(19) **DANMARK**

Patent- og
Varemærkestyrelsen

(10) **DK/EP 3841877 T5**

(12) Rettet oversættelse af
europæisk patentskrift

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(51) Int.Cl.:
A 01 K 67/027 (2006.01) *A 61 K 49/00 (2006.01)* *C 07 K 14/47 (2006.01)*
C 07 K 14/505 (2006.01) *C 07 K 14/52 (2006.01)* *C 07 K 14/535 (2006.01)*
C 07 K 14/54 (2006.01) *C 07 K 14/715 (2006.01)* *C 12 N 9/00 (2006.01)*
C 12 N 9/14 (2006.01) *C 12 Q 1/18 (2006.01)* *G 01 N 33/50 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2024-08-19**

(80) Dato for Den Europæiske Patentmyndigheds
bekendtgørelse om meddelelse af patentet: **2023-09-06**

(86) Europæisk ansøgning nr.: **20209073.4**

(86) Europæisk indleveringsdag: **2015-05-18**

(87) Den europæiske ansøgnings publiceringsdag: **2021-06-30**

(30) Prioritet: **2014-05-19 US 201462000460 P**

(62) Stamansøgningsnr: **18214077.2**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

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(54) Benævnelse: **Genetisk modificeret mus, der eksprimerer human EPO**

(56) Fremdragne publikationer:
WO-A1-2012/051572
WO-A2-2014/071397
ASHLEY M VAUGHAN ET AL: "Development of humanized mouse models to study human malaria parasite infection", FUTURE MICROBIOLOGY, vol. 7, no. 5, May 2012 (2012-05), pages 657-665, XP055234288, GB ISSN: 1746-0913, DOI: 10.2217/fmb.12.27
G. L. SEMENZA ET AL: "Polycythemia in transgenic mice expressing the human erythropoietin gene.", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 86, no. 7, April 1989 (1989-04), pages 2301-2305, XP055233828, US ISSN: 0027-8424, DOI: 10.1073/pnas.86.7.2301
GREGG L SEMENZA ET AL: "Cell-type-specific and hypoxia-inducible expression of the human erythropoietin gene in transgenic mice", GENETICS, vol. 88, October 1991 (1991-10), pages 8725-8729, XP055148842,

Fortsættes ...

LEONARD D. SHULTZ ET AL: "Humanized mice for immune system investigation: progress, promise and challenges", NATURE REVIEWS IMMUNOLOGY, vol. 12, no. 11, November 2012 (2012-11), pages 786-798, XP055064740, ISSN: 1474-1733, DOI: 10.1038/nri3311

Z. HU ET AL: "Macrophages prevent human red blood cell reconstitution in immunodeficient mice", BLOOD, vol. 118, no. 22, 24 November 2011 (2011-11-24), pages 5938-5946, XP055233818, US ISSN: 0006-4971, DOI: 10.1182/blood-2010-11-321414

ASHLEY M VAUGHAN ET AL: "Development of humanized mouse models to study human malaria parasite infection", FUTURE MICROBIOLOGY, vol. 7, no. 5, May 2012 (2012-05-01), GB, pages 657 - 665, XP055234288, ISSN: 1746-0913, DOI: 10.2217/fmb.12.27

G. L. SEMENZA ET AL: "Polycythemia in transgenic mice expressing the human erythropoietin gene.", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 86, no. 7, April 1989 (1989-04-01), US, pages 2301 - 2305, XP055233828, ISSN: 0027-8424, DOI: 10.1073/pnas.86.7.2301

GREGG L SEMENZA ET AL: "Cell-type-specific and hypoxia-inducible expression of the human erythropoietin gene in transgenic mice", GENETICS, vol. 88, October 1991 (1991-10-01), pages 8725 - 8729, XP055148842

LEONARD D. SHULTZ ET AL: "Humanized mice for immune system investigation: progress, promise and challenges", NATURE REVIEWS IMMUNOLOGY, vol. 12, no. 11, November 2012 (2012-11-01), pages 786 - 798, XP055064740, ISSN: 1474-1733, DOI: 10.1038/nri3311

Z. HU ET AL: "Macrophages prevent human red blood cell reconstitution in immunodeficient mice", BLOOD, vol. 118, no. 22, 24 November 2011 (2011-11-24), US, pages 5938 - 5946, XP055233818, ISSN: 0006-4971, DOI: 10.1182/blood-2010-11-321414

DESCRIPTION

CROSS-REFERENCE

FIELD OF THE INVENTION

[0001] This invention pertains to the field of genetically modified mice.

INTRODUCTION

[0002] Genetically modified mice, modified and engrafted mice, and their use in modeling human diseases are known in the art. However, to date, there has been little success in generating genetically modified mice that model human infection by pathogens that target cells of the human erythroid lineage. Such pathogens, *e.g.,* protozoans of the genera *Plasmodium, Babesia, and Theileria,* may cause life-threatening diseases in humans.

[0003] For example, protozoans of the genus *Plasmodium* cause malaria. During 2010, a total of 106 countries worldwide were considered as endemic of malaria with an estimated 3.3 billion people at risk for developing the disease. The disease burden in 2010 was estimated at 216 million cases with 655,000 estimated deaths worldwide, in which, 86% were in children under 5 years of age. Currently, drugs and vaccines for the prevention and treatment of malaria are still very limited. In addition, parasite resistance to commonly used malaria therapeutics has emerged and presents a constant challenge. The development of new drugs and vaccines for the control and treatment of pathogens that target human erythrocytes are therefore urgently needed.

[0004] Since many of these pathogens do not infect the red blood cells of laboratory rodents, in vivo studies have traditionally been limited to studies of malaria caused by the rodent parasite *Plasmodium berghei* ANKA, or to studies of NOD/SCID, NOD/SCID/IL2rg^{null} (NSG), or BXN mice acutely engrafted by daily injection of large numbers of human erythrocytes and coincidentally or subsequently infected by injection of parasitized red blood cells (Angulo-Barturen et al. (2008) A murine model of falciparum-malaria by in vivo selection of competent strains in non-myelodepleted mice engrafted with human erythrocytes. PLoS One 3:e2252; Jimenez-Diaz et al. (2009) Improved murine model of malaria using Plasmodium falciparum competent strains and non-myelodepleted NOD-scid IL2Rgnull mice engrafted with human erythrocytes. Antimicrob Agents Chemother 53:4533-4536; Badell et al. (2000) Human malaria in immunocompromised mice: an in vivo model to study defense mechanisms against Plasmodium falciparum. JEM 192(11):1653-1660; Moreno et al. (2006) The course of infections and pathology in immunomodulated NOD/LtSz-SCID mice inoculated with

Plasmodium falciparum laboratory lines and clinical isolates. Int. J. Parasitol. 36:361-369). In order to study the effects of *Plasmodium* and other pathogens on humans, and to test vaccines and drugs for efficacy in preventing infection and treating humans infected with these and other pathogens, it would be useful to have a non-human animal such as a mouse that is genetically modified so that it is susceptible to infection with such a pathogen, or so that it will better support human erythrocytes that are acutely grafted into the animal prior to infection as done in traditional rodent models.

