CLAIMS

What is claimed is:

1. A method of producing NK cells, comprising:
   isolating from a biological fluid a mixture of mononuclear cells, and contacting the mixture of the mononuclear cells with an anti-CD16 antibody and N-803 to activate NK cells; and
   sequentially feeding the activated NK cells with a medium containing N-803.

2. The method of claim 1 wherein the step of isolating the mixture of the mononuclear cells is performed using density gradient centrifugation.

3. The method of any one of the preceding claims wherein the biological fluid is whole blood or cord blood.

4. The method of any one of the preceding claims wherein the mixture of mononuclear cells comprises T cells, NK cells, NKT cells, and DN T cells.

5. The method of any one of the preceding claims wherein the mixture of mononuclear cells is not further processed to enrich NK cells.

6. The method of any one of the preceding claims wherein the anti-CD16 antibody is a monoclonal antibody with specificity to human CD16.

7. The method of any one of the preceding claims wherein the mixture of the mononuclear cells contains about 100-500 x 10^6 cells.

8. The method of any one of the preceding claims wherein the step of contacting the mixture is performed in a volume of between about 100-300ml or at a cell density of about 1 x 10^6 cells/ml.

9. The method of any one of the preceding claims wherein the anti-CD16 antibody is present at a concentration of between 0.05-0.5 mcg/ml.

10. The method of any one of the preceding claims wherein the N-803 is present at a concentration of between 0.1-1.0 nM.

11. The method of any one of the preceding claims wherein the step of contacting the mixture further includes contacting the mixture of the mononuclear cells with an anti-CD3 antibody.

12. The method of claim 11 wherein the anti-CD3 antibody is present at a concentration of between 0.1-1.0 ng/ml.

13. The method of any one of the preceding claims wherein the medium containing N-803 comprises human AB serum.

14. The method of any one of the preceding claims wherein the medium containing N-803 comprises NK MACS medium.

15. The method of any one of the preceding claims wherein the step of sequentially feeding is performed about every 72 hours.

16. The method of any one of the preceding claims wherein the step of sequentially feeding is performed until a total cell number of about 0.5- 5.0 x 10^9 cells is reached.

17. The method of any one of the preceding claims wherein the step of sequentially feeding the activated NK cells is performed in single container.

18. The method of any claim 17 wherein the step of contacting the mixture of the mononuclear cells is performed in the single container.

19. The method of any one of the preceding claims wherein the step of sequentially feeding the activated NK cells is performed until NK cells are enriched to an at least 100-fold expansion.

20. The method of any one of the preceding claims wherein the step of sequentially feeding the activated NK cells is performed until NK cells constitute at least about 80% of all live cells.

21. The method of any one of the preceding claims wherein the step of sequentially feeding the activated NK cells is performed until NK cells constitute at least about 90% of all live cells.

22. A method of expanding NK cells from a mixture of mononuclear cells, comprising:

providing a mixture of the mononuclear cells that contains equal or less than 5% NK cells;

contacting the mixture of the mononuclear cells with an anti-CD16 antibody and N-803 to activate NK cells; and

feeding the activated NK cells with a medium containing N-803.

23. The method of claim 22 wherein the mixture of the mononuclear cells is obtained from whole blood or cord blood.

24. The method of claim 22 wherein the mixture of the mononuclear cells is obtained from an MHC-matched allogenic source relative to an individual that receives the NK cells.

25. The method of any one of claims 22-24 wherein the mixture of the mononuclear cells that contains equal or less than 3% NK cells.

26. The method of any one of claims 22-25 wherein the mixture of the mononuclear cells further comprises T cells, NKT cells, and DN cells.

27. The method of any one of claims 22-26 wherein the anti-CD16 antibody is a monoclonal antibody with specificity to human CD16.

28. The method of any one of claims 22-27 wherein the anti-CD16 antibody is present at a concentration of between 0.05-0.5 mcg/ml.

29. The method of any one of claims 22-28 wherein the N-803 is present at a concentration of between 0.1-1.0 nM.

30. The method of any one of claims 22-29 wherein the step of contacting the mixture further includes contacting the mixture of the mononuclear cells with an anti-CD3 antibody.

31. The method of claim 30 wherein the anti-CD3 antibody is present at a concentration of between 0.1-1.0 ng/ml.

32. The method of any one of claims 22-31 wherein the medium containing N-803 comprises human AB serum.

33. The method of any one of claims 22-32 wherein the step of feeding comprises sequentially feeding at an interval of about every 72 hours.

34. The method of any one of claims 22-33 wherein the step of feeding is performed until a total cell number of about 0.5- 5.0 x 10^9 cells is reached.

35. The method of any one of claims 22-34 wherein the step of feeding the activated NK cells is performed until NK cells are enriched to an at least 100-fold expansion.

36. The method of any one of claims 22-35 wherein the step of feeding the activated NK cells is performed in an automated manner.

37. The method of any one of claims 22-35 wherein the step of feeding the activated NK cells is performed in a single container.

38. A method of expanding NK cells in an automated bioreactor, comprising:
    incubating a mixture of mononuclear cells in an activation medium containing N-803 and an anti-CD16 antibody for a time sufficient to activate NK cells;
    wherein the mixture of mononuclear cells is contained in a cell culture container while incubating the mixture;
    measuring growth of the cells while the cells are in the container;
    automatically feeding the cells using a medium containing N-803, wherein the feeding is controlled by a predetermined schedule and/or a result from the step of measuring growth of the cells;
    terminating feeding the cells wherein the terminating is controlled by a predetermined schedule and/or a result from the step of measuring growth of the cells.

39. The method of claim 38 wherein the container has a volume of between about 200 ml and about 2,500 ml.

40. The method of any one of claims 38-39 wherein the step of measuring growth of the cells is performed through a wall of the container.

41. The method of any one of claims 38-40 wherein the step of measuring growth of the cells uses an optical measurement.

42. The method of any one of claims 38-40 wherein the activation medium contains N-803 at a concentration of between 0.1-1.0 nM and the anti-CD16 antibody at a concentration of between 0.05-0.5 mcg/ml.

43. The method of any one of claims 38-42 wherein the time sufficient to activate NK cells is between 24 hours and 96 hours.

44. The method of any one of claims 38-43 wherein the medium containing N-803 contains N-803 at a concentration of between 0.1-1.0 nM.

45. The method of any one of claims 38-44 wherein the cells are fed until a total cell number of about 0.5- 5.0 x 10^9 cells is reached.

46. The method of any one of claims 38-45 wherein the cells are fed until NK cells are enriched to an at least 100-fold expansion.

47. A cell culture container containing distinct types of immune competent cells, comprising:
a medium in which are disposed
NK cells in an amount of at least 80% of all live cells;
NKT cells in an amount of equal or less than 10% of all live cells;
T cells an amount of equal or less than 5% of all live cells; and
DN T cells an amount of equal or less than 3% of all live cells;

48. The cell culture container of claim 47 wherein the container has a volume of between about 200 ml and about 2,500 ml.

49. The cell culture container of any one of claims 47-48 wherein at least one wall of the container has an optically transparent portion.

50. The cell culture container of any one of claims 47-49 wherein the NK cells are present in an amount of at least about 90% of all live cells.