Tregs without negatively impacting Treg identity and function. Moreover, development of a simplified and adaptable protocol for Treg expansion is desirable to reduce the complexity of cell manufacturing processes and better enable process automation, while maintaining Treg phenotype of the starting cell population.

BRIEF SUMMARY

[0006] The present disclosure relates generally to the manufacture of regulatory T cells (Tregs) for use in adoptive cell therapy. In particular, the present disclosure relates to simplified approaches for the expansion of Tregs *ex vivo*. Tregs produced in this way are suitable for use in various immunotherapy regimens.

[0006a] In one aspect, the present disclosure provides a method for the production of human regulatory T cells (Tregs), comprising: a) isolating CD4+, CD25+, CD127-/low T cells from a lymphocyte-containing biological sample obtained from a human subject; and b) culturing the T cells in medium comprising a CD28 superagonist (CD28SA) antibody, interleukin-2 (IL-2), and tumor necrosis factor-alpha (TNF-alpha) ~~under conditions effective in~~to produc~~ing~~ human Tregs that are CD4+, FOXP3+, HELIOS+, and have a demethylated Treg-specific demethylation region (TSDR).

[006b] In another aspect, the present disclosure provides a pharmaceutical composition comprising from 10^7 to 10^{11} of ~~the~~ human Tregs ~~produced using the method disclosed herein~~, and a physiologically acceptable buffer, wherein the human Tregs are produced using a method comprising the steps of: a) isolating CD4+, CD25+, CD127-/low T cells from a lymphocyte-containing biological sample obtained from a human subject; and b) culturing the T cells in medium comprising a CD28 superagonist (CD28SA) antibody, interleukin-2 (IL-2), and tumor necrosis factor-alpha (TNF-alpha) to produce human Tregs that are CD4+, FOXP3+, HELIOS+, and have a demethylated Treg-specific demethylation region (TSDR), wherein step b) does not comprise use of an anti-CD3 antibody and does not comprise use of magnetic beads or Fc receptor-expressing feeder cells to cross-link CD28 and CD3 of the isolated T cells.

[006c] In yet another aspect, the present disclosure provides the pharmaceutical composition disclosed herein for use in treating or preventing a pathological immune response in a human subject in need thereof.

[006d] In one aspect, the present disclosure provides use of ~~the~~ human Tregs ~~produced using the method disclosed herein~~ in the manufacture of a medicament for inhibiting proliferation of human effector T cells (Teffs) to treat or prevent a pathological immune response in a human subject in need thereof, the use comprising: contacting human CD4+, CD25-, CD127+ Teffs with the human Tregs under conditions effective in inhibiting proliferation of the Teffs; optionally wherein the contacting is done in the presence of TNF-alpha, wherein the human Tregs are produced using a method comprising the steps of: a) isolating CD4+, CD25+, CD127-/low T cells from a lymphocyte-containing biological sample obtained from a human subject; and b) culturing the T cells in medium comprising a CD28 superagonist (CD28SA) antibody, interleukin-2 (IL-2), and tumor necrosis factor-alpha (TNF-alpha) to produce human Tregs that are CD4+, FOXP3+, HELIOS+, and have a demethylated Treg-specific demethylation region (TSDR), wherein step b) does not comprise use of an anti-CD3 antibody and does not comprise use of magnetic beads or Fc receptor-expressing feeder cells to cross-link CD28 and CD3 of the isolated T cells.

[006e] In one aspect, the present disclosure provides use of the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating or preventing a pathological immune response in a human subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. 1 provides a graph depicting the extent of expansion of human Tregs produced using a standard protocol involving anti-CD3 and anti-CD28 monoclonal antibodies conjugated to magnetic beads in comparison to the bead-free protocols of the present disclosure described in Example 1. Abbreviations are as follows: BF1 = protocol involving anti-CD28SA Ab and IL-2; BF2 = protocol involving anti-CD28SA Ab, IL-2, and IL-6; BF3 = protocol involving anti-CD28SA Ab, IL-2, and TNF-alpha; and BF4 = protocol involving anti-CD28SA Ab, IL-2, IL-6 and TNF-alpha.

