CLAIMS

What is claimed is:

1. A method for producing dendritic cells from monocytes, comprising:
   b. providing monocytes that have been incubated at a temperature of 1°C - 34°C for a period of approximately 6 to 96 hours from the time they are isolated from a subject; and
   c. inducing the differentiation of said monocytes into dendritic cells.

2. The method of claim 1, wherein the monocytes are human monocytes.

3. The method of claim 1, wherein the incubation temperature is 6°C – 28°C.

4. The method of claim 4, wherein the incubation temperature is 8°C – 26°C.

5. The method of claim 1, wherein the period of incubation is 8 to 48 hours.

6. The method of claim 5, wherein the period of incubation is 10 to 30 hours.

7. The method of claim 1, wherein the period of incubation is 26 to 72 hours.

8. The method of claim 1, wherein the period of incubation is 48 to 80 hours

9. The method of claim 1, wherein the differentiation is induced by contacting the monocytes with a culture medium comprising an effective amount of a composition that induces the differentiation of monocytes into immature dendritic cells.

10. The method of claim 9, wherein the composition is GM-CSF and IL-4; GM-CSF and IL-13; GM-CSF and IL-15; or IFNα.

11. The method of claim 1, wherein the monocytes are present together with peripheral blood mononuclear cells (PBMCs) during all or a portion of the incubation period.

12. The method of claim 11, wherein the PBMCs are isolated from the subject by leukapheresis.

13. The method of claim 11, wherein the monocytes are enriched from the PBMCs during or following the incubation period.

14. The method of claim 13, wherein the monocytes are enriched from said PBMCs by elutriation, dilute Ficoll gradient centrifugation, dilute Percoll density gradient centrifugation or magnetic bead sorting.

15. The method of claim 13, wherein the monocytes are enriched from the PBMCs following the incubation period by allowing the monocytes to adhere to plastic.

16. The method of claim 9, further comprising a step of maturing the immature dendritic cells into mature dendritic cells.

17. The method of claim 16, further comprising loading one or more antigens into said mature dendritic cells.

18. The method of claim 16, wherein the step comprises contacting the immature dendritic cells with PGE_2, TNFα, IL-6 and IL-1β.

19. The method of claim 16, wherein said step comprises signaling said immature dendritic cells with IFN-γ, followed by signaling the dendritic cells with CD40L.

20. The method of claim 19, wherein said signaling with CD40L is effected upon translation of a recombinant CD40L mRNA within the dendritic cells.

21. The method of claim 16, wherein the step comprises contacting the immature dendritic cells with PGE_2, TNFα, and IFNγ to produce mature dendritic cells.

22. The method of claim 21, further comprising transfecting said mature dendritic cells with an RNA encoding CD40L and/or RNA encoding one or more antigens or epitopes of interest.

23. The method of claim 22, wherein the RNA encodes one or more cancer cell antigens.

24. The method of claim 22, wherein the RNA encodes one or more pathogen antigens.

25. The method of claim 1, further comprising loading the dendritic cells with one or more antigens to produce an antigen-loaded dendritic cell.

26. The method of claim 25, wherein the antigen is autologous to the subject.

27. The method of claim 25, wherein said antigen is loaded by transfecting said dendritic cell with one or more RNAs encoding said antigen(s).

28. The method of claim 27, wherein the dendritic cells are transfected with the RNA by electroporation.

29. The method of claim 27, wherein said cells are transfected with approximately 1-4 μg RNA per 10^6 dendritic cells.

30. The method of claim 25, wherein said dendritic cell is immature at the time of antigen loading.

31. The method of claim 30, further comprising maturing said antigen-loaded immature dendritic cells into antigen-loaded mature dendritic cells.

32. The method of claim 25, wherein the antigen is from or derived from one or more cancer cells or pathogens.

33. The method of claim 32, wherein the cancer is selected from the group consisting of renal cell cancer, chronic lymphocytic leukemia, multiple myeloma, melanoma, prostate cancer, breast cancer, lung cancer, colon cancer, stomach cancer and pancreatic cancer.

34. The method of claim 32, wherein said pathogen is HTV or HCV.

35. Use of an antigen-loaded dendritic cell for the preparation of a frozen medicament for the treatment or prevention of cancer or pathogen infection, wherein the medicament comprises at least 2% DMSO and is ready for administration upon thawing, and wherein said cell(s) are prepared by method of claim 1.