SUMMARY

[0005] Genetically modified mice expressing human EPO from the mouse genome are provided. Also disclosed are methods for making mice expressing human EPO from the mouse genome, and methods for using mice expressing human EPO from the mouse genome. These animals and methods find many uses in the art, including, for example, in modeling human erythropoiesis and erythrocyte function; in modeling human pathogen infection of erythrocytes; in *in vivo* screens for agents that modulate erythropoiesis and/or erythrocyte function, e.g. in a healthy or a diseased state; in *in vivo* screens for agents that are toxic to erythrocytes or erythrocyte progenitors; in *in vivo* screens for agents that prevent against, mitigate, or reverse the toxic effects of toxic agents on erythrocytes or erythrocyte progenitors; in *in vivo* screens of erythrocytes or erythrocyte progenitors from an individual to predict the responsiveness of an individual to a disease therapy.

[0006] Genetically modified mice are provided that express human EPO from the mouse's genome. In other words, the mouse comprises a nucleic acid sequence encoding a human EPO protein.

[0007] The nucleic acid sequence encoding a human EPO protein is operably linked to an *EPO* gene promoter. The EPO promoter is the endogenous, i.e. mouse, *EPO* promoter and is at the mouse *EPO* gene locus. In other words the nucleic acid sequence that encodes the human EPO protein is operably linked to the mouse EPO promoter at the mouse *EPO* locus. The operable linkage results in a null mutation in the mouse *EPO* gene at the mouse EPO gene locus.

[0008] In some embodiments, the subject mouse is heterozygous for the allele comprising the nucleic acid sequence that encodes the human EPO protein. In other embodiments, the mouse is homozygous for the allele comprising the nucleic acid sequence that encodes the human EPO protein.

[0009] In some embodiments, the nucleic acid sequence that encodes the human EPO protein comprises human *EPO* genomic coding and non-coding sequence. In other embodiments, the nucleic acid sequence that encodes the human EPO protein comprises human *EPO* cDNA sequence. according to claim 1

[0010] The subject mouse according to claim 1 expresses one or more additional human proteins selected from the group consisting of: a M-CSF protein encoded by a nucleic acid under the control of an *M-csf* promoter, a IL-3 protein encoded by a nucleic acid under control of an *Il-3* promoter, a GM-CSF protein encoded by a nucleic acid under the control of a *Gm-csf* promoter, a TPO protein encoded by a nucleic acid under the control of a *TPO* promoter, and a Sirpa protein encoded by a nucleic acid under the control of a *Sirpa* promoter. In some such embodiments, the promoter is an endogenous mouse promoter at the corresponding mouse gene locus, and the mouse is heterozygous null for the mouse gene. In other embodiments, the promoter is an endogenous mouse promoter at the corresponding mouse gene locus, and the mouse is homozygous null for the mouse gene. The mouse according to claim 1 expresses human, e.g., humanized, TPO; human, e.g., humanized, IL-3; human, e.g., humanized, GM-CSF; human, e.g., humanized, EPO; and human, e.g., humanized, Sirpa.

[0011] In some embodiments, the subject genetically modified mouse has a genome that comprises a nucleic acid sequence encoding a human, e.g., humanized protein, e.g. a nucleic acid encoding a human, e.g., humanized, EPO; a human, e.g., humanized, Sirpa; a human, e.g., humanized, IL-3; a human, e.g., humanized, GM-CSF, a human, e.g., humanized, M-CSF, a human, e.g., humanized, TPO, a human, e.g., humanized, IL-6; etc. operably linked to its corresponding mouse promoter, e.g. a *Sirpa, IL-3*, *GM-CSF, M-CSF, TPO,* or *IL-6* promoter, respectively, wherein the animal expresses the encoded human protein(s) and the native mouse protein(s). In other embodiments, the subject genetically modified mouse has a genome that comprises a nucleic acid sequence encoding a human, e.g., humanized, protein, e.g. a nucleic acid encoding human, e.g., humanized, EPO; human, e.g., humanized, Sirpa; human, e.g., humanized, IL-3; human, e.g., humanized GM-CSF; human, e.g., humanized M-CSF; human, e.g., humanized, TPO, operably linked to its corresponding non-human animal promoter, e.g. a *Sirpa*, *IL-3*, *GM-CSF, M-CSF*, or *TPO* promoter, respectively, wherein the mouse expresses the encoded human protein(s) and does not express the native mouse protein(s). Thus, in some embodiments, the genetically modified mouse is heterozygous for some or all the human, e.g., humanized, genes disclosed herein. In some embodiments, the genetically modified mouse is homozygous for some or all of the human, e.g., humanized genes disclosed herein.

[0012] The subject mouse is immunodeficient for an endogenous immune system. In some such embodiments, the immunodeficiency is caused by a deficiency for one or both of Rag2 and IL2rg.

[0013] In some embodiments, the subject genetically modified immunodeficient mouse further comprises an engraftment of human hematopoietic cells. In some such embodiments, the human hematopoietic cells comprise one or more cells selected from the group consisting of a human CD34-positive cell, a human hematopoietic stem cell, a human myeloid precursor cell, a human erythroid precursor cell, a human myeloid cell, a human dendritic cell, a human monocyte, a human granulocyte, a human erythrocyte, a human neutrophil, a human mast cell, a human thymocyte, and a human B lymphocyte.

[0014] As demonstrated in the working examples herein, human hematopoietic cell-engrafted genetically modified immunodeficient mice comprising a nucleic acid sequence encoding a human EPO protein operably linked to an *EPO* promoter at the endogenous locus demonstrate high levels of human erythropoiesis in the bone marrow and a 2 to 5-fold increase in human cells of the erythroid lineage in bone marrow as compared with control mice not expressing human EPO. In some embodiments, human hematopoietic cell-engrafted genetically modified immunodeficient mice comprising a nucleic acid sequence encoding a human EPO protein operably linked to an *EPO* promoter at the endogenous locus demonstrate high levels of human erythropoiesis in the bone marrow and about a 2 to about a 10-fold increase in human cells of the erythroid lineage in bone marrow as compared with control mice not expressing human EPO, e.g., about a 2 fold, about a 3 fold, about a 4 fold, about a 5 fold, about a 6 fold, about a 7 fold, about an 8 fold, about a 9 fold, or about a 10 fold increase in human cells of the erythroid lineage in bone marrow as compared with control mice not expressing human EPO. As such, in some embodiments, the subject engrafted, genetically modified immunodeficient animal comprises bone marrow in which 20% or more of the erythroid cells (CD235+) are human erythroid cells. In some embodiments, the subject engrafted, genetically modified immunodeficient mouse comprises bone marrow in which about 10% or more, e.g., about 20% or more, about 30% or more, about 40% or more, or about 50% or more of the erythroid cells (CD235+) are human erythroid cells.

