CLAIMS

1. A single chain fusion protein, comprising from amino-terminus to carboxy-terminus:

(a) a binding domain comprising an immunoglobulin heavy chain variable region (VH) and an immunoglobulin light chain variable region (VL) that specifically binds to a TCR complex or a component thereof,

(b) a linker polypeptide,

(c) an immunoglobulin C_{H2} region polypeptide comprising

(i) an amino acid substitution at the asparagine of position 297, and at least one substitution and at least one deletion at positions 234 to 238;

(ii) one or more substitutions or deletions at positions 234 to 238, and at least one substitution at position 253, 310, 318, 320, 322, or 331; or

(iii) an amino acid substitution at the asparagine of position 297, one or more substitutions or deletions at positions 234 to 238, and at least one substitution at position 253, 310, 318, 320, 322, or 331, and

(d) an immunoglobulin C_{H3} region polypeptide,

wherein the fusion protein does not induce or induces a minimally detectable cytokine release.

2. The fusion protein of claim 1, wherein the binding domain is a single chain Fv (scFv) that specifically binds to the TCR complex or a component thereof.

3. The fusion protein of claim 1 or 2, wherein the CH2 region is derived from a human IgG, a human IgD, or a mouse IgG2a.

4. The fusion protein of any one of claims 1-3, wherein the TCR complex or a component thereof is TCRα, TCRβ, or CD3ε.

5. The fusion protein of any one of claims 1-4, wherein the binding domain is a single chain Fv (scFv) that specifically binds to the TCR complex or a component thereof and comprises an amino acid sequence selected from the group consisting of: SEQ ID NOS:258-264.

6. The fusion protein of any one of claims 1-5, wherein the linker is an immunoglobulin hinge region polypeptide.

7. The fusion protein of claim 6, wherein the immunoglobulin hinge region polypeptide is selected from the group consisting of: a wild type human IgG1 hinge, a human IgG1 hinge with at least one cysteine mutated, and a wild type mouse IGHG2c hinge.

8. The fusion protein of claim 6, wherein the immunoglobulin hinge region polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NOS:212-218, 300, 379-434, and amino acids 3-17 of SEQ ID NO:10.

9. The fusion protein of any one of claims 1-8, wherein the immunoglobulin C_{H2} region polypeptide comprises an amino acid substitution at the asparagine of position 297, and at least one substitution and at least one deletion at positions 234 to 238.

10. The fusion protein of any one of claims 1-8, wherein the immunoglobulin C_{H2} region polypeptide comprises one or more substitutions or deletions at positions 234 to 238, and at least one substitution at position 253, 310, 318, 320, 322, or 331.

11. The fusion protein of any one of claims 1-8, wherein the immunoglobulin C_{H2} region polypeptide comprises an amino acid substitution at the

asparagine of position 297, one or more substitutions or deletions at positions 234 to 238, and at least one substitution at position 253, 310, 318, 320, 322, or 331.

12. The fusion protein of any one of claims 1-11, wherein the asparagine at position 297 is substituted with an alanine.

13. The fusion protein of any one of claims 1-12, wherein the immunoglobulin C_{H2} region polypeptide comprises:

(i) an amino acid substitution at the asparagine of position 297 and one amino acid substitution at position 234, 235, 236 or 237;

(ii) an amino acid substitution at the asparagine of position 297 and amino acid substitutions at two of positions 234-237;

(iii) an amino acid substitution at the asparagine of position 297 and amino acid substitutions at three of positions 234-237;

(iv) an amino acid substitution at the asparagine of position 297, amino acid substitutions at positions 234, 235 and 237, and an amino acid deletion at position 236;

(v) amino acid substitutions at three of positions 234-237 and amino acid substitutions at positions 318, 320 and 322; or

(vi) amino acid substitutions at three of positions 234-237, an amino acid deletion at position 236, and amino acid substitutions at positions 318, 320 and 322.

14. The fusion protein of any one of claims 1-8, wherein the immunoglobulin C_{H2} region polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NOS:103, 104, and 75.

15. The fusion protein of any one of claims 1-12, wherein the immunoglobulin C_{H2} and C_{H3} region polypeptides are human IgG2 C_{H2} and C_{H3} region polypeptides or IgG4 C_{H2} and C_{H3} region polypeptides.

16. The fusion protein of any one of claims 1-8, comprising an amino acid sequence selected from the group consisting of: SEQ ID NOS:12-16, 74, 101, and 307-309.

17. The fusion protein of claim 1, comprising: an amino acid sequence selected from the group consisting of: SEQ ID NOS: 81-83, 89-91, 96-97, 266-268, 271-273, 276-278, 281-283, 286-288, 291-293, and 296-298; or an amino acid sequence selected from the group consisting of SEQ ID NOS: 234, 236, 238, 240, and 304 each without the first 22 amino acid leader sequence.

