T Cell Production from RAG Inactivated iPSCs

Field

This invention relates to methods of producing T cells from RAG inactivated progenitor cells, such as induced pluripotent stem cells (iPSCs).

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.

The expression of endogenous T cell receptors (TCRs) may cause T cells to exert off-target toxicity when administered therapeutically. Whilst the expression of endogenous TCRs may in principle be prevented by the inactivation of the recombination activating gene 1 (RAG1) and/or recombination activating gene 2 (RAG2), this inactivation also blocks the differentiation of precursor cells into lymphopoietic lineage (Mombaerts et al (1992) Cell 68 (5) 869-877).

Summary

The present inventors have recognised that the capacity of RAG inactivated progenitor cells to differentiate into T cells can be restored by the expression of an exogenous T Cell Receptor (TCR). This may be useful for example in the production of T cells for immunotherapy.

In one aspect, the present disclosure provides a method of producing a population of T cells comprising;

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

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

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

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

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

wherein the method comprises introducing a heterologous nucleic acid encoding an antigen receptor into one of the (a) iPSCs, (b) the HPCs or (c) the progenitor T cells.

In another aspect, the present disclosure provides a population of double positive T cells and/or single positive T cells produced by a method as described herein, optionally wherein the T cells express a heterologous antigen receptor, optionally wherein the heterologous antigen receptor is a TCR, CAR or NKCR, and optionally wherein the T cells are RAG inactivated and do not express an endogenous TCR.

In yet another aspect, the present disclosure provides a pharmaceutical composition comprising a population of double positive T cells and/or single positive T cells produced by a method as described herein and a pharmaceutically acceptable excipient.

In a further aspect, the present disclosure provides a population of double positive T cells and/or single positive T cells produced by a method as described herein for use in therapy. In a further aspect, the present disclosure provides a use of a population of double positive T cells and/or single positive T cells produced by a method as described herein in the manufacture of a medicament for treating cancer.

A first ~~aspect~~example of the ~~invention~~disclosure provides a method of producing a population of T cells which may comprise;

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

(ii) differentiating the MCs to produce a population of haemogenic endothelial cells (HECs),

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

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

(v) maturing the progenitor T cells to produce a population of double positive CD4+ CD8+ T cells,

wherein the method comprises

(a) inactivating RAG in one of the (a) iPSCs, (b) MCs and (c) HECs; and

(b) introducing a heterologous nucleic acid encoding an antigen receptor, such as a T Cell Receptor (TCR) or chimeric antigen receptor (CAR), into one of the RAG inactivated (a) iPSCs, (b) MCs (c) HECs (d) HPCs or (e) the progenitor T cells.

For example, a method of the first ~~aspect~~example may comprise;

(i) differentiating a population of RAG inactivated (RAG-/-) induced pluripotent stem cells (iPSCs) into mesoderm cells

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

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

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

(v) maturing the progenitor T cells to produce a population of double positive CD4+ CD8+ T cells,

wherein the method comprises introducing a heterologous nucleic acid encoding an antigen receptor, such as a T Cell Receptor (TCR) or chimeric antigen receptor (CAR), into one of the RAG inactivated cells selected from, (a) iPSCs, (b) MCs (c) HECs (d) HPCs and (e) the progenitor T cells.

A method of the first ~~aspect~~example may further comprise;

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

A second ~~aspect~~example of the ~~invention~~disclosure provides a population of T cells produced by a method of the first ~~aspect~~example. The T cells may be RAG inactivated (RAG-/-) and may display expression of a heterologous antigen receptor and no expression of an endogenous TCR.

A third ~~aspect~~example of the ~~invention~~disclosure provides a pharmaceutical composition comprising a population of T cells of the second ~~aspect~~example and a pharmaceutically acceptable excipient.

A fourth ~~aspect~~example of the ~~invention~~disclosure provides a method of treatment comprising administering a therapeutically effective dose of a population of T cells of the second ~~aspect~~example to an individual in need thereof.

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

Brief Description of Figures

Figure 1 shows a schematic view of an example of a six-stage method for generating T cells from iPSCs.

