use in cell therapy. In particular, methods are needed for the preparation and engineering of cells, e.g., a plurality of isolated cell types or sub-types, with improved efficiency, safety, variability, and conservation of resources. Provided are methods, cells, compositions, kits, and systems that meet such needs.

Summary

[0004a] In one aspect, there is provided a method for enriching CD4+ and CD8+ T cells, the method comprising: (a) performing a first immunoaffinity-based selection in a closed system, said first selection comprising enriching for one of (i) CD4+ T cells and (ii) CD8+ T cells from a sample containing primary human T cells, the enrichment thereby generating a first selected population and a non-selected population; (b) performing a second immunoaffinity-based selection in the closed system, said second selection comprising enriching for the other of (i) CD4+ T cells and (ii) CD8+ T cells from the non-selected population, the enrichment thereby generating a second selected population; and (c) combining cells of the first selected population and cells of the second selected population, at a desired ratio of between at or about 5:1 and at or about 1:5 CD4+ cells to CD8+ cells.

[0004b] Also disclosed is a kit produced by the method as described herein, comprising cells of the first selected population and cells of the second selected population at the desired CD4:CD8 ratio.

[0004c] In another aspect, there is provided a method for producing genetically engineered T cells, the method comprising: (a) performing a first immunoaffinity-based selection in a closed system, said first selection comprising enriching for one of (i) CD4+ cells and (ii) CD8+ cells from a sample containing primary human T cells, the enrichment thereby generating a first selected population and a non-selected population; and (b) performing a second immunoaffinity-based selection in the closed system, said second selection comprising enriching for the other of (i) CD4+ cells and (ii) CD8+ cells from the non-selected population, the enrichment thereby generating a second selected population; (c) incubating a culture-initiating composition, which comprises cells of the first selected population and cells of the second selected population, in a culture vessel under stimulating conditions, thereby generating stimulated cells; and (d) introducing a genetically engineered antigen receptor into stimulated cells generated in (c), wherein the method thereby generates an output composition comprising CD4+ T cells and CD8+ T cells expressing the genetically engineered antigen receptor.

[0004d] Also disclosed is a method for enriching CD4+ and CD8+ T cells, the method comprising contacting cells of a sample containing primary human T cells with a first immunoaffinity reagent that specifically binds to CD4 and a second immunoaffinity reagent that specifically binds to CD8 in an incubation composition, under conditions whereby the immunoaffinity reagents specifically bind to CD4 and CD8 molecules, respectively, on the surface of cells in the sample; and recovering cells bound to the first and/or the second immunoaffinity reagent, thereby generating an enriched composition comprising CD4+ cells and CD8+ cells at a desired ratio, wherein: the first and/or second immunoaffinity reagent are present in the incubation composition at a sub-optimal yield concentration, whereby the enriched composition contains less than 70% of the total CD4+ cells in the incubation composition and/or less than 70% of the CD8+ cells in the incubation composition.

[0004e] Also disclosed is a method for producing genetically engineered T cells, the method comprising: (a) enriching a sample for primary human T cells , the enriching comprising: contacting cells of said sample with a first immunoaffinity reagent that specifically binds to CD4 and a second immunoaffinity reagent that specifically binds to CD8 in an incubation composition, under conditions whereby the immunoaffinity reagents specifically bind to CD4 and CD8 molecules, respectively, on the surface of cells in the sample; and recovering cells bound to the first and/or the second immunoaffinity reagent, thereby generating an enriched composition comprising CD4+ cells and CD8+ cells at a culture-initiating ratio, wherein: the first and/or second immunoaffinity reagent are present in the incubation composition at a sub-optimal yield concentration, whereby the enriched composition contains less than 70% of the total CD4+ cells in the incubation composition and/or less than 70% of the CD8+ cells in the incubation composition; and (b) incubating cells of the enriched composition at or substantially at the culture-initiation ratio in a culture-initiation composition in a culture vessel under stimulating conditions; and (c) introducing a genetically engineered antigen receptor into cells stimulated in (b), thereby generating an output composition comprising CD4+ T cells and CD8+ T cells expressing the genetically engineered antigen receptor.

[0004f] Also disclosed is a composition or kit comprising cells produced by the method as described herein.

[0004g] Also disclosed is the composition or kit as described herein for use in treating a disease or condition in a subject.

[0004h] Also disclosed is use of a composition as described herein for the manufacture of a medicament for treating a disease or condition in a subject.

