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 ~~of the Invention~~

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

The ~~invention~~ disclosure also provides a method for producing a fusion protein ~~of the invention~~as defined herein, comprising the steps of (i) culturing the host cell ~~of the invention~~ 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, there is provided a fusion protein produced by the method as defined herein.

In ~~one~~ another aspect, the ~~invention~~ disclosure provides a pharmaceutical composition comprising the fusion protein ~~of the invention~~ as defined herein and a pharmaceutically acceptable carrier. The fusion protein or the pharmaceutical composition ~~of the invention~~ 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

composition as defined herein, for use in the treatment or prophylaxis of transplant rejection or graft-versus-host disease.

~~Further~~ In another aspect, there is provided ~~is~~ the use of the fusion protein ~~of the invention~~ as defined herein for the manufacture of a medicament for the treatment of a disease in an individual in need thereof. Further, the disclosure provides~~, and~~ a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the fusion protein ~~of the invention~~ as defined herein in a pharmaceutically acceptable form. In one embodiment, said disease is an autoimmune disease. In a more specific embodiment, said autoimmune disease is type 1 diabetes, multiple sclerosis (MS), systemic lupus erythematosus (SLE), inflammatory bowel disease, Crohn's disease or ulcerative colitis. In an even more specific embodiment, said autoimmune disease is type 1 diabetes. In another embodiment said disease is transplant rejection or graft-versus-host disease. In one embodiment, said individual is a mammal, particularly a human.

Further provided is the fusion protein ~~of the invention~~ as defined herein for use in selective activation of regulatory T cells *in vitro* or *in vivo*. In another aspect, there is provided the fusion protein as defined herein for use in selective activation of regulatory T cells *in vitro*. In one embodiment, said activation comprises induction of proliferation of regulatory T cells and/or induction of IL-2 receptor signaling, particularly phosphorylation of

STAT5, in regulatory T cells. In one embodiment, said use is *in vitro* and said fusion protein is used at a concentration of about 10 ng/mL or less, particularly about 1 ng/mL or less. In another embodiment, said use is *in vivo* and said fusion protein is used at a dose of about 100 µg/kg body weight or less, particularly about 25 µg/kg body weight or less, more particularly about 10 µg/kg body weight or less.

The ~~invention~~ disclosure also provides a method for selective activation of regulatory T cells *in vitro* or *in vivo*, comprising contacting said regulatory T cells with the fusion protein ~~of the invention~~as defined herein. In another aspect, there is provided a method for selective activation of regulatory T cells *in vitro*, comprising contacting said regulatory T cells with the fusion protein as defined herein. In one embodiment, said activation comprises induction of proliferation of regulatory T cells and/or induction of IL-2 receptor signaling, particularly phosphorylation of STAT5, in regulatory T cells. In one embodiment, said method is *in vitro* and said fusion protein is used at a concentration of about 10 ng/mL or less, particularly about 1 ng/mL or less. In another embodiment, said method is *in vivo* and said fusion protein is used at a dose of about 100 µg/kg body weight or less, particularly about 25 µg/kg body weight or less, more particularly about 10 µg/kg body weight or less.

Brief description of the drawings

Figure 1. Purification of DP47GS IgG-IL-2 fusion protein (see SEQ ID NOs 13, 15, 19). (A) Elution profile of the Protein A affinity chromatography step. (B) Elution profile of the size exclusion chromatography step. Yield 4 mg/L. (C) Analytical capillary electrophoresis SDS (Caliper) of the final product. The following band were observed: non-reduced – 7.5% area at 111 kDa, 92.5% area at 174 kDa; reduced – 23.6% area at 29 kDa, 23.5% area at 67 kDa, 52.9% area at 82 kDa. The product contains about 7.5% "half IgG". (D) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (91% monomer content).

Figure 2. Purification of DP47GS IgG-$(IL\text{-}2)_2$ fusion protein (see SEQ ID NOs 17, 19). (A) Elution profile of the Protein A affinity chromatography step. (B) Elution profile of the size exclusion chromatography step. Yield 13 mg/L. (C) Analytical capillary electrophoresis SDS (Caliper) of the final product. The following band were observed: non-reduced – 2.3% area at 172.5 kDa, 97.7% area at 185 kDa; reduced – 18.3% area at 27.3 kDa, 0.6% area at 29.2 kDa, 81.1% area at 78.3 kDa. (D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (100% monomer content).

Claims

1. 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, 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 is not capable of specific binding to an antigen.

5. 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.

