METHODS OF EXPANDING AND ASSESSING B CELLS AND USING EXPANDED B CELLS TO TREAT DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application claims the benefit of priority of United States Provisional Patent Application No. 61/697,663, filed September 6, 2012; United States Provisional Patent Application No. 61/707,256, filed September 28, 2012; and United States Patent Application No. 13/795,889, filed March 12, 2013, all of which are incorporated herein by reference in their entireties.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

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INTRODUCTION

It is well-known that B cells regulate immune responses by producing antigen-specific antibody. However, specific B cell subsets can also negatively regulate immune responses, validating the existence of regulatory B cells. Human and mouse regulatory B cells (B10 cells) with the ability to express the inhibitory cytokine interleukin-10 (IL-10) have been identified. Although rare, B10 cells are potent negative regulators of antigen-specific inflammation and T cell-dependent autoimmune disease in mice. B10 cell IL-10 production regulates antigen-specific immune responses *in vivo* without inducing systemic immunosuppression. B10 cells may thereby be useful in regulating or controlling inflammation or autoimmune diseases.

SUMMARY

In one aspect, there is provided a method of expanding B10 cells capable of producing IL-10 *ex vivo* comprising contacting B cells harvested from a subject with IL-21 and isolating the B10 cells.

In one aspect, there is a provided a method of expanding B cells *ex vivo* comprising:

a) Contacting B cells harvested from a subject with feeder cells expressing a CD40 agonist and a B cell survival promoter in the presence of IL-4;

b) Contacting the B cells from step (a) with feeder cells expressing a CD40 agonist and a B cell survival promoter in the presence of IL-21;

c) Isolating the B cells from step (b); and

d) Isolating the B10 cells from the B cells of step (c).

In one aspect, there is provided an *in vitro* method of assessing B10 cell function in a subject comprising:

a) Harvesting B cells from a sample from the subject;

b) Contacting the B cells with IL-21 and a CD40 agonist; and

c) Determining if the B cells are capable of producing IL-10.

In one aspect, there is provided a composition comprising the cells produced by the method as described herein.

In one aspect, there is provided a pharmaceutical composition comprising the composition as described herein and a pharmaceutically acceptable carrier.

In one aspect, there is provided the use of a therapeutically effective amount of the composition as described herein for manufacture of a medicament to treat an autoimmune disorder, to prevent or treat organ, tissue or cell transplant rejection or associated chronic graft versus host disease, to treat an allergic disorder or inflammatory disorder or to treat subjects receiving recombinant, therapeutic or xenogeneic protein(s).

Provided herein are methods of expanding B cells *ex vivo*, compositions comprising expanded B cells and methods of using the expanded B cell compositions for assessing or screening for a disease state or condition and for treating diseases as described herein. Methods of expanding B10 cells capable of producing IL-10 *ex vivo* by contacting, culturing or incubating B cells harvested and isolated from a subject with IL-21 are provided herein. The resultant

Claims

We claim:

1. A method of expanding B10 cells capable of producing IL-10 *ex vivo* comprising contacting B cells harvested from a subject with IL-21 and isolating the B10 cells.
2. The method of claim 1, further comprising contacting the cells with a CD40 agonist or contacting the B cells with feeder cells expressing a CD40 agonist and a B cell survival promoter.
3. ~~The method of claim 1, further comprising contacting the B cells with feeder cells expressing a CD40 agonist and a B cell survival promoter.~~
4. 3. The method of claim 2~~3~~, further comprising first contacting the B cells with feeder cells expressing a CD40 agonist and a B cell survival promoter in the presence of IL-4.
5. ~~The method of claim 3 or 4, wherein the B cell survival promoter is BAFF (BLyS).~~
6. 4. A method of expanding B cells *ex vivo* comprising:
   a) Contacting B cells harvested from a subject with feeder cells expressing a CD40 agonist and a B cell survival promoter in the presence of IL-4;
   b) Contacting the B cells from step (a) with feeder cells expressing a CD40 agonist and a B cell survival promoter in the presence of IL-21; ~~and~~
   c) Isolating the B cells from step (b); and
   ~~c)~~d)isolating the B10 cells from the B cells of step (c).
   ~~The method of claim 6, further comprising isolating the B10 cells from the B cells of step (c).~~
7. ~~7.~~5. The method of claim 1-~~45 or 7~~, wherein the B10 cells are isolated by selecting for cell surface expression of CD1d, CD5, CD24 or CD27.
8. ~~8.~~6. The method of any one of claims 2-5~~3-8~~, wherein the feeder cells further express VCAM-1 and CD44 or at least one marker selected from CD24, IL-7, Mst1 or Tslp.
9. ~~The method of any one of claims 3-9, wherein the feeder cells further express at least one of CD24, IL-7, Mst1 or Tslp.~~
7. The method of any one of claims 2-6~~3-10~~, wherein the feeder cells express at least two fold less of CD99, CXCR7, Dlk1, Jag1, Notch1 or TGFBI than control feeder cells.
8. An *in vitro* method of assessing B10 cell function in a subject comprising:
   a) Harvesting B cells from a sample from the subject;
   b) Contacting the B cells with IL-21 and a CD40 agonist; and
   c) Determining if the B cells are capable of producing IL-10.
9. The method of claim 8, further comprising contacting the B cells with feeder cells expressing a CD40 agonist and B cell survival promoter in the presence of IL-4 prior to culturing with IL-21.
10. The method of claim 8 or 9, wherein the cells are contacted with feeder cells expressing a CD40 agonist and B cell survival promoter in the presence of IL-21.