[0008] FIG. 2 provides a graph depicting the level of expression of Treg-lineage markers FOXP3, HELIOS and CD27 on human Tregs produced using the bead-free protocols of the present disclosure described in Example 1. Tregs were harvested on day 14. Abbreviations are as described for FIG. 1.

[0009] FIG. 3 provides flow cytometry histograms depicting the level of expression of Treg-lineage markers FOXP3, HELIOS, CD62L and CD27 on human Tregs produced using the bead-free protocols of the present disclosure described in Example 1. Tregs were harvested on day 14. Abbreviations are as described for FIG. 1.

[0010] FIG. 4 provides flow cytometry histograms depicting the level of expression of Treg-lineage markers HELIOS and CD27 on human Tregs produced using the bead-free protocols of the present disclosure described in Example 1. Tregs were harvested on day 14. Abbreviations are as described for FIG. 1

CLAIMS

We claim:

1. A method for the production of human regulatory T cells (Tregs), comprising:

a) isolating CD4+, CD25+, CD127-/low T cells from a lymphocyte-containing biological sample obtained from a human subject; and

b) culturing the T cells in medium comprising a CD28 superagonist (CD28SA) antibody, interleukin-2 (IL-2), and tumor necrosis factor-alpha (TNF-alpha) to~~under conditions effective in produc~~~~ing~~ human Tregs that are CD4+, FOXP3+, HELIOS+, and have a demethylated Treg-specific demethylation region (TSDR).

2. The method of Claim 1, wherein step b) does not comprise use of an anti-CD3 antibody.

3. The method of Claim 2, wherein step b) does not comprise use of magnetic beads or Fc receptor-expressing feeder cells to cross-link CD28 and CD3 of the isolated T cells.

4. The method of Claim 3, wherein the medium further comprises one or both of a tumor necrosis factor receptor 2 agonist (TNFR2a) and interferon-gamma (IFN-gamma).

5. The method of Claim 3, wherein the medium further comprises one or both of IL-6 and IL-1beta.

6. The method of Claim 1, wherein the lymphocyte-containing biological sample is selected from the group consisting of whole blood, a leukapheresis product, and peripheral blood mononuclear cells (PBMC).

7. The method of Claim 5, wherein the biological sample is either fresh or cryopreserved after being obtained from the human subject and subsequently thawed prior to step a).

8. The method of Claim~~s~~ 1, wherein the CD4+, CD25+, CD127-/low T cells of step a) are isolated from the biological sample by fluorescence-activated cell sorting (FACS) or magnetic-activated cell sorting (MACS).

9. The method of any one of Claims 1-7, further comprising step c) harvesting the human Tregs 7-18 days after step b) commences.

10. The method of Claim 9, wherein the human Tregs comprise from ~~about~~ 200 to ~~about~~ 2000 fold more cells than the CD4+, CD25+, CD127-/low T cells at the onset of step a).

11. A pharmaceutical composition comprising from 10^7 to 10^{11} of ~~the~~ human Tregs ~~produced using the method of any one of Claims 1-10~~, and a physiologically acceptable buffer,

wherein the human Tregs are produced using a method comprising the steps of:

a) isolating CD4+, CD25+, CD127-/low T cells from a lymphocyte-containing biological sample obtained from a human subject; and

b) culturing the T cells in medium comprising a CD28 superagonist (CD28SA) antibody, interleukin-2 (IL-2), and tumor necrosis factor-alpha (TNF-alpha) to produce human Tregs that are CD4+, FOXP3+, HELIOS+, and have a demethylated Treg-specific demethylation region (TSDR),

wherein step b) does not comprise use of an anti-CD3 antibody and does not comprise use of magnetic beads or Fc receptor-expressing feeder cells to cross-link CD28 and CD3 of the isolated T cells.