36. The use of claim 35, wherein the medicament comprises at least 10% DMSO.

37. A composition comprising dendritic cell manufactured by the method of claim 16.

38. The composition of claim 37, wherein the dendritic cells are antigen-loaded.

39. The composition of claim 38, wherein the dendritic cells are transfected with mRNA encoding CD40L.

40. The composition of claim 37, wherein the mature dendritic cells has increased levels of one or more of CD80, CD83, CD86, MHC class I molecules, or MHC class II molecules as compared to mature dendritic cells prepared from fresh monocytes.

41. A composition comprising mature monocyte derived dendritic cells as prepared by the method of claim 1, wherein the mature dendritic cells have increased levels of one or more of CD80, CD83, CD86, MHC class I molecules, or MHC class II molecules as compared to mature dendritic cells prepared from fresh monocytes.

42. The composition of claim 41, wherein the mature dendritic cells are transiently transfected with an mRNA encoding CD40L.

43. A mature monocyte derived dendritic cell as prepared by the method of claim 1, wherein the steady state ratio of ALOX 15 RNA to ß-actin RNA or GAPDH RNA in the cell is less than 1.0.

44. The dendritic cell of claim 43, wherein the ratio is between 0.2 to 0.7.

45. The dendritic cell of claim 44, wherein the ratio is between 0.4 to 0.5.

46. A mature monocyte derived dendritic cell as prepared by the method of claim 1, wherein the steady state ratio of CD52 RNA to ß-actin RNA or GAPDH in the cell is greater than 1.0.

47. The dendritic cell of claim 46, wherein the ratio is between 1.2 to 5.0.

48. The dendritic cell of claim 47, wherein the ratio is between 1.5 to 2.2.

49. The dendritic cell of claim 48, wherein the ratio is between 1.8 to 1.9.

50. A mature monocyte derived dendritic cell as prepared by the method of claim 1, wherein the steady state ratio of TLRI RNA_5 TLR2 RNA_5 IL-I ß RNA or CD69 RNA to ß-actin RNA or GAPDH RNA in the cell is less than 1.0.

51. The dendritic cell of claim 50, wherein the ratio is between 0.2 to 0.9.

52. The dendritic cell of claim 51, wherein the ratio is between 0.5 to 0.8.

53. A vaccine comprising the composition of any of claims 37-52.

CLAIMS

What is claimed is:

1. A method for producing dendritic cells from monocytes, comprising:

    a. enriching monocytes from a leukapheresis product by elutriation, wherein prior to elutriation, monocytes have been incubated at a temperature of 1°C - 14°C for a period of approximately 6 to 96 hours following the time the leukapheresis product is collected from a subject; and

    b. inducing the differentiation of said monocytes into dendritic cells.

2. The method of claim 1, wherein the monocytes are human monocytes.

3. The method of claim 1, wherein the incubation temperature is *6°C* - 8°C.

4. The method of claim 1, wherein the incubation temperature is 1°C - 8°C.

5. The method of claim 1, wherein the period of incubation is 8 to 48 hours.

6. The method of claim 5, wherein the period of incubation is 10 to 30 hours.

7. The method of claim 1, wherein the period of incubation is 26 to 72 hours.

8. The method of claim 1, wherein the period of incubation is 48 to 80 hours.

9. The method of claim 1, wherein the differentiation is induced by contacting the monocytes with a culture medium comprising an effective amount of a composition that induces the differentiation of monocytes into immature dendritic cells.

10. The method of claim 9, wherein the composition is GM-CSF and IL-4; GM-CSF and IL-13; GM-CSF and IL-15; or IFNα.

11 The method of claim 9, further comprising a step of maturing the immature dendritic cells into mature dendritic cells.

12. The method of claim 11, further comprising loading one or more antigens into said mature dendritic cells.

13. The method of claim 11, wherein the step comprises contacting the immature dendritic cells with PGE_2, TNFα, IL-6 and IL-1β.

14. The method of claim 11, wherein said step comprises signaling said immature dendritic cells with IFN-γ, followed by signaling the dendritic cells with CD40L.

15. The method of claim 14, wherein said signaling with CD40L is effected upon translation of a recombinant CD40L mRNA within the dendritic cells.

16. The method of claim 11, wherein the step comprises contacting the immature dendritic cells with PGE_2, TNFα, and IFNγ to produce mature dendritic cells.

17. The method of claim 16, further comprising transfecting said mature dendritic cells with an RNA encoding CP40L and/or RNA encoding one or more antigens or epitopes of interest.