18. The fusion protein of any one of claims 1-17, wherein the binding domain comprises a variable heavy domain selected from SEQ ID NOs: 220, 243, 244, and 245, and a variable light domain selected from SEQ ID NOs: 222, 241, and 242.

19. The fusion protein of claim 1-17, wherein the binding domain comprises the amino acid sequence as set forth in SEQ ID NO:245 and the amino acid sequence as set forth in SEQ ID NO:241.

20. The protein of claim 18 or 19, wherein the amino acids set forth in SEQ ID NOs:245 and 241 are joined by a linker comprising GlySer, Gly_2Ser (SEQ ID NO:339), Gly_3Ser (SEQ ID NO:340), Gly_4Ser (SEQ ID NO:341), and Gly_5Ser (SEQ ID NO:342), including $(Gly_3Ser)_1(Gly_4Ser)_1$ (SEQ ID NO:343), $(Gly_3Ser)_2(Gly_4Ser)_1$ (SEQ ID NO:344), $(Gly_3Ser)_3(Gly_4Ser)_1$ (SEQ ID NO:345), $(Gly_3Ser)_4(Gly_4Ser)_1$ (SEQ ID NO:346), $(Gly_3Ser)_5(Gly_4Ser)_1$ (SEQ ID NO:347), $(Gly_3Ser)_1(Gly_4Ser)_2$ (SEQ ID NO:349), $(Gly_3Ser)_1(Gly_4Ser)_3$ (SEQ ID NO:350), $(Gly_3Ser)_1(Gly_4Ser)_4$ (SEQ ID NO:351), $(Gly_3Ser)_1(Gly_4Ser)_5$ (SEQ ID NO:352), $(Gly_3Ser)_3(Gly_4Ser)_3$ (SEQ ID NO:353), $(Gly_3Ser)_4(Gly_4Ser)_4$ (SEQ ID NO:354), $(Gly_3Ser)_5(Gly_4Ser)_5$ (SEQ ID NO:355), $(Gly_4Ser)_2$ (SEQ ID NO:356), $(Gly_4Ser)_3$ (SEQ ID NO:145), $(Gly_4Ser)_4$ (SEQ ID NO:357), $(Gly_4Ser)_5$ (SEQ ID NO:358) or GGGGSGGGGSGGGGSAQ (SEQ ID NO:98).

21. The fusion protein of any one of claims 1-20, wherein the fusion protein further does not activate or minimally activates T cells.

22. The fusion protein of any one of claims 1-21, wherein the fusion protein further has at least one of the activities selected from the group consisting of: inducing calcium flux, inducing phosphorylation of a molecule in the T cell receptor signaling pathway, blocking T cell response to an alloantigen, blocking memory T cell response to an antigen, and down modulating the TCR complex.

23. A composition comprising the fusion protein of any one of claims 1-22 and a pharmaceutically acceptable carrier, diluent, or excipient.

24. A unit dose form comprising the composition of claim 23.

25. A polynucleotide encoding the fusion protein of any one of claims 1-22.

26. An expression vector comprising the polynucleotide of claim 25 operably linked to an expression control sequence.

27. A single chain fusion protein, comprising from amino-terminus to carboxy-terminus:

(a) a single chain Fv (scFv) binding domain polypeptide comprising the amino acid sequence of SEQ ID NO:263;

(b) an immunoglobulin hinge region polypeptide comprising amino acids 3-17 of SEQ ID NO:10;

(c) an immunoglobulin C_{H2} region polypeptide comprising

(i) an amino acid substitution at the asparagine of position 297, and one or more substitutions or deletions at positions 234 to 238;

(ii) one or more substitutions or deletions at positions 234 to 238, and at least one substitution at position 253, 310, 318, 320, 322, or 331; or

(iii) an amino acid substitution at the asparagine of position 297, one or more substitutions or deletions at positions 234 to 238, and at least one substitution at position 253, 310, 318, 320, 322, or 331 and

(d) an immunoglobulin C_{H3} region polypeptide.

28. The fusion protein of claim 27, wherein the immunoglobulin C_{H3} region polypeptide consists of the amino acid sequence of SEQ ID NO:69.

29. Use of the fusion protein of any one of claims 1-22, 27, or 28, or the composition of claim 23, or the unit dose of claim 24 in the manufacture of a medicament for reducing rejection of solid organ transplant in a solid organ transplant recipient.

