CLAIMS

1. A method for engineering of immune cell function in an immune cell comprising intracellular delivery of a compound by passing a suspension comprising said immune cell through a microfluidic device comprising a constriction and contacting the immune cell with said compound to deliver said compound into the cytosol of said immune cell, wherein the constriction has a diameter of 2 μm to 10 μm, and wherein said compound:

a) comprises a differentiation factor, a self-amplifying RNA that encodes for continual production of an encoded protein, a chimeric antigen receptor, a nucleic acid encoding a chimeric antigen receptor, a tolerogenic factor, an adjuvant, a virus, a virus-like particle, a nucleic acid that is a plasmid, a nucleic acid that is a transposon, or a nucleic acid that encodes an MHC complex; or

b) confers the expression of a homing phenotype to the immune cell; or

c) induces the differentiation of the immune cell into a cell with a tolerogenic phenotype; or

d) enhances immune cell function, wherein the immune cell is a T cell, and wherein the compound that enhances T cell function is an immune checkpoint pathway inhibitor.

2. The method of claim 1, wherein the compound comprises a differentiation factor.

3. The method of claim 1, wherein said membrane is disrupted by passing said immune cell through a constriction having a diameter of 3 μm to 6 μm.

4. The method of claim 1, wherein said immune cell is a T cell, B cell, dendritic cell, macrophage, NK cell, monocyte, neutrophil, granulocyte, or innate lymphoid cell.

5. The method of claim 1, wherein said compound confers the expression of a homing phenotype to the immune cell.

6. The method of claim 1, wherein said compound induces the differentiation of the immune cell into a cell with a tolerogenic phenotype.

7. The method of claim 1 wherein said compound comprises a self-amplifying RNA that encodes for continual production of an encoded protein.

8. The method of claim 1, further comprising contacting said immune cell with an effector T cell and activating a cytotoxic T cell immune response.

9. The method of claim 1, further comprising contacting said immune cell with an effector T cell and activating a helper T cell immune response.

10. The method of claim 1, further comprising contacting said immune cell with an effector T cell and activating a tolerogenic T cell immune response.

11. The method of claim 1, wherein said compound comprises a chimeric antigen receptor.

12. The method of claim 11, wherein the chimeric antigen receptor is a chimeric T cell receptor.

13. The method of claim 1, wherein said compound enhances immune cell function.

14. The method of claim 13, wherein the immune cell is a T cell.

15. The method of claim 14, wherein the compound that enhances T cell function is an immune checkpoint pathway inhibitor.

16. The method of claim 1, wherein said compound comprises a tolerogenic factor.

17. The method of claim 1, wherein said compound comprises an adjuvant.

18. The method of claim 1, wherein said compound comprises a nucleic acid.

19. The method of claim 18, wherein said nucleic acid is or encodes an siRNA, mRNA, miRNA, lncRNA, tRNA, saRNA, or shRNA.

20. The method of claim 18, wherein said nucleic acid is a plasmid.

21. The method of claim 1, wherein the compound comprises a protein or peptide.

22. The method of claim 1, wherein the compound comprises a virus.

23. The method of claim 18, wherein the nucleic acid encodes an MHC complex.

24. The method of claim 1, wherein said immune cell is in a resting state.

25. The method of claim 24, wherein expression of one or more of the markers CD25, KLRG1, CD80, CD86, PD-1, PDL-1, CTLA-4, CD28, CD3, MHC-I, MHC-II, CD62L, CCR7, CX3CR1 or CXCR5 in the immune cell is modulated by delivery of said compound into said immune cell.

26. The method of claim 25, wherein expression of one or more of the markers is decreased and/or expression of one or more of the markers is increased.

27. The method of claim 1, wherein said immune cell is a naive immune cell or a memory immune cell.

28. The method of claim 1, wherein said cell suspension comprises whole blood, buffy coat cells, a mixed cell population, or a purified cell population.

29. The method of claim 1, wherein said cell suspension comprises peripheral blood mononuclear cells, and wherein the compound is delivered to a peripheral blood mononuclear cell of the cell suspension.

30. The method of claim 1, wherein said cell suspension comprises mammalian cells.

31. The method of claim 30, wherein said cell suspension comprises human, mouse, dog, cat, horse, monkey, or rat cells.

32. The method of claim 1, wherein said compound comprises a nucleic acid encoding a chimeric antigen receptor.

33. The method of claim 32, wherein the chimeric antigen receptor is a chimeric T cell receptor.

34. The method of claim 1, wherein said compound comprises a virus-like particle.

35. The method of claim 1, wherein said nucleic acid is a transposon.

36. The method of claim 29, wherein the peripheral blood mononuclear cells comprise one or more of the following types of cells: T cells, B cells, NK cells, monocytes, dendritic cells, and macrophages.