[0015] The subject genetically modified mouse may be an immunodeficient mouse comprising a nucleic acid encoding a human EPO protein operably linked to the mouse *EPO* promoter at the mouse *EPO* locus, a nucleic acid encoding a human TPO protein operably linked to the mouse *TPO* promoter at the mouse *TPO* locus, a nucleic acid encoding a human Il-3 protein operably linked to the mouse *Il-3* promoter at the mouse *Il-3* locus, a nucleic acid encoding a human GM-CSF protein operably linked to a *GM-CSF* promoter at the mouse *GM-CSF* locus, and a nucleic acid encoding a human M-CSF protein operably linked to the mouse *M-CSF* promoter at the mouse *M-CSF* locus, for example, a Rag2$^{-/-}$ IL2rg$^{y/-}$ Tpo$^{h/h}$ Mcsf$^{h/h}$ Il3$^{h/h}$ Gmcsf$^{h/h}$ Epo$^{h/h}$ ("MITER-G") mouse.

[0016] In some such embodiments, the subject genetically modified animal is an immunodeficient mouse comprising a nucleic acid encoding a human EPO protein operably linked to the mouse *EPO* promoter at the mouse *EPO* locus, a nucleic acid encoding a human TPO protein operably linked to the mouse animal *TPO* promoter at the mouse *TPO* locus, a nucleic acid encoding a human Il-3 protein operably linked to the mouse *Il-3* promoter at the mouse *Il-3* locus, a nucleic acid encoding a human GM-CSF protein operably linked to a *GM-CSF* promoter at the mouse *GM-CSF* locus, and a nucleic acid encoding a human M-CSF protein operably linked to the mouse *M-CSF* promoter at the mouse *M-CSF* locus, and a nucleic acid encoding a human SIRPa protein operably linked to the mouse *SIRPa* promoter randomly integrated into the mouse genome, for example, a Rag$^{2-/-}$IL2rg$^{y/-}$ Tpo$^{h/h}$ Mcsf$^{h/h}$ Il3$^{h/h}$ Gmcsf$^{h/h}$ Epo$^{h/h}$ hSIRPα^{+} ("MISTER-G") mouse. In other such embodiments, the nucleic acid encoding a human, e.g., humanized, SIRPa protein is operably linked to the mouse *SIRPa* promoter at the mouse locus, for example, a Rag2$^{-/-}$ IL2rg$^{y/-}$ Tpo$^{h/h}$ Mcsf$^{h/h}$ Il3$^{h/h}$ Gmcsf$^{h/h}$

Epo$^{h/h}$ SIRPα$^{h/h}$ ("SupER-G") mouse.

[0017] The subject genetically modified animal is an immunodeficient mouse comprising one allele of a nucleic acid encoding a human EPO protein operably linked to the mouse *EPO* promoter at the mouse *EPO* locus (i.e., the mouse is heterozygous for human EPO), a nucleic acid encoding a human, e.g., humanized, SIRPa protein operably linked to the mouse *SIRPa* promoter at the mouse *SIRPa* locus, a nucleic acid encoding a human TPO protein operably linked to the mouse *TPO* promoter at the mouse *TPO* locus, a nucleic acid encoding a human Il-3 protein operably linked to the mouse *Il-3* promoter at the mouse *Il-3* locus, and a nucleic acid encoding a human GM-CSF protein operably linked to a *GM-CSF* promoter at the mouse *GM-CSF* locus, for example, a Rag2$^{-/-}$IL2rg$^{y/-}$ Tpo$^{h/h}$ Il3$^{h/h}$ Gm-csf$^{h/h}$ Epo$^{h/m}$ SIRPα$^{h/h}$ ("TIES") mouse.

[0018] In some embodiments, human hematopoietic cell-engrafted genetically modified immunodeficient mouse comprising a nucleic acid sequence encoding a human EPO protein operably linked to an *EPO* promoter at the endogenous locus may demonstrate better survival and engraftment with human erythrocytes when they comprise only one copy of the nucleic acid sequence encoding the EPO protein, and when they comprise endogenous M-csf. This is because the high level of human myeloid cell engraftment supported by human M-CSF in the knock-in results in the destruction of mouse red blood cells, which in turn leads to anemia and death of engrafted mice. In addition, heterozygosity for the human EPO allele improves fertility, developmental competency, and viability over mice homozygous for human EPO and null for mouse EPO. As such, in some embodiments, the subject engrafted, genetically modified immunocompromised animal demonstrates improved viability over mice that constitutively express EPO, e.g. transgenic mice, or mice that comprise two copies of human EPO, e.g. EPO$^{h/h}$. In some such embodiments, the genetically modified immunodeficient mouse is the TIES mouse (Rag2$^{-/-}$ IL2rg$^{y/-}$ Tpo$^{h/h}$ Il3$^{h/h}$ Gm-csf$^{h/h}$ Epo$^{h/m}$ SIRPα$^{h/h}$)

[0019] In some embodiments, human hematopoietic cell-engrafted genetically modified immunodeficient non-human animals injected with clodronate liposomes show a 1000-fold increase in the number of human erythroid cells (CD235+) in the peripheral blood as compared to uninjected animals. In some embodiments, human hematopoietic cell-engrafted genetically modified immunodeficient non-human animals injected with clodronate liposomes show about a 10-fold or greater, about a 50 fold or greater, about a 100-fold or greater, about a 500 fold or greater, or about a 1000-fold or greater increase in the number of human erythroid cells (CD235+) in the peripheral blood as compared to uninjected animals. Of these human erythroid cells, 10% or more, 20% or more, 30% or more, 40% or more, or 50% or more may be reticulocytes (erythrocyte precursors, CD71+). As such, in some embodiments, subject engrafted, genetically modified immunodeficient animal comprises peripheral blood in which 1% or more, e.g., 5% or more or 10% or more, of the erythroid cells (CD235+) are human erythroid cells, and 10% or more, e.g., 20% or more, 30% or more, 40% or more, or 50% or more, of those human erythroid cells are human reticulocytes (CD71+). The subject engrafted genetically modified immunodeficient mouse may be the MISTER-G mouse, SupER-G mouse, or TIES mouse.

[0020] In some embodiments, the mouse further comprises an infection with a pathogen that targets human cells of the erythroid lineage. In some such embodiments, the pathogen is selected from a *Plasmodium* sp., *Babesia* sp., and a *Theileri* sp. In some embodiments, the infection is produced by injecting parasite into the mouse. In some embodiments, the infection is produced by injecting parasitized human erythroid cells into the mouse. In some embodiments, the infection is produced by injecting parasitized human erythroid cells and healthy human erythroid cells into the mouse.

[0021] Methods are disclosed for identifying an agent that inhibits an infection by a pathogen that targets human cells of the erythroid lineage.

[0022] In some examples, the method comprises administering a candidate agent to a genetically modified mouse, wherein the animal comprises a nucleic acid sequence that encodes a human EPO protein operably linked to an *EPO* gene promoter, one or more gene mutations that results in immunodeficiency in the mouse, an engraftment of-human hematopoietic cells, and an infection by a pathogen that targets human cells of the erythroid lineage; and determining whether the agent reduces the amount of the pathogen in the pathogen-infected mouse.

[0023] In some examples, the method comprises contacting a human hematopoietic cell-engrafted, genetically modified immunodeficient mouse comprising a nucleic acid sequence that encodes a human EPO protein operably linked to an EPO gene promoter with clodronate; administering a candidate agent to the clodronate-contacted mouse; injecting the genetically modified mouse with parasitized reticulocytes or erythrocytes; and determining whether the agent prevents the infection of the human reticulocytes and/or erythrocytes of the mouse.

