosphorylation levels is done by flow cytometric analysis.

In another aspect, there is provided a polynucleotide encoding the fusion protein as defined herein. Further, in another aspect, there is provided a vector, particularly an expression vector, comprising the polynucleotide as defined herein. In another aspect, the disclosure provides a host cell comprising the polynucleotide or the vector as defined herein.

The disclosure also provides a method for producing a fusion protein as defined herein, comprising the steps of (i) culturing the host cell as defined herein under conditions suitable for expression of the fusion protein, and (i) recovering the fusion protein. Also provided is a fusion protein comprising (i) an immunoglobulin molecule and (ii) two interleukin-2 (IL-2) molecules comprising an amino acid mutation that reduces affinity of the mutant IL-2 molecule to the intermediate affinity IL-2 receptor, as compared to a wild-type IL-2 molecule, produced by said method. In another aspect, there is provided a method for producing a fusion protein comprising an immunoglobulin molecule and two mutant IL-2 molecules comprising an amino acid mutation that reduces affinity of the mutant IL-2 molecule to the intermediate affinity IL-2 receptor as compared to a wild-type IL-2 molecule, comprising the steps of (i) culturing the host cell as defined herein under conditions suitable for expression of the fusion protein, and (ii) recovering the fusion protein.

In another aspect, the disclosure provides a pharmaceutical composition comprising the fusion protein as defined herein and a pharmaceutically acceptable carrier. The fusion protein or the pharmaceutical composition as defined herein 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), inflammatory bowel disease, Crohn's disease or ulcerative colitis, most specifically type 1 diabetes, or graft-versus-host disease or transplant rejection. In another aspect, there is provided the fusion protein or the pharmaceutical composition as defined herein, for use as a medicament. In another aspect, there is provided the fusion protein or the pharmaceutical composition as defined herein, for use in the treatment or prophylaxis of an autoimmune disease. In another aspect, there is provided the fusion protein or the pharmaceutical

Claims

1. A fusion protein comprising (i) an immunoglobulin molecule that is not capable of specific binding to an antigen and (ii) two mutant interleukin-2 (IL-2) molecules comprising an amino acid mutation that reduces affinity of the mutant IL-2 molecule to the intermediate affinity IL-2 receptor, as compared to a wild-type IL-2 molecule, wherein said mutant IL-2 molecules comprise the sequence of SEQ ID NO: 58.

2. The fusion protein of claim 1, wherein said immunoglobulin molecule is an IgG-class immunoglobulin molecule, particularly an IgG_1-subclass immunoglobulin molecule.

3. The fusion protein of claim 1 or 2, wherein said immunoglobulin molecule is a human immunoglobulin molecule.

4. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises a heavy chain variable region sequence based on the human Vh3-23 germline sequence.

5. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises the heavy chain variable region sequence of SEQ ID NO: 9.

6. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises a light chain variable region sequence based on the human Vk3-20 germline sequence.

7. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises the light chain variable region sequence of SEQ ID NO: 11.

8. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification.

9. The fusion protein of claim 8, wherein said Fc receptor is an Fcγ receptor, particularly a human Fcγ receptor.

10. The fusion protein of claim 8 or 9, wherein said Fc receptor is an activating Fc receptor.

11. The fusion protein of any one of claims 8 to 10, wherein said Fc receptor is selected from the group of FcγRIIIa (CD16a), FcγRI (CD64), FcγRIIa (CD32) and FcαRI (CD89).

12. The fusion protein of any one of claims 8 to 11, wherein said Fc receptor is FcγRIIIa, particularly human FcγRIIIa.

13. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises an amino acid substitution at position 329 (EU numbering) of the immunoglobulin heavy chains.

14. The fusion protein of claim 13, wherein said amino acid substitution is P329G.

15. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises amino acid substitutions at positions 234 and 235 (EU numbering) of the immunoglobulin heavy chains.

16. The fusion protein of claim 15, wherein said amino acid substitutions are L234A and L235A (LALA).

17. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises the amino acid substitutions L234A, L235A and P329G (EU numbering) in the immunoglobulin heavy chains.

18. The fusion protein of any one of the preceding claims, wherein said mutant 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.

19. The fusion protein of any one of the preceding claims, wherein said mutant IL-2 molecules are each fused to said immunoglobulin molecule through a peptide linker.

20. The fusion protein of claim 19, wherein said peptide linker comprises at least 10, particularly at least 15, amino acids.

21. The fusion protein of claim 19 or 20, wherein said peptide linker comprises the amino acid sequence $(G_4S)_3$ (SEQ ID NO: 66).

22. The fusion protein of any one of the preceding claims, wherein the fusion protein comprises the polypeptide sequences of SEQ ID NO: 19 and SEQ ID NO: 50.

23. The fusion protein of any one of the preceding claim, wherein said fusion protein selectively activates regulatory T cells.

24. A polynucleotide encoding the fusion protein of any one of the preceding claims.

25. A vector, particularly an expression vector, comprising the polynucleotide of claim 24.

26. A host cell comprising the polynucleotide of claim 24 or the vector of claim 25.

27. A method for producing a fusion protein comprising an immunoglobulin molecule and two mutant IL-2 molecules comprising an amino acid mutation that reduces affinity of the mutant IL-2 molecule to the intermediate affinity IL-2 receptor as compared to a wild-type IL-2 molecule, comprising the steps of (i) culturing the host cell of claim 26 under conditions suitable for expression of the fusion protein, and (ii) recovering the fusion protein.

28. A pharmaceutical composition comprising the fusion protein of any one of claims 1-23 and a pharmaceutically acceptable carrier.

29. The fusion protein of any one of claims 1-23, or the pharmaceutical composition of claim 28, for use as a medicament.

30. The fusion protein of any one of claims 1-23, or the pharmaceutical composition of claim 28, for use in the treatment or prophylaxis of an autoimmune disease.

31. The fusion protein or the pharmaceutical composition of claim 30, wherein said autoimmune disease is selected from the group of type 1 diabetes, systemic lupus erythematosus, inflammatory bowel disease, Crohn's disease, ulcerative colitis and multiple sclerosis.

32. The fusion protein of any one of claims 1-23, or the pharmaceutical composition of claim 28, for use in the treatment or prophylaxis of transplant rejection or graft-versus-host disease.

33. Use of the fusion protein of any one of claims 1-23 for the manufacture of a medicament for the treatment of a disease in an individual in need thereof.

34. The use of claim 33, wherein said disease is selected from the group of type 1 diabetes, systemic lupus erythematosus, inflammatory bowel disease, Crohn's disease, ulcerative colitis and multiple sclerosis.

35. The use of claim 33, wherein said disease is transplant rejection or graft-versus-host disease.

36. The use of any one of claims 33 to 35, wherein said individual is a mammal, particularly a human.

37. The fusion protein of any one of claims 1-23 for use in selective activation of regulatory T cells *in vitro*.

38. The fusion protein of claim 37, wherein said activation comprises induction of proliferation of regulatory T cells and/or induction of IL-2 receptor signaling in regulatory T cells.

39. A method for selective activation of regulatory T cells *in vitro*, comprising contacting said regulatory T cells with the fusion protein of any one of claims 1-23.

40. The method of claim 39, wherein said activation comprises induction of proliferation of regulatory T cells and/or induction of IL-2 receptor signaling in regulatory T cells.