[0004i] Also disclosed is a closed apparatus system for purification of target cells, comprising: a) a first affinity chromatography matrix comprising a first binding agent immobilized thereon, which binding agent specifically binds to a first cell surface marker present on a first cell type, said first affinity matrix not comprising a second binding agent that specifically binds to a second cell surface marker, wherein: the first affinity chromatography matrix is operably connected to a storage reservoir comprising a cell sample; and is operably connected to an output vessel; b) a second affinity chromatography matrix comprising a second binding agent immobilized thereon, which second binding agent specifically binds to a second cell surface marker present on a second cell type, wherein: the first affinity chromatography matrix is operably connected to the second affinity chromatography matrix; and the second affinity chromatography matrix is operably connected to the output vessel, and is operably linked to a waste vessel; c) the output vessel; and d) the waste vessel, wherein the system is configured to permit, within the closed system, collection of, in the output vessel in a single composition, (i) cells having bound and been recovered from the first affinity chromatography matrix and (ii) cells having passed through and not bound to the first affinity chromatography matrix and having bound and been recovered from the second chromatography matrix.

[0005] Provided are methods for the preparation and engineering of cells and populations of cells and cells and compositions produced by the methods. In some examples, the cells can be used for immunotherapy, such as in connection with adoptive immunotherapy methods. In some example, the provided methods include isolation, selection or enrichment of CD4+ and CD8+ cells, or sub-populations thereof, from the same starting sample, such as a single sample, for example a single apheresis sample, leukapheresis sample or a sample containing peripheral blood mononuclear

CLAIMS

1. A method for enriching CD4+ and CD8+ T cells, the method comprising:

(a) performing a first immunoaffinity-based selection in a closed system, said first selection comprising enriching for one of (i) CD4+ T cells and (ii) CD8+ T cells from a sample containing primary human T cells, the enrichment thereby generating a first selected population and a non-selected population;

(b) performing a second immunoaffinity-based selection in the closed system, said second selection comprising enriching for the other of (i) CD4+ T cells and (ii) CD8+ T cells from the non-selected population, the enrichment thereby generating a second selected population; and

(c) combining cells of the first selected population and cells of the second selected population, at a desired ratio of between at or about 5:1 and at or about 1:5 CD4+ cells to CD8+ cells.

2. The method of claim 1, further comprising introducing a genetically engineered antigen receptor into cells of the first selected population and/or introducing a genetically engineered antigen receptor into cells of the second selected population.

3. The method of any of claims 1-2, further comprising incubating cells of the first selected population under stimulating conditions and/or incubating cells of the second selected population under stimulating conditions.

4. The method of claim 1, further comprising:

introducing a genetically engineered antigen receptor into cells of the first selected population and/or introducing a genetically engineered antigen receptor into cells of the second selected population; and

incubating cells of the first selected population under stimulating conditions and/or incubating cells of the second selected population under stimulating conditions,

wherein the incubation of cells of the first selected population and/or of cells of the second selected population is performed prior to, during and/or subsequent to introducing the genetically engineered antigen receptor.

5. The method of any of claims 1-4, wherein said combining is performed in the closed system.

6. The method of any of claims 1-5, wherein the desired CD4+ to CD8+ ratio is between at or about 2:1 and at or about 1:2 or between 2:1 and 1:2.

7. The method of any of claims 1-6, wherein the desired ratio is 1:1 or is about 1:1.

8. A method for producing genetically engineered T cells, the method comprising:

(a) performing a first immunoaffinity-based selection in a closed system, said first selection comprising enriching for one of (i) $CD4^+$ cells and (ii) CD8+ cells from a sample containing primary human T cells, the enrichment thereby generating a first selected population and a non-selected population; and

(b) performing a second immunoaffinity-based selection in the closed system, said second selection comprising enriching for the other of (i) CD4+ cells and (ii) CD8+ cells from the non-selected population, the enrichment thereby generating a second selected population;

(c) incubating a culture-initiating composition, which comprises cells of the first selected population and cells of the second selected population, in a culture vessel under stimulating conditions, thereby generating stimulated cells; and

(d) introducing a genetically engineered antigen receptor into stimulated cells generated in (c),

wherein the method thereby generates an output composition comprising $CD4^+$ T cells and $CD8^+$ T cells expressing the genetically engineered antigen receptor.

9. The method of claim 8, further comprising, prior to step (c), combining cells of the first and second selected cell populations to produce the culture-initiating composition and/or wherein the CD4+ and CD8+ cells in the culture-initiating composition are present at a desired ratio of between at or about 10:1 and at or about 1:10 CD4+ cells to CD8+ cells.

10. The method of claim 9, wherein said combining is performed in the closed system.

11. The method of claim 9 or claim 10, wherein the desired ratio of $CD4^+$ to $CD8^+$ cells is between at or about 5:1 and at or about 1:5, or between at or about 2:1 and at or about 1:2.

12. The method of any of claims 9-11, wherein the desired ratio of CD4+ to CD8+ cells is at or about 1:1.

13. The method of any of claims 1-7 and 9-12, wherein the sample is obtained from a human subject and:

the desired ratio of $CD4^+$ to $CD8^+$ cells is different than the ratio of $CD4^+$ to $CD8^+$ cells in the sample from the subject; and/or

the desired ratio of $CD4^+$ to $CD8^+$ cells is at least 10 %, at least 20 %, at least 30 %, at least 40 %, or at least 50 % greater or less than the ratio of $CD4^+$ to $CD8^+$ cells in the sample from the subject.