37. The method of claim 29, wherein the peripheral blood mononuclear cells comprise a T cell.

38. The method of claim 29, wherein the peripheral blood mononuclear cells comprise a B cell.

39. The method of claim 29, wherein the peripheral blood mononuclear cells comprise an NK cell.

40. The method of claim 29, wherein the peripheral blood mononuclear cells comprise a monocyte.

41. The method of claim 29, wherein the peripheral blood mononuclear cells comprise a dendritic cell.

42. The method of claim 29, wherein the peripheral blood mononuclear cells comprise a macrophage.

43. The method of claim 37, wherein the compound comprises a chimeric antigen receptor.

44. The method of claim 37, wherein the compound comprises a nucleic acid encoding a chimeric antigen receptor.

45. The method of claim 37, wherein the compound comprises a nucleic acid that is a transposon.

46. The method of claim 37, wherein the compound enhances T cell function, and wherein the compound that enhances T cell function is an immune checkpoint pathway inhibitor.

47. The method of claim 37, wherein the compound comprises a virus or virus-like particle.

48. A method for engineering of immune cell function in an immune cell comprising intracellular delivery of a compound by passing a suspension comprising said immune cell through a microfluidic device comprising a constriction and contacting the immune cell with said compound to deliver said compound into the cytosol of said immune cell, wherein the constriction has a diameter of 2 μm to 10 μm, and wherein said compound comprises:

a) an antigen that comprises a full-length, unprocessed protein; or

b) a tumor antigen or viral antigen selected from the group consisting of liver cancer antigen, lung cancer antigen, bladder cancer antigen, breast cancer antigen, colon cancer antigen, rectal cancer antigen, endometrial cancer antigen, kidney cancer antigen, leukemia antigen, melanoma antigen, non-Hodgkin lymphoma antigen, pancreatic cancer antigen, prostate cancer antigen, thyroid cancer antigen, ovarian cancer antigen, uterine cancer antigen, HIV antigen, Ebola antigen, HPV antigen, and EBV antigen.

49. The method of claim 48, wherein said compound comprises an antigen having a length of greater than 7, 8, 9 or 10 amino acids and wherein said immune cell processes said antigen and displays a class I histocompatibility antigen restricted processed form of said antigen on a surface of said immune cell.

50. The method of claim 48, wherein the compound comprises an antigen, and wherein said immune cell processes said antigen and displays said antigen on a surface of said immune cell.

51. The method of claim 50, wherein the immune cell displays a class I or class II histocompatibility antigen restricted processed form of said antigen on a surface of said immune cell.

52. The method of claim 48, wherein said membrane is disrupted by passing said immune cell through a constriction having a diameter of 3 μm to 6 μm.

53. The method of claim 48, wherein said immune cell is a T cell, B cell, dendritic cell, macrophage, NK cell, monocyte, neutrophil, granulocyte, or innate lymphoid cell.

54. The method of claim 48, further comprising intracellular delivery of a compound that induces the differentiation of the immune cell into a cell with a tolerogenic phenotype.

55. The method of claim 48, wherein said antigen comprises a full-length, unprocessed protein.

56. The method of claim 48, further comprising contacting said immune cell with an effector T cell and activating a cytotoxic T cell immune response.

57. The method of claim 48, further comprising contacting said immune cell with an effector T cell and activating a helper T cell immune response.

58. The method of claim 48, further comprising contacting said immune cell with an effector T cell and activating a tolerogenic T cell immune response.

59. The method of claim 48, wherein said compound comprises a disease-associated antigen.

60. The method of claim 55, wherein said compound comprises a tumor antigen, viral antigen, bacterial antigen, self-antigen, or fungal antigen.

61. The method of claim 48, wherein said compound comprises a liver cancer antigen, lung cancer antigen, bladder cancer antigen, breast cancer antigen, colon cancer antigen, rectal cancer antigen, endometrial cancer antigen, kidney cancer antigen, leukemia antigen, melanoma antigen, non-Hodgkin lymphoma antigen, pancreatic cancer antigen, prostate cancer antigen, thyroid cancer antigen, ovarian cancer antigen, or uterine cancer antigen.

