CLAIMS

What is claimed is:

1. Use of an immune cell comprising (i) an antigen, (ii) a tolerogenic factor, or (iii) an antigen and a tolerogenic factor in the manufacture of a medicament for inducing tolerance or for suppressing an immune response to the antigen in an individual, wherein the immune cell comprising (i) the antigen, (ii) the tolerogenic factor, or (iii) the antigen and the tolerogenic factor is generated by passing a cell suspension comprising an immune cell through a constriction, wherein said constriction deforms the cell, causing a perturbation of the cell such that (i) the antigen, (ii) the tolerogenic factor, or (iii) the antigen and the tolerogenic factor contacted with the cell enters the immune cell, wherein the immune cell is a tolerogenic immune cell or an immunosuppressive immune cell, and wherein presentation of said antigen induces tolerance or suppresses an immune response to the antigen.

2. Use of a tolerogenic immune cell or an immunosuppressive immune cell comprising (i) an antigen, (ii) a tolerogenic factor, or (iii) an antigen and a tolerogenic factor in the manufacture of a medicament for inducing tolerance or for suppressing an immune response to the antigen in an individual by introducing the tolerogenic immune cell or the immunosuppressive immune cell comprising: (i) the antigen, (ii) the tolerogenic factor, or (iii) the antigen and the tolerogenic factorinto the individual, wherein presentation of said antigen induces tolerance or suppresses an immune response to the antigen, and wherein (i) the antigen, (ii) the tolerogenic factor, or (iii) the antigen and the tolerogenic factor was introduced to the tolerogenic immune cell or the immunosuppressive immune cell by passing the tolerogenic immune cell or the immunosuppressive immune cell or a precursor thereof through a constriction, wherein said constriction deformed the cell thereby causing a perturbation of the cell such that (i) the antigen, (ii) the tolerogenic factor, or (iii) the antigen and the tolerogenic factor entered the tolerogenic immune cell or the immunosuppressive immune cell or precursor thereof.

3. A method for delivering (i) an antigen, (ii) a tolerogenic factor or (iii) an antigen and a tolerogenic factor into a tolerogenic immune cell or an immunosuppressive immune cell, the method comprising passing a cell suspension comprising the tolerogenic immune cell or the immunosuppressive immune cell through a constriction, wherein said constriction deforms the

tolerogenic immune cell or the immunosuppressive immune cell, causing a perturbation of the cell such that (i) the antigen, (ii) the tolerogenic factor or (iii) the antigen and the tolerogenic factor can enter the cell, and contacting said cell suspension with (i) the antigen, (ii) the tolerogenic factor or (iii) the antigen and the tolerogenic factor.

4. The use or the method of any one of claims 1-3, wherein the tolerogenic immune cell or the immunosuppressive immune cell or a precursor thereof has not been contacted with an adjuvant.

5. The use or the method of any one of claims 1-4, wherein:
a) the tolerogenic immune cell or the immunosuppressive immune cell comprises a reduced ability to provide one or more costimulatory signals as compared to a non-tolerogenic precursor of the tolerogenic immune cell, or a non-immunosuppressive precursor of the immunosuppressive immune cell; and/or
b) the tolerogenic immune cell or the immunosuppressive immune cell comprises a reduced ability to provide one or more inflammatory signals as compared to a non-tolerogenic precursor of the tolerogenic immune cell or a non-immunosuppressive precursor of the immunosuppressive immune cell

6. The use or the method of claim 5, wherein:
(i) the one or more costimulatory signals are mediated by a molecule selected from the group consisting of CD40, CD80, CD86, CD54, CD83, CD79, and ICOS ligand; and/or
(ii) the one or more inflammatory signals are mediated by a molecule selected from the group consisting of interleukin-1 (IL-1), IL-12, IL-18, tumor necrosis factor (TNF), interferon gamma (IFN-gamma), granulocyte-macrophage colony stimulating factor (GM-CSF), NF-κB, an interferon regulatory factor (IRF), IL-4, IL-10, IL-13, IL-35, IFN-α, transforming growth factor-beta (TGFβ),and a molecule associated with the JAK-STAT signaling pathway.

7. The use or the method of any one of claims 1 to 6, wherein:
(i) the tolerogenic factor inhibits expression and/or activity of an immunostimulatory agent, enhances expression and/or activity of an immunosuppressive molecule, inhibits expression and/or activity of an inflammatory molecule, and/or enhances expression and/or activity of an anti- inflammatory molecule;

(ii) the antigen is presented by the tolerogenic immune cell or the immunosuppressive immune cell; and/or
(iii) tolerance to at least one other antigen is induced or an immune response to at least one other antigen is suppressed.