12. The pharmaceutical composition of Claim 11, wherein the medium further comprises one or both of a tumor necrosis factor receptor 2 agonist (TNFR2a) and interferon-gamma (IFN-gamma).

13. The pharmaceutical composition of Claim 11, wherein the medium further comprises one or both of IL-6 and IL-1beta.

14. The pharmaceutical composition of Claim 11, wherein the lymphocyte-containing biological sample is selected from the group consisting of whole blood, a leukapheresis product, and peripheral blood mononuclear cells (PBMC).

15. The pharmaceutical composition of Claim 13, wherein the biological sample is either fresh or cryopreserved after being obtained from the human subject and subsequently thawed prior to step a).

16. The pharmaceutical composition of Claim 11, wherein the CD4+, CD25+, CD127-/low T cells of step a) are isolated from the biological sample by fluorescence-activated cell sorting (FACS) or magnetic-activated cell sorting (MACS).

17. The pharmaceutical composition of any one of Claims 11-15, wherein the method further comprises step c) harvesting the human Tregs 7-18 days after step b) commences.

18. The pharmaceutical composition of Claim 17, wherein the human Tregs comprise from 200 to 2000 fold more cells than the CD4+, CD25+, CD127-/low T cells at the onset of step a).

~~12~~19. The pharmaceutical composition of any one of Claims 11-18 for use in treating or preventing a pathological immune response in a human subject in need thereof.

~~13~~20. The pharmaceutical composition for use according to Claim 19~~12~~, wherein the pathological immune response is an autoimmune or autoinflammatory disease.

~~14~~21. The pharmaceutical composition for use according to Claim ~~13~~20, wherein the autoimmune or autoinflammatory disease is selected from the group consisting of rheumatoid arthritis, multiple sclerosis, amyotrophic lateral sclerosis, systemic lupus erythematosus, pemphigus, psoriasis, type I diabetes, celiac disease, and inflammatory bowel disease.

~~15~~22. The pharmaceutical composition for use according to Claim 20~~13~~, wherein the composition is effective in reducing a symptom, or is effective in inhibiting progression of the autoimmune or autoinflammatory disease; optionally wherein inhibiting progression of the autoimmune or autoinflammatory disease comprises inhibiting tissue destruction.

~~16~~23. The pharmaceutical composition for use according to Claim 19~~12~~, wherein the pathological immune response is rejection of a hematopoietic allograft or a solid organ allograft.

~~17~~24. The pharmaceutical composition for use according to Claim 23~~16~~, wherein the pathological immune response is rejection of a hematopoietic allograft, and the hematopoietic allograft is a bone marrow graft or a peripheral blood stem cell graft.

~~18~~25. The pharmaceutical composition for use according to Claim 23~~16~~, wherein the pathological immune response is rejection of a solid organ allograft, and the solid organ allograft is selected from the group consisting of cardiac, lung, cardiac/lung, kidney, pancreas, kidney/pancreas, liver, intestine, pancreatic islet, and skin allografts.

~~19~~26. The pharmaceutical composition for use according to Claim 23~~16~~, wherein the composition is effective in reducing a symptom of acute and/or chronic rejection, or is effective in prolonging survival of the organ allograft.

~~20~~27. The pharmaceutical composition for use according to Claim ~~12~~19, wherein the pathological immune response is a graft versus host disease (GvHD).

~~21~~28. The pharmaceutical composition for use according to Claim 27~~20~~, wherein the composition is effective in reducing a symptom of acute and/or chronic GvHD, or is effective in inhibiting damage to skin, liver, lung, and/or gut of the host.