62. The method of claim 48, wherein said compound comprises a tumor cell lysate or cell lysate from tissue infected with a pathogen.

63. The method of claim 48, wherein said compound comprises a neoantigen.

64. The method of claim 48, wherein said compound comprises an HIV antigen, Ebola antigen, HPV antigen, or EBV antigen.

65. The method of claim 48, further comprising intracellular delivery of a compound that enhances immune cell function.

66. The method of claim 48, wherein the immune cell is a T cell.

67. The method of claim 66, further comprising intracellular delivery of a compound that enhances T cell function.

68. The method of claim 67, wherein the compound that enhances T cell function is an immune checkpoint pathway inhibitor.

69. The method of claim 48, further comprising intracellular delivery of a tolerogenic factor.

70. The method of claim 48, further comprising intracellular delivery of an adjuvant.

71. The method of claim 61, wherein the compound comprises a protein or peptide.

72. The method of claim 48, wherein the compound comprises a virus or virus-like particle.

73. The method of claim 48, wherein said immune cell is in a resting state.

74. The method of claim 73, wherein expression of one or more of the markers CD25, KLRG1, CD80, CD86, PD-1, PDL-1, CTLA-4, CD28, CD3, MHC-I, MHC-II, CD62L, CCR7, CX3CR1 or CXCR5 in the immune cell is modulated by delivery of said compound into said immune cell.

75. The method of claim 74, wherein expression of one or more of the markers is decreased and/or expression of one or more of the markers is increased.

76. The method of claim 48, wherein said immune cell is a naive immune cell or a memory immune cell.

77. The method of claim 48, wherein said cell suspension comprises whole blood, buffy coat cells, a mixed cell population, or a purified cell population.

78. The method of claim 48, wherein said cell suspension comprises peripheral blood mononuclear cells, and wherein the compound is delivered to a peripheral blood mononuclear cell of the cell suspension.

79. The method of claim 48, wherein said cell suspension comprises mammalian cells.

80. The method of claim 79, wherein said cell suspension comprises human, mouse, dog, cat, horse, monkey, or rat cells.

81. The method of claim 48, wherein said compound comprises a liver cancer antigen.

82. The method of claim 48, wherein said compound comprises a lung cancer antigen.

83. The method of claim 48, wherein said compound comprises a bladder cancer antigen.

84. The method of claim 48, wherein said compound comprises a breast cancer antigen.

85. The method of claim 48, wherein said compound comprises a colon cancer antigen.

86. The method of claim 48, wherein said compound comprises a rectal cancer antigen.

87. The method of claim 48, wherein said compound comprises an endometrial cancer antigen.

88. The method of claim 48, wherein said compound comprises a kidney cancer antigen.

89. The method of claim 48, wherein said compound comprises a leukemia antigen.

90. The method of claim 48, wherein said compound comprises a melanoma antigen.

91. The method of claim 48, wherein said compound comprises a non-Hodgkin lymphoma antigen.

92. The method of claim 48, wherein said compound comprises a pancreatic cancer antigen.

93. The method of claim 48, wherein said compound comprises a prostate cancer antigen.

94. The method of claim 48, wherein said compound comprises a thyroid cancer antigen.

95. The method of claim 48, wherein said compound comprises an ovarian cancer antigen.

96. The method of claim 48, wherein said compound comprises a uterine cancer antigen.

97. The method of claim 48, wherein said compound comprises an HIV antigen.

98. The method of claim 48, wherein said compound comprises an Ebola antigen.

99. The method of claim 48, wherein said compound comprises an HPV antigen.

100. The method of claim 48, wherein said compound comprises an EBV antigen.

101. The method of claim 64, wherein the compound comprises a protein or peptide.

102. The method of claim 78, wherein the peripheral blood mononuclear cells comprise one or more of the following types of cells: T cells, B cells, NK cells, monocytes, dendritic cells, and macrophages.

103. The method of claim 78, wherein the peripheral blood mononuclear cells comprise a T cell.

104. The method of claim 78, wherein the peripheral blood mononuclear cells comprise a B cell.

105. The method of claim 78, wherein the peripheral blood mononuclear cells comprise an NK cell.

106. The method of claim 78, wherein the peripheral blood mononuclear cells comprise a monocyte.

107. The method of claim 78, wherein the peripheral blood mononuclear cells comprise a dendritic cell.

108. The method of claim 78, wherein the peripheral blood mononuclear cells comprise a macrophage.

109. The method of claim 103, further comprising intracellular delivery of a compound that enhances immune cell function.