8. The use or the method of any one of claims 1-7, wherein at least one additional antigen is introduced into the cell.

9. The use or the method of any one of claims 1-8, wherein at least one additional tolerogenic factor is introduced into the cell.

10. The use or the method of any one of claim 1-9, wherein the constriction is
a) contained within a microfluidic channel, or
b) a pore or contained within a pore.

11. The use or the method of any one of claims 1-10, wherein the constriction size is
a) a function of the diameter of the immune cell, or
b) about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% of the cell diameter.

12. The use or the method of any one of claims 1-11, wherein the cell suspension comprises
a) a mixed cell population or a purified cell population,
b) whole blood,
c) peripheral blood mononuclear cells, or
d) mammalian cells.

13. The use or the method of any one of claims 1-12, wherein the immune cell is
a) a T cell, B cell, dendritic cell, monocyte, macrophage, NK cell, innate lymphoid cell, neutrophil, basophil, eosinophil, myeloid derived suppressor cell, or mast cell, and/or,
b) isolated from a same or from a different individual.

14. The use or the method of any one of claims 1-13, wherein the antigen is

a) a foreign antigen,
b) a self-antigen,
c) an allograft transplantation antigen,
d) a protein or polypeptide,
e) a lysate,
f) a modified antigen,
g) an antigen associated with transplanted tissue
h) an antigen associated with a virus,
i) fused with a therapeutic agent, or
j) fused with a targeting peptide.

15. The use or the method of any one of claims 1-15, wherein said cell suspension is contacted with (i) the antigen, (ii) the tolerogenic factor, or (iii) the antigen and the tolerogenic factor before, concurrently, or after passing through the constriction.

16. The use or the method of any one of claims 1-15, wherein the tolerogenic factor
a) inhibits the activity of a costimulatory molecule
b) decreases expression of a costimulatory molecule,
c) deletes nucleic acid that modulates expression of a costimulatory molecule,
d) inhibits a costimulatory molecule,
e) increases the activity of a transcriptional regulator that suppresses expression of a costimulatory molecule,
f) increases the activity of a protein inhibitor that suppresses expression of a costimulatory molecule,
g) comprises nucleic acid encoding a suppressor of the costimulatory molecule,
h) enhances the activity of an immunosuppressive factor,
i) enhances the activity of a co-inhibitory molecule,
j) increases expression of a co-inhibitory molecule,
k) encodes a co-inhibitory molecule,
l) increases the activity of a co-inhibitory molecule,
m) increases the activity of a transcriptional regulator that enhances expression of a co-inhibitory molecule,

n) increases the activity of a polypeptide that increases expression of a co-inhibitory molecule,
o) comprises nucleic acid encoding an enhancer of a co-inhibitory molecule,
p) enhances the activity of the immunosuppressive molecule,
q) increases expression of an immunosuppressive molecule,
r) encodes an immunosuppressive molecule,
s) increases the activity of an immunosuppressive molecule, increases the activity of a transcriptional regulator that enhances expression of an immunosuppressive molecule,
t) increases the activity of a polypeptide that enhances expression of an immunosuppressive molecule, or
u) comprises nucleic acid encoding an enhancer of an immunosuppressive molecule.

17. The method of any one of claims 1-16, wherein the tolerogenic factor comprises:
a) a nucleic acid or a nucleic acid encoding siRNA, mRNA, miRNA, lncRNA, tRNA, or shRNA,
b) a plasmid,
c) a protein-nucleic acid complex,
d) a Cas9 polypeptide and a guide RNA or donor DNA,
e) a nucleic acid encoding for a Cas9 polypeptide and a guide RNA or donor DNA,
f) a polypeptide or a polypeptide that encodes a nuclease, TALEN protein, Zinc finger nuclease, mega nuclease, CRE recombinase, transposase or integrase enzyme, an antibody, or a transcription factor,
g) a small molecule, or
h) a nanoparticle.

18. The use or the method of any one of claims 1-17, wherein the immune response is suppressed by at least about 10%, about 15%, about 20%, about 25%, about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 90%, or about 100%.

19. The use or the method of any one of claims 1-18, wherein the suppressed immune response or the induced tolerance comprises
a) a decreased production and/or secretion of one or more inflammatory cytokines,
b) an increased production and/or secretion of one or more anti-inflammatory cytokines,

c) a decreased T cell response,

d) a decreased T cell activation,

e) a decreased T cell survival,

f) a decreased T cell proliferation,

g) a decreased T cell functionality,

h) an enhanced Treg response,

i) a decreased cytokine production,

j) a decreased autoimmune response, or

k) a decreased allergic response,

20. The use or the method of any one of claims 1-19, wherein the method is repeated at least 1, 2, 3, 4, 5, or 6 times.