[0024] In some examples, the pathogen may be selected from a *Plasmodium* sp., *Babesia* sp., and a *Theileri* sp. For example, the pathogen is selected from *P. falciparum* and *P. vivax.*

[0025] Methods are disclosed for making a mouse expressing human EPO. In some embodiments, the method comprises contacting a mouse pluripotent stem cell with a nucleic acid sequence comprising coding sequence for a human EPO protein or a fragment thereof operably linked to *EPO* promoter sequence, wherein the coding sequence and *EPO* promoter sequence form a cassette that is flanked by sequences that are homologous to the endogenous mouse *EPO* locus; culturing the pluripotent stem cell under conditions that promote the integration of the nucleic acid sequence into the mouse genome at the endogenous mouse *EPO* locus by homologous recombination; and making a mouse from the mouse pluripotent stem cell that comprises the nucleic acid sequence encoding a human EPO protein.

[0026] In some examples, the mouse pluripotent stem cell is an embryonic stem (ES) cell or an induced pluripotent stem (iPS) cell. The mouse pluripotent stem cell may be deficient for Rag2 and/or IL2rg. The *EPO* promoter sequence may be the sequence for the human *EPO* promoter. The *EPO* promoter sequence may be the sequence for the endogenous mouse *EPO* promoter. the integration results in a replacement of the non-human *EPO* gene at the mouse EPO gene locus. The nucleic acid sequence that encodes the human EPO protein may comprise human EPO genomic coding and non-coding sequence. The nucleic acid sequence that encodes the human EPO protein may comprise human

[0027] Methods are disclosed for making a mouse expressing a human EPO protein and comprising a human hematopoietic system. In some examples, the methods comprise transplanting a population of cells comprising human hematopoietic progenitor cells into the genetically modified immunodeficient mouse made by methods of the present disclosure. In some examples, the transplanting comprises tail-vein injection, fetal liver injection, or retro-orbital injection. In some examples, the genetically modified immunodeficient mouse is sublethally irradiated prior to transplantation. In some embodiments, the human hematopoietic progenitor cells that are transplanted are CD34+ cells. In some examples, the human hematopoietic progenitor cells are from fetal liver, adult bone marrow, or umbilical cord blood.

[0028] Methods are disclosed for making a mouse that is infected with a human pathogen that targets human cells of the erythroid lineage. The methods may comprise making a mouse expressing a human EPO protein and comprising a human hematopoietic system according to methods of the present disclosure, injecting the engrafted mouse with clodronate, and injecting the clodronate-injected mouse with parasitized human red blood cells (PRBCs). The method may further comprise injecting into the mouse healthy human red blood cells. The parasite may be selected from a *Plasmodium* sp *abesia* sp., and a *Theileri* sp. The *Plasmodium* sp may be seleted from *P. falciparum* and *P. vivax.*

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

Figure 1 provides a protein alignment of the mouse Epo (SEQ ID NO:2) to human EPO (SEQ ID NO:4). Underlined residues are residues that are conserved between species.

Figure 2 provides schematics of the wild type mouse EPO locus before and after knock-in of a nucleic acid sequence encoding human EPO.

Figure 3 provides a schematic of the human EPO knock-in allele.

Figure 4 provides schematics of the wild type mouse Sirpa locus before (above) and after (below) knock in of a nucleic acid sequence encoding humanized Sirpa.

Figure 5, Panels A and B, show the frequency of human erythroid cells in HSC-engrafted mice. (Panel A) Human CD235a^{+} erythrocyte engraftment in the bone marrow 6-8 weeks after HSC engraftment into Rag2$^{-/-}$Il2rg$^{-/-}$Tpo$^{h/h}$IL3$^{h/h}$ Gmcsf$^{h/h}$Mcsf$^{h/h}$ mice ("MITRG mouse") comprising the indicated combinations of human EPO expressed from the mouse EPO locus ("hEPO+") and/or human SIRPa expressed as a random integrant into the mouse genome ("hSIRPa+"). (Panel B) Frequency of human CD235a^{+} erythrocytes in the peripheral blood in the presence versus absence of hEPO 6-8 weeks after HSC engraftment into Rag2$^{-/-}$ Il2rg^{null}Tpo$^{h/h}$Mcsf$^{h/h}$Il3$^{h/h}$Gmcsf$^{h/h}$Epo$^{h/h}$SIRPα$^{h/h}$ ("SupER-G") mice versus Rag2$^{-/-}$ Il2rg^{null}Tpo$^{h/h}$Il3$^{h/h}$Gmcsf$^{h/h}$Sirpa$^{h/h}$ ("RGSKI-TI") control mice.

Figure 6, Panels A and B, demonstrate that clodronate treatment increases circulating human erythroid cells and reticulocytes in HSC-engrafted mice. Seven weeks after HSC-engraftment, SupER-G mice were treated with daily retro-orbital injection of 50µl clodronate liposome for three to five consecutive days. Frequency of human CD235^{+} cells (erythrocytes and reticulocytes) (panel A) and CD235^{+}/CD71^{+} cells (reticulocytes) (panel B) in the peripheral blood was measured by FACS. Panel B: three different mice after treatment with clodronate.

Figure 7, Panels A-C, illustrate how human RBCs produced from engrafted mice are susceptible to *P. falciparum* infection. SupER-G mice were engrafted with fetal liver or adult HSCs. Seven weeks after engraftment, mice were treated with daily retro-orbital injection of 50µl clodronate liposomes for three consecutive days before blood collection. Blood samples were then cocultured with purified *P. falciparum* 3D7 blood stage parasites infected RBC (99% purity). Fresh human RBCs were added into the culture 48 hours post infection and infection culture was maintained for additional 10 days. Giemsa staining and quantitative PCR were performed to quantify parasitemia. Human RBCs control: human RBCs; Mouse RBCs control: RBCs from unengrafted mice; Mouse RBCs spiked control: RBCs from unengrafted mice spiked with 0.1% hRBCs. In (Panel A), red: anti-human Band3; Blue: Hoechst. In Panel C, the legend identifiers listed top to bottom correspond to the bar graph x axis from left to right.

Figure 8 shows the destruction of human RBCs in the mouse peripheral blood in the absence of clodronate. Unengrafted mice were treated with clodronate or PBS. For clodronate treatment, mice received daily retro-orbital injection of 50µl clodronate for three consecutive days. For PBS treatment, 500µl PBS only was delivered one hour before human RBC transfusion. Human RBCs were transfused into pre-treated mice and peripheral blood was collected at indicated time points. Clodronate and PBS curves are shown.

DETAILED DESCRIPTION

[0030] Genetically modified mice expressing human EPO from the mouse genome are provided. Also disclosed are methods for making mice expressing human EPO from the mouse genome, and methods for using mice expressing human EPO from the mouse genome. These mice and methods find many uses in the art, including, for example, in modeling human erythropoiesis and erythrocyte function; in modeling human pathogen infection of erythrocytes; in *in vivo* screens for agents that modulate erythropoiesis and/or erythrocyte function, e.g. in a healthy or a diseased state; in *in vivo* screens for agents that are toxic to erythrocytes or erythrocyte progenitors; in *in vivo* screens for agents that prevent against, mitigate, or reverse the toxic effects of toxic agents on erythrocytes or erythrocyte progenitors; in *in vivo* screens of erythrocytes or erythrocyte progenitors from an individual to predict the responsiveness of an individual to a disease therapy. These and other objects, advantages, and features of the invention will become apparent to those persons skilled in the art upon reading the details of the compositions and methods as more fully described below.

