Background

[0004] Various strategies are available for stimulating T cell populations *in vitro*, including for expanding antigen-specific T cells *in vitro* for use in adoptive cellular immunotherapy or cancer therapy in which infusions of such T cells have been shown to have anti-tumor reactivity in a tumor-bearing host or for use to treat viral infections. Improved strategies are needed for expanding cell populations *in vitro*, including for research, diagnostic and therapeutic purposes. Provided are reagents, methods, articles of manufacture and kits that meet such needs.

Summary

[0004a] In one aspect, the present disclosure refers to a method for culturing T cells, the method comprising:

(a) incubating a composition comprising primary T cells in the presence of a receptor-binding agent that is a stimulatory agent, wherein the receptor-binding agent i) is reversibly bound to a soluble reagent comprising a plurality of binding sites capable of reversibly binding to the receptor-binding agent and ii) specifically binds to a molecule on the surface of the T cells in a manner that induces or modulates a signal in T cells in the composition; and

(b) within 5 days after initiation of said incubation, disrupting the reversible binding between the receptor-binding agent and the reagent, thereby generating cultured T cells that are stimulated.

[0004b] In another aspect, the present disclosure refers to a method for culturing T cells, the method comprising:

(a) incubating a composition comprising T cells in the presence of:

i) a first receptor-binding agent that is a stimulatory agent that specifically binds to a first molecule expressed on the surface of the T cells in a manner that induces or modulates a TCR/CD3 complex-associated signal in the T cell; and

ii) a second receptor-binding agent, wherein the second receptor-binding agent i) is reversibly bound to a soluble reagent comprising a plurality of binding sites capable of reversibly binding to the second receptor-binding agent that is a stimulatory agent and ii) specifically binds to a second molecule on the surface of the T cells in a manner to induce or modulate a second signal in the T cells; and

(b) within 5 days after initiation of said incubation, disrupting the reversible binding between the second receptor-binding agent and the reagent, thereby generating cultured T cells that are stimulated.

CLAIMS

WHAT IS CLAIMED:

1. A method for culturing T cells, the method comprising:

(a) incubating a composition comprising primary T cells in the presence of a receptor-binding agent that is a stimulatory agent, wherein the receptor-binding agent i) is reversibly bound to a soluble reagent comprising a plurality of binding sites capable of reversibly binding to the receptor-binding agent and ii) specifically binds to a molecule on the surface of the T cells in a manner that induces or modulates a signal in T cells in the composition; and

(b) within 5 days after initiation of said incubation, disrupting the reversible binding between the receptor-binding agent and the reagent, thereby generating cultured T cells that are stimulated.

2. The method of claim 1, wherein:

binding of the receptor-binding agent to the molecule initiates a TCR/CD3 complex-associated signal in the T cells; and/or

the receptor-binding agent specifically binds to a member of a TCR/CD3 complex; and/or

the receptor-binding agent specifically binds to CD3.

3. The method of claim 1 or claim 2, wherein the molecule is a component of the TCR/CD3 complex or is CD3.

4. The method of any of claims 1-3, wherein the receptor-binding agent is a first receptor-binding agent and the signal is a first signal, and the incubation is further carried out in the presence of a second receptor-binding agent, which is capable of specifically binding to a second molecule on the surface of one or more of the T cells.

5. The method of claim 4, wherein the binding of the second receptor-binding agent to the second molecule induces or modulates a second signal in the T cells that enhances, dampens, or modifies the first signal delivered through the first molecule in the T cells.

6. The method of claim 4 or claim 5, wherein the reagent comprises a plurality of binding sites capable of reversibly binding to the second receptor-binding agent, whereby the second receptor-binding agent is reversibly bound to the reagent.

7. The method of claim 4 or claim 5, wherein the reagent is a first reagent and the incubation is carried out in the presence of at least a second reagent which is reversibly bound to the second receptor-binding agent.

8. The method of any of claims 4-7, wherein said disruption comprises introducing to the cells a composition comprising a substance capable of reversing the bond between the first receptor-binding agent and the reagent and/or the second receptor-binding agent and the reagent.

9. A method for culturing T cells, the method comprising:

(a) incubating a composition comprising T cells in the presence of:

i) a first receptor-binding agent that is a stimulatory agent that specifically binds to a first molecule expressed on the surface of the T cells in a manner that induces or modulates a TCR/CD3 complex-associated signal in the T cell; and

ii) a second receptor-binding agent, wherein the second receptor-binding agent i) is reversibly bound to a soluble reagent comprising a plurality of binding sites capable of reversibly binding to the second receptor-binding agent that is a stimulatory agent and ii) specifically binds to a second molecule on the surface of the T cells in a manner to induce or modulate a second signal in the T cells; and

(b) within 5 days after initiation of said incubation, disrupting the reversible binding between the second receptor-binding agent and the reagent, thereby generating cultured T cells that are stimulated.

