CLAIMS

1. A method for preparing a composition of mature CD4+ CD8−, or CD8ab+ CD4− T cells from stem or progenitor cells, the method comprising culturing a three-dimensional (3D) cell aggregate comprising:

a) a selected cell line of stromal cells that express an exogenous Notch ligand;

b) a selected population of stem or progenitor cells;

wherein the 3D cell aggregate is cultured in a serum-free medium comprising insulin, FLT3 ligand (FLT3L), IL-7, and ascorbic acid for a time period sufficient for the *in vitro* differentiation of the stem or progenitor cells to said mature T cells, wherein the 3D cell aggregate does not comprise an exogenous matrix or a scaffold.

2. A method for preparing a composition of innate-like CD4−CD8− or CD4−CD8aa+ T cells from stem or progenitor cells, the method comprising culturing a three-dimensional (3D) cell aggregate comprising:

a) a selected cell line of stromal cells that express an exogenous Notch ligand;

b) a selected population of stem or progenitor cells;

wherein the 3D cell aggregate is cultured in a serum-free medium comprising insulin, FLT3 ligand (FLT3L), IL-7 and ascorbic acid for a time period sufficient for the *in vitro* differentiation of the stem or progenitor cells to said innate-like T cells, wherein the 3D cell aggregate does not comprise an exogenous matrix or a scaffold.

3. The method of claim 1 or 2, wherein the method further comprises centrifugation of the stem or progenitor cells and the stromal cells to form the 3D cell aggregate.

4. The method of claim 1 or 2, wherein the stromal cells have an exogenous nucleotide sequence encoding an intact, partial, or modified Notch ligand, said Notch ligand is DLL4, DLLl, JAG1, JAG2, or a combination thereof.

5. The method of claim 1 or 2, wherein the stem or progenitor cells are selected from embryonic stem cells (ESCs), induced pluripotent stem cells (iPSC), human embryonic mesodermal progenitor cells, hematopoietic stem or progenitor cells, cells isolated from bone marrow, cells isolated from cord blood, cells isolated from peripheral blood or mobilized

blood, cells isolated from thymus, or cells that have been differentiated from ESC or iPSC *in vitro.*

6. The method of claim 1 or 2, wherein the stromal cells are a murine stromal cell line, a human stromal cell line, a selected population of stromal cells differentiated from pluripotent stem cells *in vitro*, or a combination thereof.

7. The method of claim 1 or 2, wherein the stromal cells express one or more of an exogenous human major histocompatibility complex (MHC), an exogenous antigen-specific costimulatory molecule or cytokine, an exogenous antigen, an extracellular matrix protein, or a bioactive molecule or gene that modulates T cell differentiation, proliferation, or function.

8. The method of claim 1 or 2, wherein the stem or progenitor cells comprise one or more of an exogenous T cell receptor (TCR), a chimeric antigen receptor (CAR), an exogenous invariant natural killer T cell (iNKT)-associated TCR, and genetic modification of HLA expression or function.

9. The method of claim 1 or 2, wherein the T cells from the 3D cell aggregate do not express an endogenous TCR via allelic exclusion or genetic modification, or said T cells comprise an exogenous TCR gene inserted into an endogenous TCR gene loci.

10. The method of claim 1 or 2, wherein the T cells from the 3D cell aggregate express one or both of an exogenous TCR and a CAR.