11. The method of any one of claims 8-10, further comprising comparing the amount of IL-10 produced or the percentage of cells capable of producing IL-10 to a control having normal B10 cell function and determining if the B10 cell function is normal or abnormal in the subject.

12. The method of any one of claims 8-11, further comprising diagnosing the subject as having normal, deficient or over-active B10 cells.

13. The method of any one of claims 11-12, further comprising using the comparison to diagnose whether the subject has a disease or condition affecting B10 cell function, wherein the disease or condition is an autoimmune disease, graft versus host disease, transplant rejection, anti-drug antibodies, or an inflammatory disorder.

~~10.~~

~~11.~~14. The method of any one of the preceding claims, wherein the B cells are harvested from the blood, spleen, peritoneal cavity, lymph nodes, bone marrow, site of autoimmune disease, site of inflammation or tissue undergoing transplant rejection of the subject.

~~12.~~15. The method of any one of the preceding claims, wherein the B cells are isolated by removal of non-B cells, or selection for cell surface IgM, IgD, IgG, IgA, IgE, CD19,

CD20, CD21, CD22, CD24, CD40, CD72, CD79a, CD79b, CD1d, CD5, CD9, CD10, CD23, CD27, CD38, CD48, CD80, CD86, CD138, CD148 or combinations thereof prior to use in the method.

~~13.~~16. The method of any one of claims 2-15~~13~~, wherein the CD40 agonist is selected from the group consisting of CD154, a fragment of CD154, ander an antibody, aptamer or polypeptide, or fragment thereof reactive with CD40.

~~14.~~17. The method of any one of claims 2~~3~~-16~~14~~, wherein the B cell survival promoter is selected from the group consisting of at least one of feeder cells, BAFF (BLyS), BAFF fragments, APRIL, CD22 ligand, CD22 monoclonal antibody, andor fragments thereof.

~~15.~~18. The method of any one of claims 2~~3~~-17~~15~~, wherein the feeder cells are fibroblast, endothelial cells, epithelial cells, keratinocytes, melanocytes, or other mesenchymal or stromal cells.

~~16.~~19. The method of any one of claims 3-18~~4-16~~, wherein the B cells are contacted with IL-4 for three to ten days.

~~17.~~20. The method of any one of the preceding claims, wherein the B cells are contacted with IL-21 for four to eight days.

~~18.~~21. The method of any one of claims 1-7 or 14-20~~5 or 7-18~~, wherein the B10 cells are producing IL-10, or are isolated based on differential expression of cell surface molecules including IgM, IgD, IgG, IgA, IgE, CD19, CD20, CD21, CD22, CD24, CD40, CD72, CD79a, CD79b, CD1d, CD5, CD9, CD10, CD1d, CD23, CD27, CD38, CD48, CD80, CD86, CD138, CD148 or combinations thereof.

~~19. The method of any one of claims 1-5 or 7-19, wherein the B10 cells are 10% or more of the total B cells isolated after the method.~~

~~20.~~22. The method of any one of claims 1-7 or 14-21~~5 or 7-19~~, wherein the isolated B10 cells are more than 50% B10 cells capable of producing IL-10.

~~21.~~23. A composition comprising the cells produced by the method of any one of claims 1-7 or 14-22~~21~~.

~~22. The composition of claim 22, wherein the cells are more than 90% B10 cells.~~

~~23.~~24. A pharmaceutical composition comprising the composition of claim 22 ~~or~~ 23 and a pharmaceutically acceptable carrier.

~~24.~~25. Use of a therapeutically effective amount of the composition of any one of claims 23-24 for manufacture of a medicament ~~A method of treating a subject having~~ to treat an autoimmune disorder, to prevent or treat organ, tissue or cell transplant rejection or associated chronic graft versus host disease, to treat an allergic disorder or inflammatory disorder or to treat subjects receiving recombinant, therapeutic or xenogeneic protein(s) ~~comprising administering a therapeutically effective amount of the composition of any one of claims 22-24 to a subject in need of treatment for an autoimmune disorder~~.

~~25.~~26. The ~~use~~method of claim 25, wherein the autoimmune disease is selected from multiple sclerosis, lupus, arthritis, inflammatory bowel disease and scleroderma.