30. Use of the fusion protein of any one of claims 1-22, 27, or 28, or the composition of claim 23, or the unit dose of claim 24 in the manufacture of a medicament for treating an autoimmune disease selected from the group consisting of: inflammatory bowel disease, Crohn's disease, ulcerative colitis, diabetes mellitus, asthma, and arthritis.

31. An *in vitro* method for detecting cytokine release induced by a protein that comprises a binding domain that specifically binds to a TCR complex or a component thereof, comprising:

(a) providing mitogen-primed T cells,

(b) treating the primed T cells of step (a) with the protein that comprises a binding domain that specifically binding to a TCR complex or a component thereof, and

(c) detecting release of a cytokine from the primed T cells treated in step (b), wherein the protein is the fusion protein according to any one of claims 1-22, 27, or 28, or an antibody.

32. An *in vitro* method for detecting T cell activation induced by a protein that comprises a binding domain that specifically binds to a TCR complex or a component thereof, comprising:

(a) providing mitogen-primed T cells,

(b) treating the primed T cells of step (a) with the protein that comprises a binding domain that specifically binding to a TCR complex or a component thereof, and

(c) detecting activation of the primed T cells treated in step (b), wherein the protein is the fusion protein according to any one of claims 1-22, 27, or 28, or an antibody.

CLAIMS

1. A single chain fusion protein, comprising:

a binding domain that specifically binds to a TCR complex or a component thereof, wherein the binding domain comprises an immunoglobulin heavy chain variable region (V_H) comprising an amino acid sequence that is at least 95% identical to the amino acid sequence as set forth in SEQ ID NO: 245 and an immunoglobulin light chain variable region (V_L) comprising an amino acid sequence that is at least 95% identical to the amino acid sequence as set forth in SEQ ID NO: 241;

a linker polypeptide linking said light chain variable region (V_L) with said heavy chain variable region (V_H);

an immunoglobulin C_{H2} region polypeptide; and

an immunoglobulin C_{H3} region polypeptide,

wherein the fusion protein does not induce or induces reduced cytokine release as compared to an OKT3 antibody.

2. The fusion protein of claim 1, wherein the binding domain is a single chain Fv (scFv) that specifically binds to the TCR complex or a component thereof.

3. The fusion protein of claim 1 or claim 2, wherein the C_{H2} region is derived from a human IgG, a human IgD, or a mouse IgG2a.

4. The fusion protein of any one of claims 1-3, wherein the TCR complex or a component thereof is TCRα, TCRβ, or CD3ε.

5. The fusion protein of any one of claims 1-4, wherein the binding domain is a single chain Fv (scFv) that specifically binds to the TCR complex or a component thereof and comprises the amino acid sequence of: SEQ ID NO: 263.

6. The fusion protein of any one of claims 1-5, wherein the linker is an immunoglobulin hinge region polypeptide.

7. The fusion protein of claim 6, wherein the immunoglobulin hinge region polypeptide is selected from the group consisting of: a wild type human IgG1 hinge, a human IgG1 hinge with at least one cysteine mutated, and a wild type mouse IGHG2c hinge.

8. The fusion protein of claim 6, wherein the immunoglobulin hinge region polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NOS:212-218, 300, 379-434, and amino acids 3-17 of SEQ ID NO:10.

9. The fusion protein of any one of claims 1-8, wherein the immunoglobulin C_{H2} region polypeptide comprises:

(i) an amino acid substitution at the asparagine of position 297, and one or more substitutions or deletions at positions 234 to 238; or

(ii) one or more substitutions or deletions at positions 234 to 238, and at least one substitution at position 253, 310, 318, 320, 322, or 331; or

(iii) an amino acid substitution at the asparagine of position 297, one or more substitutions or deletions at positions 234 to 238, and at least one substitution at position 253, 310, 318, 320, 322, or 331.

10. The fusion protein of claim 9, wherein the asparagine at position 297 is substituted with an alanine.

11. The fusion protein of claim 9, wherein the immunoglobulin C_{H2} region polypeptide comprises:

(i) an amino acid substitution at the asparagine of position 297 and one amino acid substitution at position 234, 235, 236 or 237;

(ii) an amino acid substitution at the asparagine of position 297 and amino acid substitutions at two of positions 234-237;

(iii) an amino acid substitution at the asparagine of position 297 and amino acid substitutions at three of positions 234-237;

(iv) an amino acid substitution at the asparagine of position 297, amino acid substitutions at positions 234, 235 and 237, and an amino acid deletion at position 236;

(v) amino acid substitutions at three of positions 234-237 and amino acid substitutions at positions 318, 320 and 322; or

(vi) amino acid substitutions at three of positions 234-237, an amino acid deletion at position 236, and amino acid substitutions at positions 318, 320 and 322.