14. The method of any of claims 1-13, wherein the first and/or second immununoaffinity-based selection comprises:

contacting cells with an antibody capable of specifically binding to a cell surface marker, wherein the antibody is immobilized on a magnetic particle; and

recovering cells bound to the antibody, thereby effecting positive selection, or recovering cells not bound to the antibody, thereby effecting negative selection, wherein the recovered cells are enriched for the CD4+ cells or the CD8+ cells.

15. The method of any of claims 1-14, wherein the first selection and second selection are carried out in separate separation vessels, which are operably connected.

16. The method of any of claims 1-13 and 15, wherein the immunoaffinity-based selection in the first and/or second selection comprises: contacting cells with an antibody immobilized on or attached to an affinity chromatography matrix, said antibody capable of specifically binding to a cell surface marker to effect positive or negative selection of CD4+ or CD8+ cells; and

recovering cells from the affinity chromatography matrix enriched for the CD4+ cells or the CD8+ cells.

17. The method of claim 16, wherein:

the antibody comprises one or more binding partners capable of forming a reversible bond with a binding reagent immobilized on the affinity chromatography matrix, whereby the antibody is reversibly bound to said matrix during said contacting; and

cells expressing a cell surface marker specifically bound by the antibody on said affinity chromatography matrix are capable of being recovered from the affinity chromatography matrix by disrupting the reversible binding between the binding reagent and binding partner.

18. The method of any of claim 16 or claim 17, wherein the affinity chromatography matrix of the first and/or second selection is packed in a separation vessel, which is a column.

19. The method of any of claims 16-18, wherein the affinity chromatography matrix adsorbs and/or is capable of selecting at least or at least about 50 x 10^6 cells/mL, 100 x 10^6 cells/mL, 200 x 10^6 cells/mL or 400 x 10^6 cells/mL.

20. The method of any of claims 1-19, wherein the enriching for the CD4+ cells comprises positive selection based on surface expression of CD4 and/or the enriching for the CD8+ cells comprises positive selection based on surface expression of CD8.

21. The method of any of claims 1-20, wherein the one of the first and second selection that comprises enriching for the CD8+ cells further comprises enriching for central memory T

(T_{CM}) cells and/or enriching for cells expressing a marker selected from among CD28, CD62L, CCR7, CD127 and CD27.

22. The method of claim 17, wherein:

the binding partner is selected from among biotin, a biotin analog, or a peptide capable of binding to the binding reagent; and

the binding reagent is selected from among streptavidin, a streptavidin analog or mutein that binds biotin and/or a streptavidin binding peptide, avidin, and an avidin analog or mutein that binds biotin and/or a streptavidin binding peptide.

23. The method of claim 22, wherein:

the binding partner comprises a peptide comprising the sequence of amino acids set forth in SEQ ID NO:6-10 and/or

the binding reagent is streptavidin mutein comprising the sequence of amino acids set forth in SEQ ID NO: 12, 13, 15 or 16.

24. The method of claim 22 or claim 23, wherein the competition reagent is biotin or a biotin analog.

25. The method of any of claims 1-7 and 9-24, comprising:

prior to performing the first selection and/or second selection, determining the ratio of CD4+ to CD8+ T cells in the sample; and

based on the ratio of CD4+ to CD8+ T cells in the sample, adjusting the first and/or second selection to enrich CD4+ cells and CD8+ cells that, when combined, are at the desired ratio.

26. The method of claim 25, wherein:

the first and/or second selection comprises immunoaffinity-based selection comprising an affinity chromatography matrix; and

adjusting the first and/or second selection comprises choosing the amount of affinity chromatography matrix in the first and/or second selection sufficient to achieve the desired ratio.

27. The method of claim 8, wherein the method results in an output composition comprising a ratio of $CD4^+$ to $CD8^+$ cells that is between at or about 2:1 and at or about 1:5.

28. The method of claim 27, wherein the ratio of $CD4^+$ to $CD8^+$ cells in the output composition is 1:1 or is about 1:1.

29. The method of any of claims 1-28, wherein the sample is obtained from a subject.

30. The method of any of claims 1-29, wherein the sample is an apheresis, peripheral blood mononuclear cell (PBMC), or leukapheresis sample.

31. The method of any of claims 3-30, wherein: the stimulating condition comprises an agent capable of activating one or more intracellular signaling domains of one or more components of a TCR complex; and/or comprises the presence of an anti-CD3 antibody, and anti-CD28 antibody, anti-4-1BB antibody, and/or a cytokine.

32. The method of any of claims 2-31, wherein: the genetically engineered antigen receptor comprises a T cell receptor (TCR) or a functional non-TCR antigen receptor; or the genetically engineered antigen receptor comprises a chimeric antigen receptor (CAR).