~~26. A method of treating a subject to prevent or treat organ, tissue or cell transplant rejection or associated chronic graft versus host disease comprising administering a therapeutically effective amount of the composition of any one of claims 22-24 to a subject in need of treatment for transplant rejection or graft versus host disease.~~

~~27. A method of treating a subject having an allergic disorder or inflammatory disorder comprising administering a therapeutically effective amount of the composition of any one of claims 22-24 to a subject in need of treatment for allergies or inflammation.~~

~~28.~~27. The ~~use~~method of claim 2~~5~~8, wherein the inflammatory disorder is selected from asthma, encephilitis, inflammatory bowel disease, chronic obstructive pulmonary disease (COPD), allergic disorders, septic shock, pulmonary fibrosis, undifferentitated spondyloarthropathy, undifferentiated arthropathy, arthritis, inflammatory osteolysis, and chronic inflammation resulting from chronic viral or bacterial infections.

~~29. A method of treating a subject receiving recombinant, therapeutic or xenogeneic protein(s) comprising administering a therapeutically effective amount of the composition of any one of claims 22-24 to a subject in need of treatment for a genetic, transplantation, allergy, inflammation, or autoimmune disorder.~~

~~30.~~28. The ~~use~~method of any one of claims 2~~5~~-27~~5~~-~~30~~, wherein the composition comprises autologous B cells.

~~31.~~29. The ~~use~~method of any one of claims 2~~5~~-28~~5~~-~~31~~, wherein between 10^6 and 10^{10} B cells are administered to the subject in the composition.

~~32. The method of any one of claims 25-32, wherein the composition is administered before, during or after the onset of symptoms in the subject.~~

~~33. A method of assessing B10 cell function in a subject comprising:~~

~~a) Harvesting B cells from a sample from the subject;~~

~~b) Contacting the B cells with IL-21 and a CD40 agonist; and~~

~~c) Determining if the B cells are capable of producing IL-10.~~

~~34. The method of claim 34, further comprising contacting the B cells with feeder cells expressing a CD40 agonist and B cell survival promoter in the presence of IL-4 prior to culturing with IL-21.~~

35. The method of claim 34 or 35, wherein the cells are contacted with feeder cells expressing a CD40 agonist and B cell survival promoter in the presence of IL-21.

36. The method of any one of claims 34-36, further comprising comparing the amount of IL-10 produced or the percentage of cells capable of producing IL-10 to a control *having normal B10 cell function and determining if the B10 cell function is normal or* abnormal in the subject.

37. The method of any one of claims 34-37, further comprising diagnosing the subject as having normal, deficient or over-active B10 cells.

38. The method of claim 38, wherein the B10 cells are normal if the percentage of B10 cells capable of producing IL-10 in step (c) is within 2 standard deviations of the average percentage of B10 cells capable of producing IL-10 in a normal population, wherein the B10 cells are deficient if the percentage of B10 cells capable of producing IL-10 in step (c) is more than 2 standard deviations lower than the average percentage of B10 cells capable of producing IL-10 in a normal population, and wherein the B10 cells are over-active if the percentage of B10 cells capable of producing IL-10 in step (c) is more than 2 standard deviations higher than the average percentage of B10 cells capable of producing IL-10 in a normal population.

39. The method of any one of claims 34-39, wherein the B cells are harvested from the blood, spleen, peritoneal cavity, lymph nodes, bone marrow, site of autoimmune disease, site of inflammation or tissue undergoing transplant rejection of the subject.

40. The method of any one of claims 34-40, wherein the B cells are isolated by removal of non-B cells, or selection for cell surface IgM, IgD, IgG, IgA, IgE, CD19, CD20, CD21, CD22, CD24, CD40, CD72, CD79a, CD79b, CD1d, CD5, CD9, CD10, CD23, CD27, CD38, CD48, CD80, CD86, CD138, CD148 or combinations thereof.

41. The method of any one of claims 34-41, wherein the CD40 agonist is CD154, a fragment of CD154, or an antibody, aptamer, polypeptide or fragment thereof reactive with CD40.

42. The method of any one of claims 34-42, wherein the B cell survival promoter is selected from at least one of feeder cells, BAFF (BLyS), BAFF fragments, APRIL, CD22 ligand, CD22 monoclonal antibody, or fragments thereof.

43. The method of any one of claims 34-43, wherein the feeder cells are fibroblasts, endothelial cells, epithelial cells, keratinocytes, melanocytes, or other mesenchymal or stromal cells.

44. The method of any one of claims 35-44, wherein the B cells are contacted with IL-4 for three to ten days.

45. The method of any one of claims 35-45, wherein the B cells are contacted with IL-21 for four to eight days.

46. The method of any one of claims 38-46, further comprising using the comparison to diagnose whether the subject has a disease or condition affecting B10 cell function.

47. The method of claim 47, wherein the disease or condition is an autoimmune disease, graft versus host disease, transplant rejection, anti-drug antibodies, or an inflammatory disorder.

48. The methods and compositions substantially as disclosed and described herein.