TITLE: STIMULATORY ANTIGEN PARTICLES, METHODS OF PRODUCTION, AND METHODS OF USE THEREOF

FIELD

[0001] The present invention generally relates to cell stimulatory particles, and methods of use thereof.

BACKGROUND OF THE DISCLOSURE

[0002] Various feeder cell-based methods are available for the generation of T lineage cells from stem/progenitor cells. However, the expression of cell surface proteins by the supportive stromal cells in feeder cell-based systems is heterogenous, leading to diverse outcomes. Further, feeder cell-based systems are not easily scalable to meet the needs of clinical manufacturing.

[0003] Immobilized Notch signalling ligands, such as DL4, in combination with VCAM-1, have been shown to promote *in vitro* generation of progenitor T cells in a feeder-free and serum-free culture system (Shukla et al., 2017). Microbeads modified to present DL4 have also been shown to support *in vitro* differentiation of T-lineage cells, albeit with limited progression to mature lineages such as CD4-CD8+ cells (Trotman-Grant et al., 2021). Short-term stimulation of T cell progenitors with a CD3 antibody in the absence of Notch signalling followed by maturation without CD3 antibody has been shown to promote generation of CD8αβ+ T cells (Iriguchi et al., 2021).

[0004] Immobilized and soluble antibodies have previously been used to stimulate and expand primary (i.e., donor-derived) T cells, such as through stimulation of a chimeric antigen receptor (CAR) (Philipson et al., 2020). Antigens presented on particles and by antigen-presenting cells have also previously been used to stimulate and expand CAR+ primary T cells (Wu et al. US 20180223255).

[0005] It is desirable to obviate or mitigate one or more of the above deficiencies.

SUMMARY OF THE DISCLOSURE

[0006] In an example, a method of generating CD4-CD8+ T cells is provided. The method comprises contacting a population of CD4+CD8+ cells expressing an antigen receptor with an antigen immobilized to a substrate. The antigen binds to the antigen receptor. In one aspect, the disclosure provides a method of generating CD4-CD8+ T cells, comprising: contacting a population of CD4+CD8+ cells expressing an antigen receptor with an antigen immobilized to a substrate, wherein the antigen binds to the antigen receptor.

[0007] In an example, the CD4+CD8+ cells are derived in vitro from hematopoietic stem/progenitor cells.

[0008] In an example, the hematopoietic stem/progenitor cells are derived in vitro from pluripotent stem cells.

[0009] In an example, the CD4+CD8+ cells are derived according to a method comprising contacting a population of hematopoietic stem/progenitor cells with an immobilized Notch signalling ligand.

[0010] In an example, the step of contacting the population of hematopoietic stem/progenitor cells with the immobilized Notch signalling ligand further comprises contacting the population of hematopoietic stem/progenitor cells with the immobilized Notch signalling ligand at a first ligand concentration, generating a population of progenitor T cells; and contacting the population of progenitor T cells with the immobilized Notch signalling ligand at a second ligand concentration, lower than the first ligand concentration.

[0011] In an example, the first ligand concentration is 3.15 $x10^{11}$ to 1.26 $x10^{12}$ molecules/mL and the second ligand concentration is 3.94 $x10^{10}$ to 6.31 $x10^{11}$ molecules/mL.

[0012] In an example, the method further comprises a step of enriching the CD4+CD8+ cells for CD8α or CD8β.

[0013] In an example, the immobilized Notch signalling ligand is DL4.

[0014] In an example, the antigen is a CD19 polypeptide and the antigen receptor binds the CD19 polypeptide.

[0015] In an example, the CD19 polypeptide is an engineered variant of CD19.

[0016] In an example, the antigen is a polypeptide having the sequence of SEQ ID NO:8.

[0017] In an example, the substrate is a particle.

[0018] In an example, the particle is comprised of a material selected from a group consisting of polystyrene, iron oxide and gold.

[0019] In an example, the particle is comprised of polystyrene and magnetizable iron oxides.