10. The method of claim 9, wherein the second signal enhances, dampens or modifies a signal delivered through the first molecule in the T cells.

11. The method of claim 9 or claim 10, wherein said disruption is carried out greater than 30 minutes after initiation of said incubation.

12. The method of any of claims 9-11, wherein:

said disruption is carried out between 1 hour and 4 days after initiation of said incubation, between 6 hours and 3 days after initiation of said incubation, between 12 hours and 2 days after initiation of said incubation or between 1 day and 3 days after initiation of said incubation; or

said disruption is carried out between about 1 hour and about 4 days after initiation of said incubation, between about 6 hours and about 3 days after initiation of said incubation, between about 12 hours and about 2 days after initiation of said incubation or between about 1 day and about 3 days after initiation of said incubation; or

said disruption is carried out greater than or equal to about 1 hour after initiation of said incubation and within 1 day, 2 days, 3 days or 4 days after initiation of said incubation.

13. The method of any of claims 5-12, wherein:

the second signal is a signal other than a TCR/CD3 complex-associated signal;

the second signal is capable of enhancing or potentiating a TCR/CD3 complex-associated signal; or

the second molecule is a costimulatory molecule, accessory molecule, cytokine receptor, chemokine receptor, immune checkpoint molecule or is a member of the TNF family or the TNF receptor family.

14. The method of any of claims 4-13, wherein the second molecule is selected from among CD28, CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40 and HVEM.

15. The method of any of claims 4-14, wherein the second molecule is CD28.

16. The method of any of claims 1-15, comprising, after said disruption, further incubating the composition comprising the T cells.

17. The method of claim 16, wherein:

the incubation and further incubation are carried out in the same vessel; and/or

the further incubation is carried out in the presence of the substance; and/or

the method does not comprise removing the substance, receptor-binding agent, second receptor-binding agent and/or reagent from the cell composition prior to the further incubation; and/or

the incubation and/or further incubation is carried out at or about 37 °C ± 2 °C; and/or

the incubation and/or further incubation is carried out in the presence of a further agent that is capable of delivering a signal to T cells.

18. The method of claim 16 or claim 17, wherein the incubation or further incubation is carried out in the presence of a further agent capable of enhancing or inducing proliferation of T cells, CD4+ T cells and/or CD8+ T cells.

19. The method of claim 18, wherein the further agent is a cytokine selected from among IL-2, IL-15 and IL-7.

20. The method of any of claims 16-19, wherein the further incubation is carried out for a time that is no more than 14 days, no more than 12 days, no more than 10 days, no more than 8 days or no more than 6 days.

21. The method of any of claims 1-20, wherein the T cells are primary cells from a subject.

22. The method of any of claims 1-21, wherein the T cells are unfractionated T cells, are enriched or isolated CD3+ T cells, are enriched or isolated CD4+ T cells or are enriched or isolated CD8+ T cells.

23. The method of any of claims 1-22, wherein the T cells are human cells.

24. The method of any of claims 1-23, wherein the each receptor-binding agent individually is selected from among an antibody fragment, a monovalent antibody fragment, a proteinaceous binding molecule with antibody-like binding properties, a molecule containing Ig domains, a cytokine, a chemokine, an aptamer, and MHC molecule and binding fragments thereof.

25. The method of any of claims 1-24, wherein:

the receptor-binding agent comprise an antibody fragment;

the receptor-binding agent comprise a Fab fragment;

the receptor-binding agent is a divalent antibody fragment selected from among a $F(ab')_2$-fragment and a divalent single-chain Fv (scFv) fragment;

the receptor-binding agent is a monovalent antibody fragment selected from among a Fab fragment, an Fv fragment and an scFv fragment; and/or

the receptor-binding agent is a proteinaceous binding molecule with antibody-like binding properties selected from among aptamers, muteins based on a polypeptide of the lipocalin family, glubodies, proteins based on the ankyrin scaffold, proteins based on the crystalline scaffold, adnectins and avimers.

26. The method of any of claims 1-25, wherein the reagent is or comprises streptavidin, avidin, mutein of streptavidin that reversibly binds biotin, a biotin analog or a biologically active fragment thereof; mutein of avidin or streptavidin that reversibly binds a streptavidin-binding peptide; a reagent that comprises at least two chelating groups K, wherein the at least two chelating groups are capable of binding to a transition metal ion; an agent capable of binding to an oligohistidine affinity tag; an agent capable of binding to a glutathione-S-transferase; calmodulin or an analog thereof; an agent capable of binding to calmodulin binding peptide (CBP); an agent capable of binding to a FLAG-peptide; an agent capable of binding to an HA-tag; an agent capable of binding to maltose binding protein (MBP); an agent capable of binding to an HSV epitope; an agent capable of binding to a myc epitope; or an agent capable of binding to a biotinylated carrier protein.

