the main mechanisms (Chatenoud(2003) Nature Reviews:123-132). It is further believed that the induction of cytokine storm and *in vivo* T cell activation are required for efficacy of CD3/TCR-directed therapy (Carpenter *et al.* (2000) J. Immunology 165:6205-13). Finally, second generation anti-CD3 monoclonal antibodies reported to be "non-activating" *in vitro* have still induced a cytokine storm *in vivo*.

A number of anti-CD3 directed antibodies are currently being tested in the clinic for use in autoimmune disease, inflammatory disease, and transplant patient. These antibodies include hOKT3γ1(Ala-Ala) (Macrogenics), visilizumab (Nuvion®, PDL), TRX-4 (Tolerx), and NI-0401 (NovImmune). However, patients treated with each of these antibodies have experienced cytokine-release associated adverse events (moderate to severe) and sometimes viral reactivation above that typically observed in the patient population.

Given the cytokine-release associated adverse events related to current T cell antibody and other biologic therapies, there is a continuing need for alternative therapies. The present invention meets such needs, and further provides other related advantages.

BRIEF SUMMARY

The present disclosure provide fusion proteins that bind to a TCR complex or a component thereof, compositions and unit dosage forms comprising such fusion proteins, polynucleotides and expression vectors that encode such fusion proteins, methods for reducing rejection of solid organ transplant or treating an autoimmune disease, and methods for detecting T cell activation.

In one aspect, the present disclosure provides 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. Also disclosed is a fusion protein, comprising, consisting essentially of, or consisting of, from amino-terminus to carboxy-terminus: (a) a binding domain that specifically binds to a TCR complex or a component thereof, (b) a linker polypeptide, (c) optionally an immunoglobulin C_{H2} region polypeptide comprising (i) an amino acid

substitution at the asparagine of position 297; (ii) one or more amino acid substitutions or deletions at positions 234-238; (iii) at least one amino acid substitution or deletion at positions 253, 310, 318, 320, 322, or 331; (iv) an amino acid substitution at the asparagine of position 297p and one or more substitutions or deletions at positions 234-238; (v) an amino acid substitution at the asparagine of position 297 and at least one substitution or deletion at position 253, 310, 318, 320, 322, or 331; (vi) one or more amino acid substitutions or deletions at positions 234-238, and at least one amino acid substitution or deletion at position 253, 310, 318, 320, 322, or 331; or (vi) an amino acid substitution at the asparagine of position 297, one or more amino acid substitutions or deletions at positions 234-238, and at least one amino acid substitution or deletion at position 253, 310, 318, 320, 322, or 331, and (d) an immunoglobulin C_{H3} region polypeptide, wherein the fusion protein does not induce a cytokine storm or induces a minimally detectable cytokine release, and wherein the amino acid residues in the immunoglobulin C_{H2} region are numbered by the EU numbering system. Additional fusion proteins are provided according to claims 2 to 20 and described herein.

In another aspect, the present disclosure provides a composition comprising a fusion protein provided herein and a pharmaceutically acceptable carrier, diluent, or excipient.

In another aspect, the present disclosure provides a unit dose form comprising the above-noted pharmaceutical composition.

In another aspect, the present disclosure provides a polynucleotide encoding a fusion protein provided herein.

In another aspect, the present disclosure provides an expression vector comprising a polynucleotide encoding a fusion protein provided herein that is operably linked to an expression control sequence.

In another aspect, the present disclosure provides use of the fusion protein as described herein, or the composition as described herein, or the unit dose as described herein in the manufacture of a medicament for reducing rejection of solid organ transplant in a solid organ transplant recipient. Also disclosed is a method of reducing rejection of solid organ transplant, comprising administering to a solid organ transplant recipient an effective amount of a fusion protein provided herein.

In another aspect, the present disclosure provides use of the fusion protein as described herein, or the composition as described herein, or the unit dose as described herein 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. Also disclosed is a method for treating an autoimmune disease *(e.g.,* inflammatory bowel diseases, including Crohn's disease and ulcerative colitis, diabetes mellitus, asthma and arthritis), comprising administering to a patient in need thereof an effective amount of a fusion protein provided herein.

In another aspect, the present disclosure provides 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 as described herein, or an antibody. Also disclosed is a 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 binds to a TCR complex or a component thereof *(e.g.,* a fusion protein and an antibody), and (c) detecting release of a cytokine from the primed T cells that have been treated in step (b).

In another aspect, the present disclosure provides 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 as described herein, or an antibody. Also disclosed is a 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 *(e.g.,* a fusion protein and an antibody), and (c) detecting activation of the primed T cells that have been treated in step (b).

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the percentage of activated T cells resulting from treating PHA-primed human T cells with various antibodies and small modular immunopharmaceutical (SMIP™) products. "No Rx" refers to no treatment, which 10 was used as a negative control.

Figure 2 shows the percentage of activated T cells resulting from treating responder cells with various antibodies and SMIP fusion proteins in a mixed lymphocyte reaction assay. "MLR" refers to mixed lymphocyte reaction without any additional treatment. "Responder only" refers to a reaction where

15