[0020] In an example, the antigen is covalently conjugated to the particle.

[0021] In an example, the step of contacting the population of CD4+CD8+ cells expressing the antigen receptor with the antigen coupled to the particle is conducted at a particle:cell ratio of 1:1.

[0022] In a second example of the disclosure, a population of CD4-CD8+ T cells made according to the methods of the first example is provided. In one aspect, the disclosure provides a population of CD4-CD8+ T cells made according to the methods.

[0023] In an example, the population of CD4-CD8+ T cells express a chimeric antigen receptor (CAR).

[0024] In an example, the CAR is a CD19 CAR, and wherein the antigen is a CD19 polypeptide.

[0025] In an example, the population of CD4-CD8+ T cells is derived in vitro from pluripotent stem cells.

[0026] In an example, the population of CD4-CD8+ T cells is TRAC-/- and/or CD3+.

[0027] In an example, the population of CD4-CD8+ cells is greater than 50% CD8αα+.

[0028] In a third example, a method of inducing cytotoxicity of a tumor cell is provided. The method comprises exposing the tumor cell to a population of CD4-CD8+ T cells. The population of CD4-CD8+ T cells is made according to the method of the first example. In one aspect, the disclosure provides a method of inducing cytotoxicity of a tumor cell comprising exposing the tumor cell to a population of CD4-CD8+ T cells, wherein the population of CD4-CD8+ T cells is made according to the method as disclosed herein.

[0029] In a fourth example, a method of treating a disease or condition in a subject is provided. The method comprises generating CD4-CD8+ T cells according to the method of the first example and administering an effective amount of the CD4-CD8+ T cells to a subject in need thereof. In one aspect, the disclosure provides an effective amount of the CD4-CD8+ T cells for use in therapy, wherein the CD4-CD8+ T cells are generated by a method as disclosed herein.

[0030] In a fifth example, a use of CD4-CD8+ T cells in the manufacture of a medicament for the treatment of a disease or condition is provided. The CD4-CD8+ T cells are generated by a method according to the first example. In one aspect, the disclosure provides a use of CD4-CD8+ T cells in the manufacture of a medicament for the treatment of a disease or condition, wherein the CD4-CD8+ T cells are generated by a method as disclosed here.

[0031] In a sixth example, a cell stimulatory particle is provided. The cell stimulatory particle comprises a particle and a modified antigen immobilized on a surface of the particle. The modified antigen comprises one or more amino acid substitutions from a native antigen. The modified antigen has greater solubility and/or stability in vitro in comparison to the native antigen. In one aspect, the disclosure provides a cell stimulatory particle, comprising a particle and a modified antigen immobilized on a surface of the particle, wherein the modified antigen comprises one or more amino acid substitutions from a native antigen, and wherein the modified antigen has greater solubility and/or stability in vitro in comparison to the native antigen.

[0032] In an example, the modified antigen comprises an extracellular domain of an antigen.

[0033] In an example, the modified antigen is covalently conjugated to the particle.

[0034] In an example, the particle is comprised of a material selected from a group consisting of polystyrene, iron oxide and gold.

[0035] In an example, the particle is comprised of polystyrene and magnetizable iron oxide.

[0036] In an example, the modified antigen is a CD19 antigen.

[0037] In an example, the modified antigen is a polypeptide having the sequence of SEQ ID NO:8.

BRIEF DESCRIPTION OF THE DRAWINGS

[0038] In order that the subject matter may be readily understood, embodiments are illustrated by way of non-limiting examples in the accompanying drawings.

[0039] Figure 1A is an annotated image of a sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel of CD19 protein variants under both non-reducing (-) and reducing (+) conditions.

[0040] Figure 1B is a graph quantifying the binding of the CD19 antibody FMC63 on 2D-coated CD19-Fc and CD19.1-Fc, as measured by direct enzyme-linked immunosorbent assay (ELISA).

[0041] Figure 1C is a graph quantifying the thermal melting curve of CD19 protein variants (top) and the first derivative plot of the melting curve (bottom).