

CLAIMS

1. A humanized immunodeficient M-CSF mouse, comprising:
a nucleic acid sequence incorporated into the genome of the humanized M-CSF mouse, which sequence encodes a human M-CSF protein and is operably linked to the endogenous promoter of the mouse M-CSF gene at the mouse M-CSF locus.
2. The humanized immunodeficient M-CSF mouse of claim 1, wherein the mouse comprises two copies of the nucleic acid sequence.
3. The humanized immunodeficient M-CSF mouse according to claim 1 or 2, wherein the mouse comprises a null mutation in at least one mouse M-CSF allele.
4. The humanized immunodeficient M-CSF mouse according to claim 3, wherein the null mutation is a deletion of mouse M-CSF exons 2-9.
5. The humanized immunodeficient M-CSF mouse according to claim 1, wherein the mouse comprises human cells.
6. The humanized immunodeficient M-CSF mouse according to claim 5, wherein the human cells are hematopoietic cells.
7. The humanized immunodeficient M-CSF mouse according to claim 6, wherein the mouse is infected by a human pathogen.
8. The humanized immunodeficient M-CSF mouse according to claim 7, wherein the pathogen is selected from a virus, a fungus, and a bacterium.
9. The humanized immunodeficient M-CSF mouse according to claim 8, wherein the bacterium is a mycobacterium or an enterobacterium.
10. The humanized immunodeficient M-CSF mouse according to any one of claims 1-9, wherein the mouse is a NOD-*scid* $\gamma c^{-/-}$ mouse.

11. The humanized immunodeficient M-CSF mouse according to any one of claims 1-9, wherein the mouse is a NOD/Shi-*scid* $\gamma\text{C}^{-/-}$ mouse.

12. The humanized immunodeficient M-CSF mouse according to any one of claims 1-9, wherein the mouse is a Balb/c Rag2 $^{-/-}$ $\gamma\text{C}^{-/-}$ mouse.

13. The humanized immunodeficient M-CSF mouse according to any one of claims 1-9, wherein the mouse is a sublethally irradiated mouse.

14. A method of screening a candidate agent for toxicity to human hematopoietic cells, the method comprising:

(a) contacting a first humanized M-CSF mouse with the candidate agent, wherein the first humanized M-CSF mouse is engrafted with human hematopoietic cells; and

(b) comparing the viability of the human hematopoietic cells in the first humanized M-CSF mouse contacted with the agent to the viability and/or function of the human hematopoietic cells in a second humanized M-CSF mouse, engrafted with human hematopoietic cells and not contacted with the agent, wherein each of the first and second humanized M-CSF mice is immunodeficient and comprises a nucleic acid sequence incorporated into the genome of the humanized M-CSF mouse, which sequence encodes a human M-CSF protein and is operably linked to the endogenous promoter of the mouse M-CSF gene at the mouse M-CSF locus,

wherein a decrease in the viability of the hematopoietic cells in the first mouse contacted with the candidate agent indicates that the candidate agent is toxic to the hematopoietic cells.

15. A method of screening a candidate agent for the ability to protect human hematopoietic cells from a toxic agent, mitigate the effects of a toxic agent on human hematopoietic cells, or reverse the effects of a toxic agent on human hematopoietic cells, the method comprising:

(a) contacting a first humanized M-CSF mouse engrafted with human hematopoietic cells with a toxic agent;

(b) contacting a second humanized M-CSF mouse engrafted with human hematopoietic cells with a toxic agent;

(c) contacting the first humanized M-CSF mouse with a candidate agent; and

(d) comparing the viability and/or function of the human hematopoietic cells in the first humanized M-CSF mouse contacted with the candidate agent to the viability and/or function of human hematopoietic cells in the second humanized M-CSF mouse not contacted with the candidate agent, wherein each of the first and second humanized M-CSF mice is immunodeficient and comprises

a nucleic acid sequence incorporated into the genome of the humanized M-CSF mouse, which sequence encodes a human M-CSF protein and is operably linked to the endogenous promoter of the mouse M-CSF gene at the mouse M-CSF locus, and

wherein an enhancement in the viability and/or function of the hematopoietic cells in the first mouse contacted with the candidate agent indicates that the candidate agent protects hematopoietic cells from the toxic agent, mitigates the effects of the toxic agent on human hematopoietic cells, or reverses the effects of the toxic agent on human hematopoietic cells.

16. The method of claim 14 or 15, wherein the first and second humanized M-CSF mice each comprises a Rag2 gene knock-out and an IL2rg gene knock-out.

17. The method of claim 14 or 15, wherein each of the first mouse and the second mouse is a NOD-*scid* γ c^{-/-} mouse.

18. The method of claim 14 or 15, wherein each of the first mouse and the second mouse is a NOD/Shi-*scid* γ c^{-/-} mouse.

19. The method of claim 14 or 15, wherein each of the first mouse and the second mouse is a Balb/c Rag2^{-/-} γ c^{-/-} mouse.

20. The method of claim 14 or 15, wherein each of the first mouse and the second mouse is a sublethally irradiated mouse.

21. The method of any one of claims 14-20, wherein the first and second humanized M-CSF mice each comprise two copies of the nucleic acid sequence.

22. The method of any one of claims 14-21, wherein the first and second humanized M-CSF mice each comprise the same null mutation in at least one mouse M-CSF allele.

23. The method of claim 22, wherein the null mutation is a deletion of mouse M-CSF exons 2-9.

24. The method of any one of claims 14-23, wherein the contacting of the first mouse with the toxic agent occurs after the contacting of the first mouse with the candidate agent.