[0031] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0032] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, some potential and preferred methods and materials are now described.

[0033] As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present invention. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

[0034] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the peptide" includes reference to one or more peptides and equivalents thereof, e.g. polypeptides, known to those skilled in the art, and so forth.

[0035] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

GENETICALLY MODIFIED MICE

[0036] Mice are provided that are genetically modified to express one or more human proteins from their genome. The human protein is a human erythropoietin (hEPO) protein (SEQ ID NO:4). In other words, the genetically modified mouse comprises in its genome a nucleic acid sequence encoding a human EPO (hEPO) protein. For example, the mouse may comprise in its genome a nucleic acid sequence that comprises human *EPO* genomic coding and non-coding sequence, e.g. sequence at chromosome 7, nucleotides 100318423-100321323 or a fraction thereof. Alternatively, the mouse may comprise in its genome a nucleic acid sequence that comprises human *EPO* cDNA sequence (SEQ ID NO:3) or a fraction thereof. The mouse is further genetically modified so as to express one or more additional human proteins from the mouse's genome. These one or more additional human proteins are human proteins that promote human hematopoietic cell development and/or function, including, a human signal-regulatory protein alpha (hSIRPa) protein (NCBI Gene ID: 140885, GenBank Accession Nos. NM_080792.2, NM_001040022.1, NM_001040023.1), a human interleukin 3 (hIL-3) protein (NCBI Gene ID: 3562, GenBank Accession No. NM_000588.3), a human colony stimulating factor 2 (granulocyte-macrophage) (hGM-CSF) protein (NCBI Gene ID: 1437, GenBank Accession No. NM_000758.3), a human colony stimulating factor 1 (macrophage) (hM-CSF) protein (NCBI Gene ID: 1435, GenBank Accession No. NM_000757.5, NM_172210.2, NM_172211.3, and NM_172212.2), a human thrombopoietin (hTPO) protein (NCBI Gene ID: 7066, GenBank Accession Nos. NM_000460.3, NM_001177597.2, NM_001177598.2, NM_001289997.1, NM_001290003.1, NM_001290022.1, NM_001290026.1, NM_001290027.1, NM_001290027.1), a human interleukin 6 (hIL6) protein (NCBI Gene ID: 3569,GenBank Accession No. NM_000600.3), and the like.

[0037] The skilled artisan will appreciate that in addition to "wild type" or "native" human nucleic acids and proteins, the terms "a human nucleic acid" and "a human protein" encompass variants of wild type human nucleic acids and proteins as well. As used herein, the term "variant" defines either an isolated naturally occurring genetic mutant of a human polypeptide or nucleic acid sequence or a recombinantly prepared variation of a human polypeptide or nucleic acid sequence, each of which contain one or more mutations compared with the corresponding wild-type human nucleic acid or polypeptide sequence. For example, such mutations can be one or more amino acid substitutions, additions, and/or deletions. The term "variant" also includes human homologs and orthologues. In some embodiments, a variant polypeptide of the present invention has 70% or more identity, e.g. 75%, 80%, or 85% or more identity to a wild-type human polypeptide, e.g. 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a wild-type human polypeptide.

[0038] The percent identity between two sequences may be determined using any convenient technique in the art, for example, aligning the sequences using, e.g., publicly available software. Mutations can be introduced using standard molecular biology techniques, such as site-directed mutagenesis, PCR-mediated mutagenesis, directed evolution, and the like. One of skill in the art will recognize that one or more nucleic acid substitutions can be introduced without altering the amino acid sequence, and that one or more amino acid mutations can be introduced without altering the functional properties of the human protein.

[0039] Conservative amino acid substitutions can be made in human proteins to produce human protein variants. By conservative amino acid substitutions it is meant art-recognized substitutions of one amino acid for another amino acid having similar characteristics. For example, each amino acid may be described as having one or more of the following characteristics: electropositive, electronegative, aliphatic, aromatic, polar, hydrophobic and hydrophilic. A conservative substitution is a substitution of one amino acid having a specified structural or functional characteristic for another amino acid having the same characteristic. Acidic amino acids include aspartate, glutamate; basic amino acids include histidine, lysine, arginine; aliphatic amino acids include isoleucine, leucine and valine; aromatic amino acids include phenylalanine, glycine, tyrosine and tryptophan; polar amino acids include aspartate, glutamate, histidine, lysine, asparagine, glutamine, arginine, serine, threonine and tyrosine; and hydrophobic amino acids include alanine, cysteine, phenylalanine, glycine, isoleucine, leucine, methionine, proline, valine and tryptophan; and conservative substitutions include substitution among amino acids within each group. Amino acids may also be described in terms of relative size, alanine, cysteine, aspartate, glycine, asparagine, proline, threonine, serine, valine, all typically considered to be small.

[0040] Human variants can include synthetic amino acid analogs, amino acid derivatives and/or non-standard amino acids, illustratively including, without limitation, alpha-aminobutyric acid, citrulline, canavanine, cyanoalanine, diaminobutyric acid, diaminopimelic acid, dihydroxy-phenylalanine, djenkolic acid, homoarginine, hydroxyproline, norleucine, norvaline, 3-phosphoserine, homoserine, 5-hydroxytryptophan, 1-methylhistidine, methylhistidine, and ornithine.

[0041] Human variants will typically be encoded by nucleic acids having a high degree of identity with a nucleic acid encoding the wild-type human protein. In some embodiments, the complement of a nucleic acid encoding a human variant specifically hybridizes with a nucleic acid encoding a wild-type human under high stringency conditions. Nucleic acids encoding a human variant can be isolated or generated recombinantly or synthetically using well-known methodology.

[0042] In addition to "wild type" or "native" human proteins, and "variants" thereof, the term "human protein", as used herein, e.g., in the context of a "human EPO protein (hEPO)", a "human SIRPa protein (hSIRPa)", a "human IL-3 protein (hIL-3)", a "human GM-CSF protein (hGM-CSF)", a "human M-CSF protein (hM-CSF)", a "human TPO protein (hTPO)", and a "human IL-6 protein (hIL-6)", encompasses fusion proteins, i.e., chimeric proteins, which include one or more fragments of a wild-type human protein (or a variant thereof) and which retain one or more functions, e.g., one or more signaling and/or receptor functions, of the wild-type human protein. A fusion protein which includes one or more fragments of a wild-type human protein (or a variant thereof), e.g., in combination with one or more non-human peptides or polypeptides, may also be referred to herein as a "humanized protein". Thus, for example, a protein which includes an amino acid sequence of an extracellular domain of a wild-type human SIRPα protein fused with a signaling domain of a wild-type mouse SIRPα protein is encompassed by the term "human SIRPα protein".

