CLAIMS

What is claimed is:

1. A method for producing dendritic cells from monocytes, comprising:
enriching monocytes from a leukapheresis product by elutriation, wherein prior to elutriation, monocytes have been incubated without culturing at a temperature maintained at 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
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 or claim 2, wherein the incubation temperature is maintained at 1°C - 8°C.

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

5. The method of claim 4, wherein the period of incubation is 10 to 30 hours.

6. The method of any of claims 1 to 3, wherein the period of incubation is 26 to 72 hours.

7. The method of any of claims 1 to 3, wherein the period of incubation is 48 to 80 hours.

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

9. The method of claim 1, wherein the enriched monocytes are frozen and thawed prior to culturing.

10. The method of claim 8, further comprising a step of maturing the immature dendritic cells into mature dendritic cells.

11. The method of claim 10, further comprising loading one or more antigens into said mature dendritic cells.

12. The method of claim 10 or claim 11, wherein the step comprises contacting the immature dendritic cells with PGE_2, TNFα, IL-6 and IL-lβ or signaling said immature dendritic cells with IFN-γ, followed by signaling the dendritic cells with CD40L.

13. The method of claim 12, wherein said signaling with CD40L is effected upon translation of a recombinant CD40L mRNA within the dendritic cells.

14. The method of claim 10, wherein the step comprises contacting the immature dendritic cells with PGE_2, TNFα, and IFNγ to produce mature dendritic cells.

15. The method of claim 14, further comprising transfecting said mature dendritic cells with an RNA encoding CD40L and/or RNA encoding one or more antigens or epitopes of interest.

16. The method of claim 15, wherein the RNA encodes one or more cancer cell antigens and/or one or more pathogen antigens.

17. The method of any preceding claims, further comprising loading the dendritic cells with one or more antigens to produce an antigen-loaded dendritic cell.

18. The method of claim 17, wherein the antigen is autologous to the subject.

19. The method of claim 17 or claim 18, wherein said antigen is loaded by transfecting said dendritic cells with one or more RNAs encoding said antigen(s).

20. The method of claim 19, wherein the dendritic cells are transfected with the RNA by electroporation.

21. The method of claim 19 or claim 20, wherein said cells are transfected with approximately 1 -4 μg RNA per 10^6 dendritic cells.

22. The method of any of claims 17 to 21, wherein said dendritic cells are immature at the time of antigen loading.

23. The method of claim 22, further comprising maturing said antigen-loaded immature dendritic cells into antigen-loaded mature dendritic cells.

24. The method of any of claims 17 to 23, wherein the antigen is from, or derived from, one or more cancer cells or pathogens.

25. The method of claim 24, 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.

26. The method of claim 24, wherein said pathogen is HIV or HCV.

27. The method of any preceding claims, wherein said monocytes are subjected to occasional or continuous motion during the incubation period.

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

29. The method of claim 27, wherein said motion is associated with shipping.

30. 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, wherein said cells have a steady state ratio of ALOX15 RNA, TLR1 RNA, TLR2 RNA, IL-1β RNA and/or CD69 RNA to β-actin RNA or GAPDH RNA of less than 1.0, and/or a steady state ratio of CD52 RNA to β-actin RNA or GAPDH of greater than 1.0.

31. The use of claim 30, wherein the medicament comprises at least 10% DMSO.

32. An antigen-loaded dendritic cell obtainable by the method of any of claims 1 to 29 for use as a pharmaceutical e.g. as a vaccine, wherein said cells have a steady state ratio of ALOX15 RNA, TLR1 RNA, TLR2 RNA, IL-1β RNA and/or CD69 RNA to β-actin RNA or GAPDH RNA of less than 1.0, and/or a steady state ratio of CD52 RNA to β-actin RNA or GAPDH of greater than 1.0.

33. A composition comprising dendritic cells obtainable by the method of any of claims 9 to 29, wherein said cells have a steady state ratio of ALOX15 RNA, TLR1 RNA, TLR2 RNA, IL-1β RNA and/or CD69 RNA to β-actin RNA or GAPDH RNA of less than 1.0, and/or a steady state ratio of CD52 RNA to β-actin RNA or GAPDH of greater than 1.0.

34. The composition of claim 33, wherein the dendritic cells are antigen-loaded.

35. The composition of claim 33 or claim 34, wherein the dendritic cells are transfected with mRNA encoding CD40L.

36. The composition of any of claims 33 to 35, 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.

37. A vaccine comprising the composition of any of claims 33-36.