

Methods of Producing Haemogenic Progenitor cells from Pluripotent Stem Cells

Field

This invention relates to the production of haemogenic progenitor cells, such as haemogenic endothelial cells (HECs) and haematopoietic progenitor cells (HPCs), for example for use in generating T cells for immunotherapy.

Background

Immunotherapeutics are poised to transform the cancer treatment landscape with the promise of long-term survival (McDermott et al., Cancer Treat Rev. 2014 Oct; 40(9): 1056-64). There is a clear unmet medical need for new immunomodulatory drugs to expand patient population and range of tumor types. In addition, new agents are needed to enhance the magnitude and duration of anti-tumor responses. The development of these agents has been possible because of the in-depth understanding of the basic principles controlling T-cell immunity over the last two decades (Sharma and Allison, Cell. 2015 Apr 9; 161(2): 205-14). This typically requires tumor specific CD4+ and CD8+ T-cells recognising tumor-associated peptide antigens presented by MHC molecules. Different vaccination strategies and adoptive transfer of ex vivo expanded tumor infiltrated lymphocytes have in some cases demonstrated the ability of tumor specific T-cells to treat late stage cancer (Rosenberg et al., Nat Med. 2004 Sep; 10(9): 909-15).

However, current adoptive T cell therapies are limited by a lack of suitable patient and tumor-specific T cells and there is a need for therapeutically sufficient and functional antigen-specific T cells for effective use in immunotherapy.

Summary

The present inventors have unexpectedly found that haemogenic progenitor cells, such as haemogenic endothelial cells (HECs) or haematopoietic progenitor cells (HPCs), that are capable of differentiation into T cells, may be produced from induced pluripotent stem cells (iPSCs) in a single culture vessel without intermediate purification or isolation steps. In addition, the haemogenic progenitor cells may be produced without the use of serum or stromal co-culture. This may be useful for example in the production of clinical grade blood cells, such as T cells, for use in immunotherapy.

A first aspect of the invention provides a method of producing a population of haemogenic progenitor cells comprising;

(i) differentiating a population of induced pluripotent stem cells (iPSCs) into mesoderm cells and;

(ii) differentiating the mesoderm cells to produce a population of haemogenic progenitor cells

wherein steps (i) and (ii) are performed without purification or isolation of cells in the population.

Haemogenic progenitor cells may include haemogenic endothelial cells (HECs). ~~A method of the first aspect~~In another example, the present disclosure may comprise;

(i) differentiating a population of induced pluripotent stem cells (iPSCs) into mesoderm cells and;

(ii) differentiating the mesoderm cells to produce a population of HECs,

wherein steps (i) and (ii) are performed without purification or isolation of cells in the population.

Haemogenic progenitor cells may include haematopoietic progenitor cells (HPCs). ~~A method of the first aspect~~In another example, the present disclosure may comprise;

(i) differentiating a population of induced pluripotent stem cells (iPSCs) into mesoderm cells

(ii) differentiating the mesoderm cells into HECs, and

(iii) differentiating the HECs into a population of haematopoietic progenitor cells (HPCs),

wherein steps (i), (ii) and (iii) are performed without purification or isolation of cells in the population.

Preferably, the iPSCs are differentiated into haemogenic progenitor cells, such as HECs or HPCs, in steps (i), (ii) and (iii) above in defined culture media in the absence of feeder cells, stromal cells, serum or other undefined media supplements.

In some preferred ~~embodiment~~examples, HPCs as described herein may be used in the production of T cells. ~~A method of the first aspect~~In another example, the present disclosure may further comprise;

(iv) differentiating the population of HPCs into T cell progenitor cells; and

(v) maturing the progenitor T cells to produce a population of DP CD4+CD8+ T cells.

~~A method of the first aspect~~In another example, the present disclosure may further comprise;

(vi) activating and expanding the DP CD4+CD8+ T cells to produce a population of SP CD8+ T cells or a population of SP CD4+ T cells.

In other ~~embodiment~~examples, HPCs are described herein may be used in the production of NK cells. ~~A method of the first aspect~~In a further example, the present disclosure may further comprise;

(iv) differentiating the population of HPCs into NK cells.

In other ~~embodiment~~examples, HPCs are described herein may be used in the production of B cells. ~~A method of the first aspect~~In a further example, the present disclosure may further comprise;

(iv) differentiating the population of HPCs into B cells.

These and other ~~aspect~~examples and ~~embodiments~~ of the ~~invention~~disclosure are described in more detail below.