[0043] A nucleic acid sequence that encodes a human protein is, therefore, a polynucleotide that includes a coding sequence for a human protein, e.g., a wild-type human protein, a variant of a wild-type human protein, a fragment of a wild-type human protein (or a variant thereof) which retains one or more functions, e.g., one or more signaling and/or receptor functions, of the wild-type human protein, or a fusion protein, e.g., a chimeric protein, which includes one or more fragments of a wild-type human protein (or a variant thereof) and which retain one or more functions, e.g., one or more signaling and/or receptor functions of the wild-type human protein.

[0044] Typically, in the genetically modified mice of the present disclosure, the nucleic acid encoding the human protein, e.g., the hEPO protein, the hSIRPa protein, the hIL-3 protein, the hGM-CSF protein, the hM-CSF protein, the hTPO protein, the hIL-6 protein, etc. is operably linked to one or more DNA regulatory elements. DNA regulatory elements include transcriptional and translational control sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for and/or regulate expression of a coding sequence in a host cell. For example, a "promoter" or "promoter sequence" refers to a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. The promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site, as well as protein binding domains responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Of particular interest to the present disclosure are DNA regulatory elements, e.g. promoters, which promote the transcription of the human protein in the same spatial and temporal expression pattern, i.e. in the same cells and tissues and at the same times, as would be observed of the corresponding endogenous protein.

[0045] The nucleic acid sequence encoding the human protein in the subject mouse may be operably linked to the human promoter for the gene, for example, if the human promoter promotes the correct spatial and temporal expression of the human protein in the mouse. Alternatively, the nucleic acid sequence encoding the human protein in the subject non-human animal may be operably linked to the mouse promoter for the corresponding mouse gene. Thus, for example, with regard to a mouse expressing a hEPO protein, the nucleic acid encoding the human EPO protein is operably linked to endogenous mouse *EPO* promoter.

[0046] In some instances, the human protein is expressed from the corresponding gene locus in the mouse. In certain instances, the nucleic acid sequence encoding the human protein replaces the nucleic acid sequence encoding the corresponding mouse protein. In other instances, the human protein is expressed from a genomic site in the mouse other than the locus for the corresponding mouse gene. Thus, for example, with regard to mice expressing a hEPO protein, the hEPO protein is expressed from the EPO locus of the mouse genome. The mouse comprises a replacement of nucleic acid sequence encoding endogenous EPO with nucleic acid sequence encoding the hEPO protein.

[0047] In some instances, the genetically modified mouse comprises one copy of the nucleic acid sequence encoding the human protein. For example, the mouse may be heterozygous for the nucleic acid sequence encoding the human protein, *i.e.,* one allele at a locus is genetically modified, while the other allele is the endogenous allele. In other instances, the genetically modified mouse comprises two copies of the nucleic acid sequence encoding the human protein. For example, the mouse may be homozygous for the nucleic acid sequence encoding the human protein, *i.e.,* both alleles for a locus in the diploid genome are genetically modified to encode the human protein, e.g. both alleles comprise a replacement of nucleic acid sequence encoding the endogenous protein with nucleic acid sequence encoding the human protein. Thus, for example, with regard to mice expressing a hEPO protein, as discussed above, the nucleic acid sequence encoding a hEPO may be integrated into the mouse *EPO* locus. In some such embodiments, the genetically modified mouse is heterozygous for the nucleic acid sequence encoding hEPO protein, *i.e.* the genetically modified mouse comprises one allele comprising the nucleic acid encoding hEPO and one allele encoding endogenous EPO. In other words, the animal is an $EPO^{h/m}$ animal, where "h" represents the allele comprising the human sequence and "m" represents the endogenous allele. In other such embodiments, the genetically modified mouse is homozygous for the nucleic acid sequence encoding the hEPO protein, *i.e.* both alleles for a locus in the diploid genome will comprise the nucleic acid sequence encoding the hEPO protein. In other words, the mouse is an $EPO^{h/h}$ mouse.

[0048] In some instances, the mouse also expresses the corresponding mouse protein. For example, the nucleic acid sequence encoding the human protein, e.g. hEPO, may be located at the corresponding mouse gene locus, e.g., mEPO locus, wherein it is integrated into the mouse gene locus in a manner that allows for the continued expression of the mouse coding sequence, e.g. the human coding sequence is inserted upstream or downstream of the mouse coding sequence and a 2A peptide sequence or IRES sequence is included between the two coding sequences. As a second example, the nucleic acid sequence encoding the human protein, e.g. hEPO, may be located at the corresponding mouse gene locus, e.g., mEPO locus, in a way that disrupts the expression of the mouse coding sequence, e.g. as a replacement of some or all of the mouse coding sequence, but the mouse is bred to be heterozygous for the insertion, i.e. "knock-in", allele, i.e. carrying one knock-in allele and one wild type allele. In other instances, the mouse does not express the corresponding mouse protein. For example, the nucleic acid sequence encoding the human protein, e.g. hEPO, may be located at the corresponding mouse gene locus, e.g., mEPO locus, in a way that disrupts the expression of the mouse coding sequence, e.g. as a replacement of some or all of the mouse coding sequence, e.g. by replacing mouse coding sequence, and the mouse is homozygous for the insertion, i.e. "knock-in", allele.

[0049] Any mouse may be genetically modified according to the subject disclosure.

[0050] In an embodiment, the subject genetically modified mouse is a mouse of a C57BL strain (e.g. C57BL/A, C57BL/An, C57BL/GrFa, C57BL/KaLwN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, C57BL/Ola, etc.); a mouse of the 129 strain (e.g. 129P1, 129P2, 129P3,129X1, 129S1 (e.g., 129S1/SV, 129S1/Svlm),129S2, 129S4, 129S5, 129S9/SvEvH, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, 129T2); a mouse of the BALB strain; e.g., BALB/c; and the like. See, e.g., Festing et al. (1999) Mammalian Genome 10:836, see also, Auerbach et al (2000) Establishment and Chimera Analysis of 129/SvEv- and C57BL/6-Derived Mouse Embryonic Stem Cell Lines). In one embodiment, the genetically modified mouse is a mix of an aforementioned 129 strain and an aforementioned C57BL/6 strain. In another embodiment, the mouse is a mix of aforementioned 129 strains, or a mix of aforementioned BL/6 strains. In yet another embodiment, the mouse is a mix of a BALB strain and another aforementioned strain.

[0051] The subject genetically modified mouse is immunodeficient. "Immunodeficient," includes deficiencies in one or more aspects of an animal's native, or endogenous, immune system, e.g. the animal is deficient for one or more types of functioning host immune cells, e.g. deficient for non-human B cell number and/or function, non-human T cell number and/or function, non-human NK cell number and/or function, etc.