110. The method of claim 103, further comprising intracellular delivery of a compound that enhances T cell function.

111. The method of claim 110, wherein the compound that enhances T cell function is an immune checkpoint pathway inhibitor.

112. The method of claim 103, further comprising intracellular delivery of a tolerogenic factor.

113. The method of claim 103, further comprising intracellular delivery of an adjuvant.

114. A method for engineering of immune cell function in an immune cell comprising intracellular delivery of a compound by passing a suspension comprising said immune cell through a microfluidic device comprising a constriction and contacting the immune cell with said compound to deliver said compound into the cytosol of said immune cell, wherein the constriction has a diameter of 2 μm to 10 μm, wherein the compound comprises a protein comprising a TALEN protein, Zinc finger nuclease, or transcription factor.

115. The method of claim 114, wherein the immune cell displays a class I or class II histocompatibility antigen restricted processed form of an antigen on a surface of said immune cell.

116. The method of claim 114, wherein said membrane is disrupted by passing said immune cell through a constriction having a diameter of 3 μm to 6 μm.

117. The method of claim 114, wherein said immune cell is a T cell, B cell, dendritic cell, macrophage, NK cell, monocyte, neutrophil, granulocyte, or innate lymphoid cell.

118. The method of claim 114, wherein said compound confers the expression of a homing phenotype to the immune cell.

119. The method of claim 114, wherein said compound induces the differentiation of the immune cell into a cell with a tolerogenic phenotype.

120. The method of claim 114, further comprising contacting said immune cell with an effector T cell and activating a cytotoxic T cell immune response.

121. The method of claim 114, further comprising contacting said immune cell with an effector T cell and activating a helper T cell immune response.

122. The method of claim 114, further comprising contacting said immune cell with an effector T cell and activating a tolerogenic T cell immune response.

123. The method of claim 114, wherein said compound enhances immune cell function.

124. The method of claim 123, wherein the immune cell is a T cell.

125. The method of claim 124, wherein the compound that enhances T cell function is an immune checkpoint pathway inhibitor.

126. The method of claim 114, wherein said compound comprises a tolerogenic factor.

127. The method of claim 114, wherein the compound comprises a peptide.

128. The method of claim 114, wherein said immune cell is in a resting state.

129. The method of claim 128, wherein expression of one or more of the markers CD25, KLRG1, CD80, CD86, PD-1, PDL-1, CTLA-4, CD28, CD3, MHC-I, MHC-II, CD62L, CCR7, CX3CR1 or CXCR5 in the immune cell is modulated by delivery of said compound into said immune cell.

130. The method of claim 129, wherein expression of one or more of the markers is decreased and/or expression of one or more of the markers is increased.

131. The method of claim 114, wherein said immune cell is a naive immune cell or a memory immune cell.

132. The method of claim 114, wherein said cell suspension comprises whole blood, buffy coat cells, a mixed cell population, or a purified cell population.

133. The method of claim 114, wherein said cell suspension comprises peripheral blood mononuclear cells, and wherein the compound is delivered to a peripheral blood mononuclear cell of the cell suspension.

134. The method of claim 114, wherein said cell suspension comprises mammalian cells.

135. The method of claim 134, wherein said cell suspension comprises human, mouse, dog, cat, horse, monkey, or rat cells.

136. The method of claim 114, wherein the compound comprises a protein comprising a TALEN protein.

137. The method of claim 114, wherein the compound comprises a protein comprising a Zinc finger nuclease.

138. The method of claim 114, wherein the compound comprises a protein comprising a transcription factor.

139. The method of claim 133, wherein the peripheral blood mononuclear cells comprise one or more of the following types of cells: T cells, B cells, NK cells, monocytes, dendritic cells, and macrophages.

140. The method of claim 133, wherein the peripheral blood mononuclear cells comprise a T cell.

141. The method of claim 133, wherein the peripheral blood mononuclear cells comprise a B cell.

142. The method of claim 133, wherein the peripheral blood mononuclear cells comprise an NK cell.

143. The method of claim 133, wherein the peripheral blood mononuclear cells comprise a monocyte.

144. The method of claim 133, wherein the peripheral blood mononuclear cells comprise a dendritic cell.

145. The method of claim 133, wherein the peripheral blood mononuclear cells comprise a macrophage.