

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.

In one aspect, the disclosure 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). In another example, the present disclosure may comprise;

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

5 (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.

10 Haemogenic progenitor cells may include haematopoietic progenitor cells (HPCs). 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

15 (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.

20 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 examples, HPCs as described herein may be used in the production of T cells. In another example, the present disclosure may further comprise;

25 (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.

In another example, the present disclosure may further comprise;

30 (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 examples, HPCs as described herein may be used in the production of NK cells. In a further example, the present disclosure may further comprise;

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

35 In other examples, HPCs as described herein may be used in the production of B cells. In a further example, the present disclosure may further comprise;

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

These and other examples of the 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) the haemogenic progenitor cells are haemogenic endothelial cells (HECs) or haematopoietic progenitor cells (HPCs).
3. A method according to claim 1 or claim 2 wherein the iPSCs are differentiated into mesoderm cells by culturing the population of iPSCs under suitable conditions to promote mesodermal differentiation.
4. A method according to any one of claims 1 to 3 wherein the iPSCs are cultured sequentially in first, second and third mesoderm induction media to induce differentiation into mesoderm cells, optionally wherein
 - (a) the first mesoderm induction medium
 - (i) stimulates SMAD2 and SMAD3 mediated signalling pathways; and/or
 - (ii) comprises activin; and/or
 - (iii) 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) 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) comprises activin, BMP, and FGF; and/or
 - (iv) 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) 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) comprises activin, BMP, FGF, and a GSK3 inhibitor; and/or
- (v) 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 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) mesoderm cells in the population are not purified following culture in the first, second and third mesoderm induction media.

5. 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) the mesoderm cells are cultured in an HE induction medium to induce differentiation into HECs, optionally 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) comprises SCF and/or VEGF; and/or
 - (iv) 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 wherein (i) the HECs display a CD34⁺ phenotype, and/or (ii) HECs in the population are not purified following culture in the HE induction medium.

6. 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) the HECs are cultured in an haematopoietic induction medium to induce differentiation into HPCs, and/or
 - (c) the haematopoietic induction medium stimulates
 - (i) 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) 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) 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 wherein the HPCs display a CD34⁺ CD45⁺ phenotype.

7. A method according to claim 6 comprising purifying the population of HPCs.

8. 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) the HPCs are differentiated by a method comprising culturing the population of HPCs in a lymphoid expansion medium to produce the progenitor T cells.

9. A method according to claim 8 wherein the progenitor T cells have a CD5⁺ CD7⁺ phenotype.

10. A method according to claim 8 or claim 9 further comprising maturing the progenitor T cells to produce a population of T cells, optionally 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.

11. A method according to claim 10 wherein the T cells have a CD8⁺ CD4⁺ phenotype.

12. A method according to claim 10 or claim 11 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.

13. A method according to any one of claims 10 to 12 wherein the T cells specifically bind to cells expressing a target antigen, optionally wherein the target antigen is a tumour antigen, optionally wherein the T cells specifically bind to cancer cells expressing the tumour antigen.

14. A method according to any one of the preceding claims wherein the iPSCs are derived from T cells obtained from a donor individual, optionally wherein (i) the T cells obtained from the donor individual are specific for the target antigen; and/or (ii) the T cells obtained from the donor individual are tumour-infiltrating lymphocytes (TILs).

15. A method according to any one of claims 1 to 13 wherein the method further comprises introducing heterologous nucleic acid encoding an antigen receptor into the iPSCs, HPCs or progenitor T cells.
16. A method according to claim 15 wherein the heterologous nucleic acid encoding the antigen receptor is comprised in an expression vector, optionally wherein the expression vector is a lentiviral vector or adeno-associated viral (AAV) vector.
17. A method according to claim 15 or 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 wherein the gene editing system is CRISPR/Cas9 or AAV.
18. A method according to any one of claims 15 to 17 wherein the antigen receptor is a TCR; optionally wherein (i) the TCR is an affinity enhanced TCR; and/or (ii) the TCR is a non-MHC restricted TCR; and/or (iii) the TCR binds specifically to an MHC displaying a peptide fragment of a target antigen expressed by cells; and/or (iv) the TCR binds specifically to an MHC displaying a peptide fragment of a tumour antigen expressed by the cancer cells.
19. A method according to any one of claims 15 to 17 wherein the antigen receptor is a chimeric antigen receptor (CAR); and optionally wherein (i) the CAR binds specifically to a target antigen expressed by cells; and/or (ii) the CAR binds specifically to an MHC displaying a peptide fragment of a tumour antigen expressed by cancer cells.