25. The method of any one of claims 14-23, wherein the contacting of the first mouse with the toxic agent occurs before the contacting of the first mouse with the candidate agent.

26. The method of any one of claims 14-23, wherein the contacting of the first mouse with the toxic agent occurs simultaneously with the contacting of the first mouse with the candidate agent.

27. A method for making a human immune cell from a humanized M-CSF mouse, the methods comprising the steps of:

exposing the humanized M-CSF mouse to an immune stimulant under conditions sufficient for the human immune cell in the mouse to become biologically activated; and
isolating the biologically activated human immune cell from the mouse,

wherein the mouse comprises a nucleic acid sequence incorporated into the genome of the mouse, which sequence encodes a human M-CSF protein and is operably linked to the endogenous promoter of the mouse M-CSF gene at the mouse M-CSF locus, wherein the mouse comprises a Rag2 gene knock-out and an IL2rg gene knock-out, or is selected from the group consisting of a NOD-*scid* $\gamma\text{C}^{-/-}$ mouse, a NOD/Shi-*scid* $\gamma\text{C}^{-/-}$ mouse, and a Balb/c Rag2^{-/-} $\gamma\text{C}^{-/-}$ mouse,

wherein the mouse comprises human hematopoietic cells,
wherein the mouse expresses M-CSF RNA encoded by the nucleic acid sequence in bone marrow, spleen, blood, liver, brain, lung, testis and kidney, and
wherein the human immune cell is (i) a human monocyte, (ii) a human macrophage, or (iii) a human monocyte and a human macrophage.

28. The method of claim 27, wherein the immune stimulant is an endotoxin.

29. The method of claim 28, wherein the endotoxin is LPS.

30. A method of making biologically active human M-CSF protein from a humanized M-CSF mouse, the method comprising:

- (i) purifying biologically active human M-CSF protein from blood of the mouse; or
- (ii) isolating a cell that expresses biologically active human M-CSF protein from the mouse; culturing the cell under conditions sufficient for the cell to express and secrete biologically active human M-CSF protein; and isolating biologically active human M-CSF protein secreted from the cell,

wherein the mouse comprises a nucleic acid sequence incorporated into the genome of the mouse, which sequence encodes a human M-CSF protein and is operably linked to the endogenous promoter of the mouse M-CSF gene at the mouse M-CSF locus,

wherein the mouse comprises a Rag2 gene knock-out and an IL2rg gene knock-out or is selected from the group consisting of a NOD-*scid* γ c^{-/-} mouse, a NOD/Shi-*scid* γ c^{-/-} mouse, and a Balb/c Rag2^{-/-} γ c^{-/-} mouse, and

wherein the mouse expresses M-CSF RNA encoded by the nucleic acid sequence in bone marrow, spleen, blood, liver, brain, lung, testis and kidney.

31. A method of isolating a nucleotide sequence encoding a human immunoglobulin sequence from a humanized M-CSF mouse, the method comprising the steps of:

- (i) exposing the mouse to an immune stimulant;
- (ii) isolating a biologically active human immune cell from the mouse; and
- (iii) isolating a nucleotide sequence encoding a human immunoglobulin sequence from the human immune cell,

wherein the mouse comprises a nucleic acid sequence incorporated into the genome of the mouse, which sequence encodes a human M-CSF protein and is operably linked to the endogenous promoter of the mouse M-CSF gene at the mouse M-CSF locus,

wherein the mouse comprises a Rag2 gene knock-out and an IL2rg gene knock-out or is selected from the group consisting of a NOD-*scid* γ c^{-/-} mouse, a NOD/Shi-*scid* γ c^{-/-} mouse, and a Balb/c Rag2^{-/-} γ c^{-/-} mouse,

wherein the mouse comprises human hematopoietic cells,

wherein the mouse expresses M-CSF RNA encoded by the nucleic acid sequence in bone marrow, spleen, blood, liver, brain, lung, testis and kidney, and

wherein the human immune cell is a human B cell.

32. A mouse embryonic stem (ES) cell, comprising:
a nucleic acid sequence incorporated into the genome of the mouse ES cell, which sequence encodes a biologically active human Macrophage colony-stimulating factor (hM-CSF) protein and is operably linked to the endogenous promoter of the mouse M-CSF gene at the mouse M-CSF locus.

33. The mouse ES cell of claim 32, wherein the mouse ES cell comprises two copies of the nucleic acid sequence.

34. The mouse ES cell of claim 32 or 33, wherein the mouse ES cell comprises a null mutation in at least one mouse M-CSF allele.

35. The mouse ES cell of any one of claims 32 to 34, wherein the null mutation is a deletion of mouse M-CSF exons 2-9.

36. The mouse ES cell of any one of claims 32 to 35, wherein the mouse ES cell is homozygous null for Rag2.

37. The mouse ES cell of claim 36, wherein the mouse ES cell is homozygous null for IL2rg.

38. A mouse embryo comprising the mouse ES cell of any one of claims 32 to 37.

39. A targeting construct for targeting a mouse M-CSF gene, comprising:
(a) upstream and downstream targeting arms that are complementary or substantially complementary to upstream and downstream nucleotide sequences of either (i) a nucleotide sequence encoding a mouse M-CSF protein, or, (ii) a nucleotide sequence complementary to a nucleotide sequence encoding a mouse M-CSF protein;
(b) a nucleotide sequence encoding a biologically active human M-CSF protein, or a complement of a nucleotide sequence encoding a biologically active human M-CSF protein; and

(c) a marker and/or a selection cassette.