18. The method of claim 17, wherein the RNA encodes one or more cancer cell antigens.

19. The method of claim 17, wherein the RNA encodes one or more pathogen antigens.

20. The method of claim 1, further comprising loading the dendritic cells with one or more antigens to produce an antigen -loaded dendritic cell.

21. The method of claim 20, wherein the antigen is autologous to the subject.

22. The method of claim 20, wherein said antigen is loaded by transfecting said dendritic cell with one or more RNAs encoding said antigen(s).

23. The method of claim 22, wherein the dendritic cells are transfected with the RNA by electroporation.

24. The method of claim 22, wherein said cells are transfected with approximately 1 -4 μg RNA per 10^6 dendritic cells.

25. The method of claim 20, wherein said dendritic cell is immature at the time of antigen loading.

26. The method of claim 25, further comprising maturing said antigen-loaded immature dendritic cells into antigen-loaded mature dendritic cells.

27. The method of claim 20, wherein the antigen is from or derived from one or more cancer cells or pathogens.

28. The method of claim 27, wherein the cancer is selected from the group consisting of renal cell cancer, chronic lymphocytic leukemia, multiple myeloma, melanoma, prostate cancer, breast cancer, lung cancer, colon cancer, stomach cancer and pancreatic cancer.

29. The method of claim 27, wherein said pathogen is HTV or HCV.

30. The method of claim 1, further comprising the step of freezing said enriched monocytes.

31. The method of claim 1, wherein the period of incubation is 26 to 96 hours.

32. The method of claim 1, wherein said monocytes are subjected to occasional or continuous motion during the incubation period.

33. The method of any one of claims 1 to 32, wherein said monocytes are subjected to occasional or continuous motion associated with shipping during the incubation period.

34. The method of claim 32, wherein said motion is associated with shipping.

35. The method of claim 32, wherein said motion may prevent cell damage associated with compaction during settling.

36. Use of an antigen-loaded dendritic cell for the preparation of a frozen medicament for the treatment or prevention of cancer or pathogen infection, wherein the medicament comprises at least 2% DMSO and is ready for administration upon thawing, and wherein said cell(s) are prepared by method of claim 1.

37. The use of claim 36, wherein the medicament comprises at least 10% DMSO.

38. A composition comprising dendritic cell manufactured by the method of claim 11.

39. The composition of claim 38, wherein the dendritic cells are antigen-loaded.

40. The composition of claim 39, wherein the dendritic cells are transfected with mRNA encoding CD40L

41. The composition of claim 38, wherein the mature dendritic cells has increased levels of one or more of CD80, CD83, CD86, MHC class I molecules, or MHC class II molecules as *compared* to mature dendritic cells prepared from fresh monocytes.

42. A composition comprising mature monocyte derived dendritic cells as prepared by the method of claim 1, wherein the mature dendritic cells have increased levels of one or more of CD80, CD83, CD86, MHC class I molecules, or MHC class II molecules as compared to mature dendritic cells prepared from fresh monocytes.

43. The composition of claim 42, wherein the mature dendritic cells are transiently transfected with an mRNA encoding CD40L.

44. A mature monocyte derived dendritic cell as prepared by the method of claim 1, wherein the steady state ratio of ALOX 15 RNA to ß-actin RNA or GAPDH RNA in the cell is less than 1.0.

45. The dendritic cell of claim 44, wherein the ratio is between 0.2 to 0.7.

46. The dendritic cell of claim 45, wherein the ratio is between 0.4 to 0.5.

47. A mature monocyte derived dendritic cell as prepared by the method of claim 1, wherein the steady state ratio of CD52 RNA to ß-actin RNA or GAPDH in the cell is greater than 1.0.

48. The dendritic cell of claim 47, wherein the ratio is between 1.2 to 5.0.

49. The dendritic cell of claim 48, wherein the ratio is between 1.5 to 2.2.

50. The dendritic cell of claim 49, wherein the ratio is between 1.8 to 1.9.

51. A mature monocyte derived dendritic cell as prepared by the method of claim 1, wherein the steady state ratio of TLRI RNA_5 TLR2 RNA_5 IL-I ß RNA or CD69 RNA to ß-actin RNA or GAPDH RNA in the cell is less than 1.0.

52. The dendritic cell of claim 51, wherein the ratio is between 0.2 to 0.9.

53. The dendritic cell of claim 52, wherein the ratio is between 0.5 to 0.8.

54. A vaccine comprising the composition of any of claims 38-53.