

CLAIMS

1. A method of making a humanized M-CSF mouse, the method comprising:
 - contacting a mouse pluripotent stem cell with a nucleic acid sequence comprising a coding sequence for a human M-CSF protein;
 - culturing the pluripotent stem cell under conditions that promote the integration of the coding sequence into the genome of the pluripotent stem cell such that the coding sequence is operably linked to the endogenous promoter of the mouse M-CSF gene at the mouse M-CSF locus;
 - making a humanized M-CSF mouse from the mouse pluripotent stem cell, wherein the humanized M-CSF mouse comprises the coding sequence for the human M-CSF protein; and
 - maintaining the humanized M-CSF mouse under conditions sufficient for the humanized M-CSF mouse to express M-CSF RNA encoded by the coding sequence in bone marrow, spleen, blood, liver, brain, lung, testis and kidney, wherein the humanized M-CSF mouse comprises a Rag2 gene knock-out and an IL2rg gene knock-out.
2. The method of claim 1, wherein the humanized M-CSF mouse comprises two copies of the coding sequence.
3. The method of any one of claims 1-2, wherein the humanized M-CSF mouse comprises a null mutation in at least one mouse M-CSF allele.

4. The method of claim 3, wherein the null mutation is a deletion of mouse M-CSF exons 2-9.
5. The method of any one of claims 1-4, further comprising transplanting human cells into the humanized M-CSF mouse.
6. The method of claim 5, wherein the human cells are hematopoietic cells.
7. The method of claim 6, further comprising infecting the humanized M-CSF mouse with a human pathogen.
8. The method of claim 7, wherein the human pathogen is selected from a virus, a fungus, and a bacterium.
9. The method according to claim 8, wherein the bacterium is a mycobacterium or an enterobacterium.
10. A method of screening an agent for activity in modulating human hematopoietic cell function in a humanized M-CSF mouse, the method comprising:
 - (a) providing or receiving a first humanized M-CSF mouse and a second humanized M-CSF mouse, each of the first and second humanized M-CSF mice 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; and

human hematopoietic cells, wherein each of the first and second humanized M-CSF mice comprises a Rag2 gene knock-out and an IL2rg gene knock-out, and wherein each of the first and second humanized M-CSF mice expresses M-CSF RNA encoded by the nucleic acid sequence in bone marrow, spleen, blood, liver, brain, lung, testis and kidney;

(b) contacting the first humanized M-CSF mouse with the agent; and

(c) comparing the first humanized M-CSF mouse contacted with the agent to the second humanized M-CSF mouse in the absence of the agent to determine the *in-vivo* effect of the agent.

11. The method of claim 10, wherein the comparing comprises comparing the function of the human hematopoietic cells in the first humanized M-CSF mouse contacted with the agent to the function of the human hematopoietic cells in the second humanized M-CSF mouse in the absence of the agent, wherein a modulation in the function of the human hematopoietic cells in the first humanized M-CSF mouse relative to the second humanized M-CSF mouse indicates that the agent modulates human hematopoietic cell function.
12. The method of claim 11, wherein the first humanized M-CSF mouse and the second humanized M-CSF mouse are the same mouse, wherein the absence of the agent occurs prior to the contacting, and wherein a modulation in the function of the human hematopoietic cells after the contacting relative to the

function of the human hematopoietic cells prior to the contacting indicates that the agent modulates human hematopoietic cell function.

13. The method of claim 11, wherein the first humanized M-CSF mouse and the second humanized M-CSF mouse are different mice.
14. The method of any one of claims 10-13, comprising exposing the first and second humanized M-CSF mice to an amount of a human pathogen sufficient to produce an infection in the first and second humanized M-CSF mice, and measuring a parameter of the infection in the first humanized M-CSF mouse over time and a parameter of the infection in the second humanized M-CSF mouse over time, wherein the comparing comprises comparing the measured parameters to determine the *in-vivo* effect of the agent on the human pathogen.
15. The method of claim 14, wherein the first humanized M-CSF mouse is contacted with the agent prior to exposing the first and second humanized M-CSF mice to the human pathogen.
16. The method of claim 14, wherein the first humanized M-CSF mouse is contacted with the agent concurrently with the exposure to the human pathogen.
17. The method of claim 14, wherein the first humanized M-CSF mouse is contacted with the agent subsequent to exposing the first and second humanized M-CSF mice to the human pathogen.

18. The method of any one of claims 10-17, wherein the first and second humanized M-CSF mice each comprise two copies of the coding sequence.
19. The method of any one of claims 10-17, wherein the first and second humanized M-CSF mice each comprise the same null mutation in at least one mouse M-CSF allele.
20. The method of claim 19, wherein the null mutation is a deletion of mouse M-CSF exons 2-9.
21. A humanized M-CSF mouse, comprising:
 - a Rag2 gene knock-out;
 - an IL2rg gene knock-out; and
 - 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 the mouse expresses M-CSF RNA encoded by the nucleic acid sequence in bone marrow, spleen, blood, liver, brain, lung, testis and kidney.
22. The humanized M-CSF mouse according to claim 21, wherein the mouse comprises two copies of the nucleic acid sequence.
23. The humanized M-CSF mouse according to claim 21, wherein the mouse comprises a null mutation in at least one mouse M-CSF allele.