Figure 2 shows virus titration of ADP-A2M4 Lentivirus on peripheral blood lymphocytes (PBL) and Stage 5 iPSC-derived T cells. Flow cytometry plots show % Vα24+CD3+ T cells with increasing dilutions of ADP-A2M4 lentivirus in a) PBL and b) iPSC-derived T cells (HCP547). Populations are gated on total live cells. c) Surface expression of CD3 correlates with ADP-A2M4 transduction efficiency in iPSC-derived T

Claims:

1. A method of producing a population of T cells comprising;

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

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

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

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

(v) maturing the progenitor T cells to produce a population of double positive CD4+ CD8+ T cells,

wherein the method comprises introducing a heterologous nucleic acid encoding an antigen receptor into one of the (a) iPSCs, (b) the HPCs or (c) the progenitor T cells.

2. A method according to claim 1 further comprising;

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

~~3. A method according to claim 1 or claim 2 wherein the method comprises introducing the heterologous nucleic acid into the iPSCs.~~

~~4. A method according to claim 1 or claim 2 wherein the method comprises introducing the heterologous nucleic acid into the HPCs.~~

~~5. A method according to claim 1 or claim 2 wherein the method comprises introducing the heterologous nucleic acid into the progenitor T cells.~~

~~6~~3. A method according to ~~any one of the preceding~~ claim~~s~~ 1 or claim 2 wherein the heterologous nucleic acid encoding the TCR is comprised in an expression vector~~.~~, optionally

~~7. A method according to claim 6~~ wherein the expression vector is a lentiviral vector or adeno-associated viral (AAV) vector~~.~~, and optionally

~~8. A method according to claim 6~~ wherein the lentiviral vector is a VSVg-pseudotyped viral vector.

~~9~~4. A method according to any one of the preceding claims wherein the antigen receptor is a T cell receptor (TCR)~~.~~, optionally

~~10. A method according to claim 9~~ wherein

(i) the TCR is an affinity enhanced TCR~~.~~;

(ii)

~~11. A method according to claim 9 or claim 10 wherein~~ the TCR is a αβ TCR or γδ TCR~~.~~, and/or

(iii)

~~12. A method according to any one of claims 9 to 11 wherein~~ the TCR binds specifically to an MHC displaying a peptide fragment of a target antigen expressed by cells~~.~~;

optionally

~~13. A method according to claim 12~~ wherein the TCR binds specifically to an MHC displaying a peptide fragment of a tumour antigen expressed by the cancer cells, optionally wherein the tumour antigen is alpha-fetoprotein (AFP), NY-ESO1, MAGE-A10 or MAGE-A4.

~~14~~5. A method according to any one of claims 1 to ~~3~~8 wherein the antigen receptor is a chimeric antigen receptor (CAR)~~.~~, optionally

~~15. A method according to claim 14~~ wherein the CAR binds specifically to a target antigen expressed by cells~~.~~, and optionally

~~16. A method according to claim 15~~ wherein the ~~TCR~~ CAR binds specifically to a tumour antigen expressed by the cancer cells, optionally wherein the tumour antigen is alpha-fetoprotein (AFP), NY-ESO1, MAGE-A10 or MAGE-A4.

~~17~~6. A method according to any one of claims 1 to ~~3~~8 wherein the antigen receptor is an NK cell receptor (NKCR)~~.~~, optionally

~~18. A method according to claim 17~~ wherein the NKCR binds specifically to an MHC displaying a peptide fragment of a target antigen expressed by cells~~.~~, optionally

~~19. A method according to claim 18~~ wherein the NKCR receptor binds specifically to an MHC displaying a peptide fragment of a tumour antigen expressed by the cancer cells~~.~~, optionally

~~20. A method according to any one of claims 13, 16 or 19~~ wherein the tumour antigen is alpha-fetoprotein (AFP), NY-ESO1, MAGE-A10 or MAGE-A4.

~~21~~7. A method according to any one of the preceding claims wherein the RAG inactivated iPSCs are differentiated into mesoderm cells by culturing the population of iPSCs under suitable conditions to promote mesodermal differentiation.

228. A method according to any one of the preceding claims wherein the RAG inactivated iPSCs are cultured sequentially in first, second and third mesoderm induction media to induce differentiation into mesoderm cells.; optionally

23. A method according to claim 22 wherein:

(a) the first mesoderm induction medium

(i) stimulates SMAD2 and SMAD3 mediated signalling pathways.; and/or

(ii)

24. A method according to claim 23 wherein the first mesoderm induction medium comprises activin., and/or

(iii)

25. A method according to claim 23 or claim 24 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.

26. A method according to any one of claims 15 to 18 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)

27. A method according to claim 26 wherein the second mesoderm induction medium comprises activin, BMP, and FGF, and/or

(iv).

