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 producing 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.

[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.

[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) under conditions effective in producing 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 Claims 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.

12. The pharmaceutical composition of Claim 11 for use in treating or preventing a pathological immune response in a human subject in need thereof.

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

14. The pharmaceutical composition for use according to Claim 13, 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. The pharmaceutical composition for use according to Claim 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. The pharmaceutical composition for use according to Claim 12, wherein the pathological immune response is rejection of a hematopoietic allograft or a solid organ allograft.

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

21. The pharmaceutical composition for use according to Claim 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. 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.

24. Use of the pharmaceutical composition of Claim 11 in the manufacture of a medicament for treating or preventing a pathological immune response in a human subject in need thereof.