24. The humanized M-CSF mouse according to claim 23, wherein the null mutation is a deletion of mouse M-CSF exons 2-9.
25. The humanized M-CSF mouse according to claim 21, wherein the mouse comprises human cells.
26. A mouse model of the human hematopoietic system, comprising:
 - a mouse which comprises:
 - a Rag2 gene knock-out;
 - an IL2rg gene knock-out;
 - 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; and
 - 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.
27. The mouse model according to claim 26, wherein the mouse comprises a null deletion for at least one mouse M-CSF allele.
28. The mouse model according to claim 27, wherein the null deletion is a deletion of mouse M-CSF exons 2-9.

29. The mouse model according to claim 27, wherein the mouse exhibits one or more characteristics selected from:
- a. exhibits a frequency of $\text{hCD14}^+\text{CD33}^+$ cells of spleen that is 2- to 6-fold higher than $\text{hCD14}^+\text{CD33}^+$ in an engrafted mouse that does not express hM-CSF;
 - b. exhibits a frequency in $\text{hCD14}^+\text{CD33}^+$ cells of peripheral blood that is 2- to 8-fold higher than $\text{hCD14}^+\text{CD33}^+$ in an engrafted mouse that does not express hM-CSF;
 - c. exhibits a level of $\text{hCD14}^+\text{CD33}^+$ monocyte/macrophage lineage cells in blood of about 15 to about 40%;
 - d. exhibits a level of $\text{hCD14}^+\text{CD33}^+$ monocyte/macrophage lineage cells in blood of about 5 to about 15% at about 20 weeks of age;
 - e. exhibits a response to LPS injection that is about 1.5- to about 6-fold greater with respect to percentage of $\text{hCD14}^+\text{CD33}^+$ cells in liver than mice that lack a human M-CSF;
 - f. exhibits an enhanced production of $\text{hCD14}^+\text{CD33}^+\text{hCD45}^+$ cells in spleen about 48 hours following LPS injection, wherein the enhancement is about 2- to about 5-fold over an engrafted mouse that lacks hM-CSF;
 - g. exhibits an enhanced production of serum human IL-6 in response to LPS, wherein the level of hIL-6 about 6 hours after LPS injection is enhanced about 2- to about 5-fold over an engrafted mouse that lacks a hM-CSF;
 - h. exhibits in vitro secretion by a monocyte and/or macrophage upon LPS stimulation that is about 2- to 3-fold higher with respect to $\text{hTNF}\alpha$ than an engrafted mouse that lacks a hM-CSF gene;

- i. exhibits in vitro secretion by a monocyte and/or macrophage upon LPS stimulation that is about 2- to 4-fold higher with respect to hIL-6 than an engrafted mouse that lacks a hM-CSF gene;
 - j. exhibits in vitro secretion by a monocyte and/or macrophage upon poly I:C stimulation that is about 3- to 6-fold higher with respect to hIFN α than an engrafted mouse that lacks a hM-CSF gene;
 - k. exhibits in vitro secretion by a monocyte and/or macrophage upon poly I:C stimulation that is about 2- to 3-fold higher with respect to hIFN β than an engrafted mouse that lacks a hM-CSF gene;
 - l. exhibits enhanced phagocytosis as compared with a genetically modified and engrafted mouse that lacks a hM-CSF gene;
 - m. exhibits enhanced chemotaxis in vitro in response to Mip3 β as compared with a genetically modified engrafted mouse that lacks a hM-CSF gene; and
 - n. exhibits upregulation in vitro of a co-stimulatory molecule in response to LPS stimulation, wherein the co-stimulatory molecule is selected from human CD40, human CD80, human CD86, human HLA-DR, and a combination thereof.
30. The mouse model according to claim 28, wherein the mouse exhibits two or more of said characteristics.
31. The mouse model according to claim 28, wherein the mouse exhibits three or more of said characteristics.
32. A mouse model of a human pathogen infection, comprising:

a mouse, wherein the mouse comprises

- a Rag2 gene knock-out;
- an IL2rg gene knock-out;
- 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;
- human hematopoietic cells; and
- an infection by a human pathogen, wherein the mouse expresses M-CSF RNA encoded by the nucleic acid sequence in bone marrow, spleen, blood, liver, brain, lung, testis and kidney.

33. The mouse model according to claim 32, wherein the pathogen is selected from a virus, a fungus, and a bacterium.

34. The mouse model according to claim 33, wherein the bacterium is a mycobacterium or an enterobacterium.