Claims:

1. A method of producing a population of haemogenic progenitor cells comprising;
 - (i) differentiating a population of induced pluripotent stem cells (iPSCs) into mesoderm cells and;
 - (ii) differentiating the mesoderm cells to produce a population of haemogenic progenitor cells
 wherein steps (i) and (ii) are performed without purification or isolation of cells in the population.
2. A method according to claim 1 wherein (a) steps (i) and (ii) are performed in the absence of stromal cells or serum; or (b)
- ~~3. A method according to claim 1 wherein~~ the haemogenic progenitor cells are haemogenic endothelial cells (HECs) or haematopoietic progenitor cells (HPCs).
- ~~43.~~ A method according to ~~any one of claims 1 to or claim 23~~ wherein the iPSCs are differentiated into mesoderm cells by culturing the population of iPSCs under suitable conditions to promote mesodermal differentiation.
- ~~54.~~ A method according to any one of claims 1 to ~~34~~ wherein the iPSCs are cultured sequentially in first, second and third mesoderm induction media to induce differentiation into mesoderm cells; ~~1~~
- ~~6. A method according to claim 5 optionally~~ wherein
 - (a) the first mesoderm induction medium
 - (i) stimulates SMAD2 and SMAD3 mediated signalling pathways; and/or
 - (ii)
- ~~7. A method according to claim 6 wherein the first mesoderm induction medium~~ comprises activin; and/or
 - (iii)
- ~~8. A method according to claim 6 or claim 7 wherein the first mesoderm induction medium~~ consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of activin; and/or optionally wherein
 - (b)
- ~~9. A method according to any one of claims 4 to 7 wherein~~ the second mesoderm induction medium

(i) stimulates SMAD1, SMAD2, SMAD3, SMAD5 and SMAD9 mediated signalling pathways and

(ii) has fibroblast growth factor (FGF) activity; and/or

(iii)

~~10. A method according to claim 9 wherein the second mesoderm induction medium~~ comprises activin, BMP, and FGF; and/or

(iv)

~~11. A method according to claim 9 or claim 10 wherein the second mesoderm induction medium~~ consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of activin, BMP, and FGF; and/or optionally wherein

(c)

~~12. A method according to any one of claims 5 to 11 wherein~~ the third mesoderm induction medium

(i) stimulates SMAD1, SMAD2, SMAD3, SMAD5 and SMAD9 mediated signalling pathways

(ii) has fibroblast growth factor (FGF) activity and

(iii) inhibits glycogen synthase kinase 3 β ; and/or

(iv)

~~13. A method according to claim 12 wherein the third mesoderm induction medium~~ comprises activin, BMP, FGF, and a GSK3 inhibitor; and/or

(v)

~~14. A method according to claim 13 wherein the third mesoderm induction medium~~ consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of activin, BMP, FGF, and a GSK3 inhibitor;

optionally

~~15. A method according to any one of claims 5 to 14 wherein~~ (i) the mesoderm cells express one or more mesoderm markers selected from Brachyury Goosecoid, Mixl1, KDR, FoxA2, GATA6, and PDGF α R; and/or (ii)

~~16. A method according to any one of claims 5 to 16 wherein~~ mesoderm cells in the population are not purified following culture in the first, second and third mesoderm induction media.

- ~~175.~~ A method according to any one of the preceding claims wherein
 (a) the mesoderm cells are differentiated into HECs by culturing the population of mesoderm cells under suitable conditions to promote haemogenic endothelial (HE) differentiation; and/or
 (b)
- ~~18.~~ ~~A method according to any one of the preceding claims wherein~~ the mesoderm cells are cultured in an HE induction medium to induce differentiation into HECs; optionally
- ~~19.~~ ~~A method according to claim 18~~ wherein the HE induction medium
 (i) stimulates cKIT receptor (CD117; KIT receptor tyrosine kinase) mediated signalling pathways; and/or
 (ii) stimulates VEGFR mediated signalling pathways; and/or
 (iii)
- ~~20.~~ ~~A method according to claim 19 wherein the HE induction medium~~ comprises SCF and/or VEGF; and/or
 (iv)
- ~~21.~~ ~~A method according to claim 20 wherein the HE induction medium~~ consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of SCF and VEGF; optionally
- ~~22.~~ ~~A method according to any of claims 18 to 21~~ wherein (i) the HECs display a CD34+ phenotype; and/or (ii)
- ~~23.~~ ~~A method according to any of claims 18 to 22 wherein~~ HECs in the population are not purified following culture in the HE induction medium.
- ~~246.~~ A method according to any one of the preceding claims wherein
 (a) the HECs are differentiated into HPCs by culturing the population of HECs under suitable conditions to promote haematopoietic differentiation; and/or
 (b)
- ~~25.~~ ~~A method according to any one of the preceding claims wherein~~ the HECs are cultured in an haematopoietic induction medium to induce differentiation into HPCs; and/or
 (c)
- ~~26.~~ ~~A method according to claim 25 wherein~~ the haematopoietic induction medium stimulates
 (i) ~~stimulates~~ cKIT receptor (CD117) mediated signalling pathways;

- (ii) VEGFR mediated signalling pathways;
- (iii) MPL (CD110) mediated signalling pathways;
- (iv) FLT3 mediated signalling pathways;
- (v) IGF1R mediated signalling pathways;
- (vi) SMAD1, 5 and 9 mediated signalling pathways;
- (vii) Hedgehog signalling pathways;
- (viii) EpoR mediated signalling pathway; and
- (ix) AGTR2 mediated signalling pathways; inhibits the AGTR1 signalling pathway and displays IL and FGF activity; and/or

(d)