[0052] One method to achieve immunodeficiency in the subject mice is sublethal irradiation. Alternatively, immunodeficiency may be achieved by any one of a number of gene mutations known in the art, any of which may be bred either alone or in combination into the subject genetically modified mice of the present disclosure or which may be used as the source of stem cells into which the genetic modifications of the subject disclosure may be introduced. Non-limiting examples include X-linked SCID, associated with IL2RG gene mutations and characterized by the lymphocyte phenotype T(-) B(+) NK(-); autosomal recessive SCID associated with Jak3 gene mutations and characterized by the lymphocyte phenotype T(-) B(+) NK(-); ADA gene mutations characterized by the lymphocyte phenotype T(-) B(-) NK(-); IL-7R alpha-chain mutations characterized by the lymphocyte phenotype T(-) B(+) NK(+); CD3 delta or epsilon mutations characterized by the lymphocyte phenotype T(-) B(+) NK(+); RAG1 and RAG2 mutations characterized by the lymphocyte phenotype T(-) B(-) NK(+); Artemis gene mutations characterized by the lymphocyte phenotype T(-) B(-) NK(+), CD45 gene mutations characterized by the lymphocyte phenotype T(-) B(+) NK(+); and Prkdc^{scid} mutations characterized by the lymphocyte phenotype T(-), B(-). As such, in some embodiments, the genetically modified immunodeficient mouse has one or more deficiencies selected from an IL2 receptor gamma chain (Il2rγ $^{y/-}$) deficiency, a Jak3 deficiency, an ADA deficiency, an IL7R deficiency, a CD3 deficiency, a RAG1 and/or RAG2 deficiency, an Artemis deficiency, a CD45 deficiency, and a Prkdc deficiency. These and other animal models of immunodeficiency will be known to the ordinarily skilled artisan, any of which may be used to generate immunodeficient mice of the present disclosure.

[0053] In some embodiments, genetically modified mice in accordance with the invention find use as recipients of human hematopoietic cells that are capable of developing human immune cells from engrafted human hematopoietic cells. As such, in some aspects of the invention, the subject genetically modified mouse is a genetically modified, immunodeficient, mouse that is engrafted with human hematopoietic cells.

[0054] Any source of human hematopoietic cells, human hematopoietic stem cells (HSCs) and/or hematopoietic stem progenitor cells (HSPC) as known in the art or described herein may be transplanted into the genetically modified immunodeficient mice of the present disclosure. One suitable source of human hematopoietic cells known in the art is human umbilical cord blood cells, in particular CD34-positive ($CD34^+$) cells. Another source of human hematopoietic cells is human fetal liver. Another source is human bone marrow. Also encompassed are induced pluripotent stem cells (iPSC) and induced hematopoietic stem cells (iHSC) produced by the de-differentiation of somatic cells, e.g., by methods known in the art. Methods for the transplantation of human cells into non-human animals are well-described in the art and elsewhere herein, any of which may be employed by the ordinarily skilled artisan to arrive at the subject genetically modified engrafted non-human animals.

[0055] The transplanted human hematopoietic cells give rise in the genetically modified mouse to one or more engrafted human cells selected from a human CD34-positive cell, a human hematopoietic stem cell, a human hematopoietic cell, a myeloid progenitor cell, an erythroid progenitor cell, a myeloid cell, a dendritic cell, a monocyte, a neutrophil, a mast cell, an erythrocyte, and a combination thereof. In one embodiment, the human cell is present at 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after engraftment. In a specific embodiment, the human cells comprise cells of the erythroid lineage.

[0056] In some embodiments, the transplanted human hematopoietic cells give rise in the genetically modified mouse to an engrafted human hemato-lymphoid system that comprises human hematopoietic stem and progenitor cells, human myeloid progenitor cells, human myeloid cells, human dendritic cells, human monocytes, human granulocytes, human neutrophils, human mast cells, human erythrocytes, human thymocytes, human T cells, human B cells, and human platelets. In one embodiment, the human hemato-lymphoid system is present at 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after engraftment. In a specific embodiment, the human hemato-lymphoid system comprises cells of the erythroid lineage.

[0057] Cells of the erythroid lineage include erythrocytes and cells that give rise to erythrocytes. "Erythrocytes" include mature red blood cells, also referred to red cells or red corpuscles. Cells that give rise to erythrocytes include erythrocyte progenitor cells, i.e., proliferating multipotent cells, and erythrocyte precursors, i.e. proliferating or nonproliferating cells committed to becoming erythrocytes.

[0058] Erythrocytes are the major cellular element of the circulating blood and transport oxygen as their principal function. The number of erythrocytes per cubic millimeter of blood is usually maintained between 4.5 and 5.5 million in men and between 4.2 and 4.8 million in women. It varies with age, activity, and environmental conditions. For example, an increase to a level of 8 million/mm^3 can normally occur at over 10,000 feet above sea level. An erythrocyte normally lives for 110 to 120 days, when it is removed from the bloodstream and broken down by the reticuloendothelial system. New erythrocytes are produced at a rate of slightly more than 1% a day; thus a constant level is usually maintained. Acute blood loss, hemolytic anemia, or chronic oxygen deprivation may cause erythrocyte production to increase greatly.

[0059] Erythrocytes originate from hematopoietic stem cells in the marrow of the long bones, developing into erythrocytes through successive cell stages that include common myeloid progenitor cells (CD123+, CD34+, c-kit+, Flt3+); megakaryocyte-erythroid progenitor cells (CD34+, CD38+, CD45RA-); proerythroblasts (also called pronormoblasts if normal, or promegaloblasts if abnormal; large CD71+, EpoR+, c-kit+, Ter119+ progenitors); basophilic erythroblasts (cytoplasm is basophilic, the nucleus is large with clumped chromatin, and the nucleoli have disappeared); polychromatic erythroblasts (also called an intermediate normoblast, in which the nuclear chromatin shows increased clumping and the cytoplasm begins to acquire hemoglobin and takes on an acidophilic tint); orthochromatic normoblasts (the final stage before nuclear loss, in which the nucleus is small and ultimately becomes a blue-black, homogeneous, structureless mass); and reticulocytes (circulating CD235+, CD71+ cells; the cell is characterized by a meshlike pattern of threads and particles at the former site of the nucleus).

[0060] Mature erythrocytes appear on a peripheral smear as biconcave, round or ovoid discs about 6-8 µm in diameter. They contain hemoglobin and have a zone of central pallor due to the cell's biconcavity, and may be readily identified by flow cytometry or immunohistochemistry-based methods by the elevated expression of cell surface markers CD235 and CD59 relative to non-erythroid cells.

[0061] As demonstrated in, for example, FIG. 5, Panel A and FIG. 5, Panel B of the present disclosure, the expression of hEPO under the control of an EPO promoter from the genome of an engrafted mouse of the present disclosure increases the number of human cells of the erythroid lineage (CD235a+) in the bone marrow on average by about 2-fold (e.g., from about 11% to about 22%). The expression of human Sirpa enhances this effect, resulting in a 3-fold increase or more on average (i.e., from about 11% to about 33%) in the representation of human CD235a+ cells in the bone marrow as compared to mice that do not express either human EPO or human Sirpa (FIG. 5, Panel A). As such, engrafted, immunodeficient, genetically modified mice expressing hEPO of the present disclosure find use in the study of human erythropoiesis and the development of drugs that modulate (e.g. promote or suppress) human erythropoiesis.