~~22. The pharmaceutical composition for use according to Claim 12, wherein the composition is effective in increasing Treg percentages over baseline in the human subject.~~

~~23~~29. Use of ~~the~~ human Tregs ~~produced using the method of any one of Claims 1-10~~ in the manufacture of a medicament for inhibiting proliferation of human effector T cells (Teffs) to treat or prevent a pathological immune response in a human subject in need thereof, the use comprising: contacting human CD4+, CD25-, CD127+ Teffs with the human Tregs under conditions effective in inhibiting proliferation of the Teffs; optionally wherein the contacting is done in the presence of TNF-alpha,

wherein the human Tregs are produced using a method comprising the steps of:

a) isolating CD4+, CD25+, CD127-/low T cells from a lymphocyte-containing biological sample obtained from a human subject; and

b) culturing the T cells in medium comprising a CD28 superagonist (CD28SA) antibody, interleukin-2 (IL-2), and tumor necrosis factor-alpha (TNF-alpha) to produce human Tregs that are CD4+, FOXP3+, HELIOS+, and have a demethylated Treg-specific demethylation region (TSDR),

wherein step b) does not comprise use of an anti-CD3 antibody and does not comprise use of magnetic beads or Fc receptor-expressing feeder cells to cross-link CD28 and CD3 of the isolated T cells.

30. The use of Claim 29, wherein the medium further comprises one or both of a tumor necrosis factor receptor 2 agonist (TNFR2a) and interferon-gamma (IFN-gamma).

31. The use of Claim 29, wherein the medium further comprises one or both of IL-6 and IL-1beta.

32. The use of Claim 29, wherein the lymphocyte-containing biological sample is selected from the group consisting of whole blood, a leukapheresis product, and peripheral blood mononuclear cells (PBMC).

33. The use of Claim 31, wherein the biological sample is either fresh or cryopreserved after being obtained from the human subject and subsequently thawed prior to step a).

34. The use of Claim 29, wherein the CD4+, CD25+, CD127-/low T cells of step a) are isolated from the biological sample by fluorescence-activated cell sorting (FACS) or magnetic-activated cell sorting (MACS).

35. The use of any one of Claims 29-33, wherein the method further comprises step c) harvesting the human Tregs 7-18 days after step b) commences.

36. The use of Claim 35, wherein the human Tregs comprise from 200 to 2000 fold more cells than the CD4+, CD25+, CD127-/low T cells at the onset of step a).

~~24~~37. Use of the pharmaceutical composition of any one of Claims 11-18 in the manufacture of a medicament for treating or preventing a pathological immune response in a human subject in need thereof.

38. The use according to Claim 37, wherein the pathological immune response is an autoimmune or autoinflammatory disease.

39. The use according to Claim 38, wherein the autoimmune or autoinflammatory disease is selected from the group consisting of rheumatoid arthritis, multiple sclerosis, amyotrophic lateral sclerosis, systemic lupus erythematosus, pemphigus, psoriasis, type I diabetes, celiac disease, and inflammatory bowel disease.

40. The use according to Claim 38, wherein the composition is effective in reducing a symptom, or is effective in inhibiting progression of the autoimmune or autoinflammatory disease; optionally wherein inhibiting progression of the autoimmune or autoinflammatory disease comprises inhibiting tissue destruction.

41. The use according to Claim 37, wherein the pathological immune response is rejection of a hematopoietic allograft or a solid organ allograft.

42. The use according to Claim 41, wherein the pathological immune response is rejection of a hematopoietic allograft, and the hematopoietic allograft is a bone marrow graft or a peripheral blood stem cell graft.

43. The use according to Claim 41, wherein the pathological immune response is rejection of a solid organ allograft, and the solid organ allograft is selected from the group consisting of cardiac, lung, cardiac/lung, kidney, pancreas, kidney/pancreas, liver, intestine, pancreatic islet, and skin allografts.

44. The use according to Claim 41, wherein the composition is effective in reducing a symptom of acute and/or chronic rejection, or is effective in prolonging survival of the organ allograft.

45. The use according to Claim 37, wherein the pathological immune response is a graft versus host disease (GvHD).

46. The use according to Claim 45, wherein the composition is effective in reducing a symptom of acute and/or chronic GvHD, or is effective in inhibiting damage to skin, liver, lung, and/or gut of the host.