27. The method of any of claims 1-26, wherein the reagent is an oligomer or polymer of streptavidin, avidin, a mutein of streptavidin that reversibly binds biotin or a biologically active fragment; a streptavidin or avidin mutein that reversibly binds a streptavidin-binding peptide; a reagent that comprises at least two chelating groups K, wherein the at least two chelating groups are capable of binding to a transition metal ion; an agent capable of binding to an oligohistidine affinity tag; an agent capable of binding to a glutathione-S-transferase; calmodulin or an analog thereof; an agent capable of binding to calmodulin binding peptide

(CBP); an agent capable of binding to a FLAG-peptide; an agent capable of binding to an HA-tag; an agent capable of binding to maltose binding protein (MBP); an agent capable of binding to an HSV epitope; an agent capable of binding to a myc epitope; or an agent capable of binding to a biotinylated carrier protein.

28. The method of any of claims 1-27, wherein the reagent comprises an oligomer or polymer of a streptavidin mutein.

29. The method of claim 27 or claim 28, wherein individual molecules of the oligomer or polymer are crosslinked by a polysaccharide or a bifunctional linker.

30. The method of any of claims 1-29, wherein:

the reagent comprises a streptavidin mutein comprising the amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1;or

the streptavidin analog or mutein comprises the amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

31. The method of any of claims 1-30, wherein the streptavidin mutein comprises:

a) the sequence of amino acids set forth in any of SEQ ID NOS: 3-6, 27 and 28;

b) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS:3-6, 27 and 28 and contains the amino acid sequence corresponding to Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} and that reversibly binds to biotin or a biologically active form thereof, a biotin analog or mutein or a biologically active fragment thereof or a streptavidin-binding peptide; or

c) a functional fragment of a) or b) that reversibly binds to biotin or a biologically active form thereof, a biotin analog or mutein or a biologically active fragment thereof or a streptavidin-binding peptide.

32. The method of any of claims 26-31, wherein:

the reagent is or comprises a streptavidin mutein ; and

the streptavidin-binding peptide is selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

33. The method of any of claims 1-32, wherein said disruption comprises introducing to the cells a composition comprising a substance capable of reversing the bond between the receptor-binding agent and the reagent.

34. The method of claim 33, wherein the substance is a free binding partner and/or is a competition agent.

35. The method of any of claims 1-34, wherein:

the reagent is or comprises a streptavidin analog or mutein or biologically active fragments thereof; and

the substance comprises a streptavidin-binding peptide, biotin or a biologically active fragment, optionally a D-biotin, or a biotin analog or biologically active fragment.

36. The method of claim 35, wherein:

the substance is a streptavidin-binding peptide is selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

37. The method of any of claims 1-36, wherein:

prior to the incubation, contacting cells with a selection agent that specifically binds to a marker comprised by T cells or target cells of the composition, thereby generating or obtaining the composition comprising the T cells or target cells; or

at least a portion of the incubation is carried out further in the presence of a selection agent that specifically binds to a marker comprised by T cells or target cells of the composition, wherein the cultured T cells are enriched for T cells or target cells comprising the marker.

38. The method of claim 37, wherein:

the selection agent is reversibly bound to the reagent, said reagent further comprising a plurality of binding sites capable of specifically binding the selection agent; or

the selection agent is reversibly bound to a further reagent comprising a plurality of binding sites capable of specifically binding to the selection agent.

39. The method of any of claims 1-38, wherein the induction or modulation of the signal effects an increase in expansion and/or activation of the cultured T cells compared to incubation of T cells in the absence of the induction or modulation of the signal.

40. The method of any of claims 1-39, wherein the method increases expansion and/or proliferation of T cells in the composition, alters the metabolic profile of T cells in the composition, alters the subset of CD8+ T cells in the composition; and/or increases the percentage of long-lived memory T cells in the composition, each compared to the T cells in the composition prior to the incubation, subsequent to the incubation but in the absence of the disruption or subsequent to the incubation but in the presence of an agent that specifically binds CD28 and/or induces or modulates CD28 signaling.

41. The method of any of claims 1-40, further comprising introducing a recombinant nucleic acid molecule into T cells of the population, which nucleic acid molecule encodes a recombinant protein, whereby cells express the recombinant protein.

42. The method of claim 41, wherein the recombinant receptor is a chimeric antigen receptor or transgenic T cell receptor (TCR).