~~27. A method according to claim 26 wherein~~ the haematopoietic induction medium comprises VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IL-3, IL-6, IL-7, IL-11, IGF-1, BMP, FGF, Sonic hedgehog (SHH), erythropoietin (EPO), angiotensin II, and an angiotensin II type 1 receptor (AT₁) antagonist; and/or

(e)

~~28. A method according to claim 27 wherein~~ the haematopoietic induction medium consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IL-3, IL-6, IL-7, IL-11, IGF-1, BMP, FGF, Sonic hedgehog (SHH), erythropoietin (EPO), angiotensin II, and an angiotensin II type 1 receptor (AT₁) antagonist;

and optionally

~~29. A method according to any one of claims 24 to 28~~ wherein the HPCs display a CD34⁺ CD45⁺ phenotype.

~~307. A method according to any one of claims 24 to 29~~ comprising purifying the population of HPCs.

~~318. A method according to any one of the preceding claims wherein~~ (i) the haemogenic progenitor cells are HPCs and the method further comprises differentiating the population of HPCs into progenitor T cells; and/or (ii)

~~32. A method according to claim 31 wherein~~ the HPCs are differentiated by a method comprising culturing the population of HPCs in a lymphoid expansion medium to produce the progenitor T cells.

~~339. A method according to claim 31 or claim 32~~ wherein the progenitor T cells have a CD5⁺ CD7⁺ phenotype.

~~3410.~~ A method according to ~~any one of claims 8 or claim 9-31 to 33~~ further comprising maturing the progenitor T cells to produce a population of T cells-; optionally

~~35.~~ ~~A method according to claim 34~~ wherein the progenitor T cells are matured by a method comprising culturing the population of progenitor T cells in a T cell maturation medium to produce the T cells.

~~3611.~~ A method according to claim ~~34-10 or claim 35~~ wherein the T cells have a CD8+ CD4+ phenotype.

~~3712.~~ A method according to ~~any one of claims 34-10 or claim 11 to 36~~ comprising activating and expanding the T cells to produce a population of T cells have a CD8+ single positive phenotype or a CD4+ single positive phenotype.

~~3813.~~ A method according to any one of claims ~~34-10 to 37-12~~ wherein the T cells specifically bind to cells expressing a target antigen-; optionally

~~39.~~ ~~A method according to claim 38~~ wherein the target antigen is a tumour antigen-; optionally

~~40.~~ ~~A method according to claim 39~~ wherein the T cells specifically bind to cancer cells expressing the tumour antigen.

~~4114.~~ A method according to any one of the preceding claims wherein the iPSCs are derived from T cells obtained from a donor individual-; optionally

~~42.~~ ~~A method according to claim 41~~ wherein (i) the T cells obtained from the donor individual are specific for the target antigen-; and/or (ii)

~~43.~~ ~~A method according to claim 41 or 42 wherein~~ the T cells obtained from the donor individual are tumour-infiltrating lymphocytes (TILs).

~~4415.~~ A method according to any one of claims 1 to ~~40-13~~ wherein the method further comprises introducing heterologous nucleic acid encoding an antigen receptor into the iPSCs, HPCs or progenitor T cells.

~~4516.~~ A method according to claim ~~44-15~~ wherein the heterologous nucleic acid encoding the antigen receptor is comprised in an expression vector-; optionally

~~46.~~ ~~A method according to claim 45~~ wherein the expression vector is a lentiviral vector or adeno-associated viral (AAV) vector.

~~47~~17. A method according to claim ~~44-15~~ or ~~45-16~~ wherein the heterologous nucleic acid is incorporated into the genome of the iPSCs, HECs, haemogenic progenitor cells, or progenitor T cells using a gene editing system-; optionally

~~48~~—~~A method according to claim 47~~ wherein the gene editing system is CRISPR/Cas9 or AAV.

~~49~~18. A method according to any one of claims ~~44-15~~ to ~~48-17~~ wherein the antigen receptor is a TCR-; optionally wherein

~~50~~—~~A method according to claim 49 wherein (i)~~ the TCR is TCR is an affinity enhanced TCR-; and/or (ii)

~~51~~—~~A method according to claim 49 wherein the TCR is~~ TCR is a non-MHC restricted TCR-; and/or (iii)

~~52~~—~~A method according to one of claims 49 to 451 wherein~~ the TCR binds specifically to an MHC displaying a peptide fragment of a target antigen expressed by cells-; and/or (iv)

~~53~~—~~A method according to claim 52 wherein~~ the TCR binds specifically to an MHC displaying a peptide fragment of a tumour antigen expressed by the cancer cells.

~~54~~19. A method according to any one of claims ~~44-15~~ to ~~48-17~~ wherein the antigen receptor is a chimeric antigen receptor (CAR)-; and optionally

~~55~~—~~A method according to claim 54 wherein (i)~~ the CAR binds specifically to a target antigen expressed by cells-; and/or (ii)

~~56~~—~~A method according to claim 55 wherein~~ the CAR binds specifically to an MHC displaying a peptide fragment of a tumour antigen expressed by cancer cells.