12. The fusion protein of any one of claims 1-9, wherein the immunoglobulin C_{H2} region polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NOS:102- 104, 75, and 375-378.

13. The fusion protein of any one of claims 1-12, wherein the immunoglobulin C_{H2} and C_{H3} region polypeptides are human IgG2 C_{H2} and C_{H3} region polypeptides or IgG4 C_{H2} and C_{H3} region polypeptides.

14. The fusion protein of claim 1 wherein the binding domain comprises the amino acid sequence as set forth in SEQ ID NO:245 and the amino acid sequence as set forth in SEQ ID NO:241.

15. The protein of claim 1, wherein the linker between said variable heavy and light domains comprises: GlySer, Gly_2Ser (SEQ ID NO:339), Gly_3Ser (SEQ ID NO:340), Gly_4Ser (SEQ ID NO:341), and Gly_5Ser (SEQ ID NO:342), including $(Gly_3Ser)_1(Gly_4Ser)_1$ (SEQ ID NO:343), $(Gly_3Ser)_2(Gly_4Ser)_1$ (SEQ ID NO:344), $(Gly_3Ser)_3(Gly_4Ser)_1$ (SEQ ID NO:345), $(Gly_3Ser)_4(Gly_4Ser)_1$ (SEQ ID NO:346), $(Gly_3Ser)_5(Gly_4Ser)_1$ (SEQ ID NO:347), $(Gly_3Ser)_1(Gly_4Ser)_2$ (SEQ ID NO:349),

$(Gly_3Ser)_1(Gly_4Ser)_3$ (SEQ ID NO:350), $(Gly_3Ser)_1(Gly_4Ser)_4$ (SEQ ID NO:351), $(Gly_3Ser)_1(Gly_4Ser)_5$ (SEQ ID NO:352), $(Gly_3Ser)_3(Gly_4Ser)_3$ (SEQ ID NO:353), $(Gly_3Ser)_4(Gly_4Ser)_4$ (SEQ ID NO:354), $(Gly_3Ser)_5(Gly_4Ser)_5$ (SEQ ID NO:355), $(Gly_4Ser)_2$ (SEQ ID NO:356), $(Gly_4Ser)_3$ (SEQ ID NO:145), $(Gly_4Ser)_4$ (SEQ ID NO:357), $(Gly_4Ser)_5$ (SEQ ID NO:358) or GGGGSGGGGSGGGGSAQ (SEQ ID NO:98).

16. The fusion protein of any one of claims 1-15, wherein the fusion protein further does not activate or minimally activates T cells.

17. The fusion protein of any one of claims 1-16, wherein the fusion protein further has at least one of the activities selected from the group consisting of: inducing calcium flux, inducing phosphorylation of a molecule in the T cell receptor signaling pathway, blocking T cell response to an alloantigen, blocking memory T cell response to an antigen, and down modulating the TCR complex.

18. A composition comprising the fusion protein of any one of claims 1-17 and a pharmaceutically acceptable carrier, diluent, or excipient.

19. A unit dose form comprising the composition of claim 18.

20. A polynucleotide encoding the fusion protein of any one of claims 1-17.

21. An expression vector comprising the polynucleotide of claim 20 operably linked to an expression control sequence.

22. Use of the fusion protein of any one of claims 1-17, or the composition of claim 18, or the unit dose of claim 19 in the manufacture of a medicament for reducing rejection of solid organ transplant in a solid organ transplant recipient.

23. Use of the fusion protein of any one of claims 1-17, or the composition of claim 18, or the unit dose of claim 19 in the manufacture of a medicament for treating an autoimmune disease selected from the group consisting of: inflammatory bowel disease, Crohn's disease, ulcerative colitis, diabetes mellitus, asthma, and arthritis.

24. An *in vitro* method for detecting cytokine release induced by a protein that comprises a binding domain that specifically binds to a TCR complex or a component thereof, comprising:

(a) providing mitogen-primed T cells,

(b) treating the primed T cells of step (a) with the protein that comprises a binding domain that specifically binding to a TCR complex or a component thereof, and

(c) detecting release of a cytokine from the primed T cells treated in step (b), wherein the protein is the fusion protein according to any one of claims 1-17, or an antibody.

25. An *in vitro* method for detecting T cell activation induced by a protein that comprises a binding domain that specifically binds to a TCR complex or a component thereof, comprising:

(a) providing mitogen-primed T cells,

(b) treating the primed T cells of step (a) with the protein that comprises a binding domain that specifically binding to a TCR complex or a component thereof, and

(c) detecting activation of the primed T cells treated in step (b), wherein the protein is the fusion protein according to any one of claims 1-17, or an antibody.