6. 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.

7. 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.

8. 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.

9. 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.

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

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

12. The fusion protein of any one of claims 9 to 11, wherein said Fc receptor is selected from the group of FcγRIIIa (CD16a), FcγRI (CD64), FcγRIIa (CD32) and FcαRI (CD89).

13. The fusion protein of any one of claims 9 to 12, wherein said Fc receptor is FcγRIIIa, particularly human FcγRIIIa.

14. 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.

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

16. 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.

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

18. 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.

~~19. The fusion protein of any one of the preceding claims, wherein said mutant IL-2 molecules comprise an amino acid mutation at a position corresponding to residue 88 of human IL-2 (SEQ ID NO: 1).~~

~~20. The fusion protein of any one of the preceding claims, wherein said amino acid mutation is an amino acid substitution.~~

~~21. The fusion protein of any one of the preceding claims, wherein said mutant IL-2 molecules comprise the amino acid substitution N88D.~~

~~22. The fusion protein of any one of the preceding claims, wherein said mutant IL-2 molecules are human IL-2 molecules.~~

~~23. The fusion protein of any one of the preceding claims, wherein said mutant IL-2 molecules comprise a sequence selected from the group of SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62 and SEQ ID NO: 64, particularly the sequence of SEQ ID NO: 58.~~

~~22.~~19. 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.

~~23.~~20. 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.

~~24.~~21. The fusion protein of claim ~~25~~20, wherein said peptide linker comprises at least 10, particularly at least 15, amino acids.

~~25.~~22. The fusion protein of claim ~~25~~ 20 or ~~26~~21, wherein said peptide linker comprises the amino acid sequence $(G_4S)_3$ (SEQ ID NO: 66).

~~26.~~23. 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.

~~27.~~24. The fusion protein of any one of the preceding claim, wherein said fusion protein selectively activates regulatory T cells.

~~28.~~25. A polynucleotide encoding the fusion protein of any one of the preceding claims.

~~29.~~26. A vector, particularly an expression vector, comprising the polynucleotide of claim ~~30~~325.

~~30.~~27. A host cell comprising the polynucleotide of claim ~~30~~ 25 or the vector of claim ~~31~~26.

~~31.~~28. 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 ~~32~~ 27 under conditions suitable for expression of the fusion protein, and (ii) recovering the fusion protein.

~~32.~~29. A fusion protein produced by the method of claim ~~33~~28.

~~33.~~30. A pharmaceutical composition comprising the fusion protein of any one of claims 1-~~29~~ 24 or ~~34~~ 29 and a pharmaceutically acceptable carrier.

~~34.~~31. The fusion protein of any one of claims 1-~~29~~24 or ~~34~~29, or the pharmaceutical composition of claim ~~32~~30, for use as a medicament.

~~35.~~32. The fusion protein of any one of claims 1-~~29~~24 or ~~34~~29, or the pharmaceutical composition of claim ~~32~~30, for use in the treatment or prophylaxis of an autoimmune disease.

~~36.~~33. The fusion protein or the pharmaceutical composition of claim ~~37~~32, 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.

~~37.~~34. The fusion protein of any one of claims 1-~~29~~24 or ~~34~~29, or the pharmaceutical composition of claim ~~35~~30, for use in the treatment or prophylaxis of transplant rejection or graft-versus-host disease.

~~38.~~35. Use of the fusion protein of any one of claims 1-~~29~~24 or ~~34~~29for the manufacture of a medicament for the treatment of a disease in an individual in need thereof.

~~39.~~36. The use of claim ~~40~~35, wherein said disease is an autoimmune disease.

~~40.~~37. The use of claim ~~41~~36, 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.

~~41.~~38. The use of claim ~~40~~35, wherein said disease is transplant rejection or graft-versus-host disease.

~~42.~~39. The use of any one of claims ~~40~~35 to ~~43~~38, wherein said individual is a mammal, particularly a human.

~~43.~~40. The fusion protein of any one of claims 1-~~29~~24 or ~~34~~29for use in selective activation of regulatory T cells *in vitro*.

~~44.~~41. The fusion protein of claim ~~45~~40, wherein said activation comprises induction of proliferation of regulatory T cells and/or induction of IL-2 receptor signaling in regulatory T cells.

~~45.~~42. 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-~~29~~24 or ~~34~~29.

~~46.~~43. The method of claim ~~47~~42, wherein said activation comprises induction of proliferation of regulatory T cells and/or induction of IL-2 receptor signaling in regulatory T cells.