28. A method according to claim 26 or claim 27 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

29. A method according to any one of claims 22to 28 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 3β., and/or

(iv)

~~30. A method according to claim 29 wherein the third mesoderm induction medium~~ comprises activin, BMP, FGF, and a GSK3 inhibitor~~.~~, and/or

(v)

~~31. A method according to claim 30 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.

~~32~~9. A method according to any one of the preceding claims wherein the mesoderm cells display one or more of Brachyury+ Goosecoid+ Mixl1+ KDR+ FoxA2+ GATA6+ and PDGFαR+.

~~33~~10. A method according to any one of the preceding claims wherein the mesoderm cells are differentiated into HECs by culturing the population of mesoderm cells under suitable conditions to promote haemogenic endothelial (HE) differentiation.

~~34~~11. 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~~.~~,

~~35. A method according to claim 27~~ wherein

(a) the HE induction medium

(i) stimulates KIT (KIT proto-oncogene, receptor tyrosine kinase) mediated signalling pathways and

(ii) stimulates VEGFR mediated signalling pathways~~.~~, and/or

~~36. A method according to claim 35~~

~~wherein~~ (b) the HE induction medium comprises SCF and VEGF~~.~~, and/or

~~37. A method according to claim 36~~

~~wherein~~ (c) 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.

~~38~~12. A method according to any one of the preceding claims wherein the HPCs are differentiated into progenitor T cells under suitable conditions to promote lymphoid differentiation~~.~~, optionally

~~39. A method according to claim 38~~

(i) 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~~.~~, and/or

~~40. A method according to claim 38 or 39~~ ~~wherein~~ (ii) the progenitor T cells have a CD5+, CD7+ phenotype.

~~41~~13. A method according to any one of the preceding claims wherein the progenitor T cells are matured into T cells under suitable conditions to promote T cell maturation~~.~~, optionally

~~42. A method according to claim 41~~ 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 double positive T cells.

~~43~~14. A method according to any one of the preceding claims further comprising (i) isolating or purifying the double positive T cells and/or single positive T cells~~.~~, optionally

~~44. A method according to claim 43~~ wherein double positive T cells and/or single positive T cells are isolated by magnetic activated cell sorting~~.~~; and/or

~~45. A method according to according to any one of the preceding claims comprising~~(ii) concentrating the population of double positive T cells and/or single positive T cells~~.~~; and/or (iii)

~~46. A method according to according to any one of the preceding claims comprising~~ storing the population of double positive T cells and/or single positive T cells~~.~~; and/or (iv)

~~47. A method according to any one of the preceding claims comprising~~ formulating the population of double positive T cells and/or single positive T cells with a pharmaceutically acceptable excipient.

~~48~~15. A population of double positive T cells and/or single positive T cells produced by a method according to any one of claims 1 to ~~47~~14~~.~~, optionally

~~49. A population according to claim 48~~ wherein the T cells express a heterologous antigen receptor~~.~~, optionally

~~50. A population according to claim 49~~ wherein the heterologous antigen receptor is a TCR, CAR or NKCR~~.~~, and optionally

~~51, A population according to claim 49 or claim 50~~ wherein the T cells ~~express~~ are RAG inactivated and do not express an endogenous TCR.

~~52~~16. A pharmaceutical composition comprising a population of double positive T cells and/or single positive T cells produced by a method according to any one of claims 1 to ~~47~~ 14 and a pharmaceutically acceptable excipient.

~~53~~17. A population of double positive T cells and/or single positive T cells produced by a method according to any one of claims 1 to ~~47~~ 14 for use in ~~a method of treatment~~therapy.

~~54~~18. Use of a~~A~~ population of double positive T cells and/or single positive T cells produced by a method according to any one of claims 1 to ~~47~~ 14 in the manufacture of a medicament for ~~use in a method of treatment of~~ treating cancer.

~~55. A method of treatment of cancer comprising administering a population of double positive T cells and/or single positive T cells produced by a method according to any one of claims 1 to 47 to an individual in need thereof.~~