[0062] Moreover, as demonstrated in, e.g. FIG. 6, clodronate treatment of human HSC-engrafted animals expressing hEPO increases by a thousand-fold the number of CD235+ erythroid cells (including CD71+ reticulocytes, FIG. 6, Panel B) in the peripheral blood of these animals, i.e. to about 1% of the total red blood cells in the periphery, as compared to untreated controls. Importantly, as demonstrated in, e.g. FIG.7, human red blood cells produced at these engraftment levels in the HSC-engrafted animals expressing human EPO are susceptible to infection with *P. falciparum.* As such, genetically modified mice expressing hEPO as described herein find particular use in the generation of animal models of parasitic infections that target human cells of the erythroid lineage, e.g. pathogens that result in malaria or malaria-like diseases.

[0063] Accordingly, in some aspects of the invention, the subject genetically modified mouse is a mouse engrafted with human hematopoietic cells and comprising an infection by a human pathogen. Of particular interest in these embodiments are human pathogens that target human cells of the erythroid lineage. Non-limiting examples of such pathogens include protozoans of the genera *Plasmodium, Babesia, Theileria,* and the like. As described in greater detail below, the subject genetically modified mice engrafted with human hematopoietic cells may be infected with human pathogen using any appropriate method known in the art or described herein for infecting animals with the pathogens of interest. Animals so infected will typically show signs of parasitaemia including altered morphology by Giemsa-stained blood smear, and a severe decrease (e.g. 50%) in total erythrocyte concentration and anemia.

METHODS OF MAKING THE SUBJECT GENETICALLY MODIFIED MICE

[0064] Methods are disclosed for making the subject mice of the present disclosure. In practicing the subject methods, a mouse is generated which comprises a nucleic acid sequence that encodes a hEPO protein operably linked to an *EPO* promoter, for example, the mouse *EPO* promoter, e.g. at the *EPO* locus of the mouse genome.

[0065] The generation of a mouse comprising a nucleic acid sequence that encodes a hEPO protein operably linked to an *EPO* promoter may be accomplished using any convenient method for the making genetically modified animals, e.g. as known in the art or as described herein.

[0066] For example, a nucleic acid encoding the hEPO protein may be incorporated into a recombinant vector in a form suitable for insertion into the genome of the host cell and expression of the human protein in a mouse host cell. The recombinant vector includes the one or more regulatory sequences operatively linked to the nucleic acid encoding the human protein in a manner which allows for transcription of the nucleic acid into mRNA and translation of the mRNA into the human protein, as described above. It will be understood that the design of the vector may depend on such factors as the choice of the host cell to be transfected and/or the amount of human protein to be expressed.

[0067] Any of various methods may then be used to introduce the human nucleic acid sequence into an animal cell to produce a genetically modified animal that expresses the human gene. Such techniques are well-known in the art and include, but are not limited to, pronuclear microinjection, transformation of embryonic stem cells, homologous recombination and knock-in techniques. Methods for generating genetically modified animals that can be used include, but are not limited to, those described in Sundberg and Ichiki (2006, Genetically Engineered Mice Handbook, CRC Press), Hofker and van Deursen (2002, Genetically modified Mouse Methods and Protocols, Humana Press), Joyner (2000, Gene Targeting: A Practical Approach, Oxford University Press), Turksen (2002, Embryonic stem cells: Methods and Protocols in Methods Mol Biol., Humana Press), Meyer et al. (2010, Proc. Nat. Acad. Sci. USA 107:15022-15026), and Gibson (2004, A Primer Of Genome Science 2nd ed. Sunderland, Massachusetts: Sinauer), U.S. Pat. No. 6,586,251, Rathinam et al. (2011, Blood 118:3119-28), Willinger et al., (2011, Proc Natl Acad Sci USA, 108:2390-2395), Rongvaux et al., (2011, Proc Natl Acad Sci USA, 108:2378-83) and Valenzuela et al. (2003, Nat Biot 21:652-659).

[0068] For example, the subject genetically modified mice can be created by introducing the nucleic acid encoding the human protein into an oocyte, e.g., by microinjection, and allowing the oocyte to develop in a female foster animal. The expression is injected into fertilized oocytes. Fertilized oocytes can be collected from superovulated females the day after mating and injected with the expression construct. The injected oocytes are either cultured overnight or transferred directly into oviducts of 0.5-day p.c. pseudopregnant females. Methods for superovulation, harvesting of oocytes, expression construct injection and embryo transfer are known in the art and described in Manipulating the Mouse Embryo (2002, A Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press). Offspring can be evaluated for the presence of the introduced nucleic acid by DNA analysis (e.g., PCR, Southern blot, DNA sequencing, etc.) or by protein analysis (e.g., ELISA, Western blot, etc.).

[0069] As another example, the construct comprising the nucleic acid sequence encoding the human protein may be transfected into stem cells (e.g., ES cells or iPS cells) using well-known methods, such as electroporation, calcium-phosphate precipitation, lipofection, etc. The cells can be evaluated for the presence of the introduced nucleic acid by DNA analysis (e.g., PCR, Southern blot, DNA sequencing, etc.) or by protein analysis (e.g., ELISA, Western blot, etc.). Cells determined to have incorporated the expression construct can then be introduced into preimplantation embryos. For a detailed description of methods known in the art useful for the compositions and methods of the invention, see Nagy et al., (2002, Manipulating the Mouse Embryo: A Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press), Nagy et al. (1990, Development 110:815-821), U.S. Pat. No. 7,576,259, U.S. Pat. No. 7,659,442, U.S. Pat. No. 7,294,754, and Kraus et al. (2010, Genesis 48:394-399).

[0070] Additionally, as described in some of the Examples below, a nucleic acid construct may be constructed using VELOCIGENE® genetic engineering technology (see, e.g., Valenzuela et al. (2003) High throughput engineering of the mouse genome coupled with high-resolution expression analysis, Nature Biotech. 21(6): 652-59 and U.S. Patent No. 6,586,251), introduced into stem cells (e.g., ES cells), and correctly targeted clones determined using loss-of-allele and gain-of-allele assays (Valenzuela et al., *supra*); correctly targeted ES cells may be used as donor ES cells for introduction into an 8-cell stage mouse embryo using the VELOCIMOUSE® method (see, e.g., U.S. Pat. No. 7,294,754 and Poueymirou et al. 2007, F0 generation mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analyses Nature Biotech. 25(1):91-99).

[0071] Genetically modified founder animals can be bred to additional animals carrying the genetic modification. Genetically modified animals carrying a nucleic acid encoding the human protein(s) of the present disclosure can further be bred to other genetically modified animals carrying other genetic modifications, e.g. hSIRPa knock-in mice, hIL-3 knock-in mice, hGM-CSF knock-in mice, hM-CSF knock-in mice, hTPO knock-in mice, hIL-6 knock-in mice, and the like, or be bred to knockout animals, e.g., a non-human animal that is deficient for one or more proteins, e.g. does not express one or more of its genes, e.g. a Rag2-deficient animal, an Il2rg-deficient animal.

[0072] Stem cells, e.g., ES cells, may be generated such that they comprise several genetic modifications, e.g., humanizations or gene deletions described herein, and such stem cells may be introduced into an embryo to generate genetically modified animals with several genetic modifications.

[0073] As discussed above, the subject genetically modified mouse is an immunodeficient mouse. Genetically modified mice that are immunodeficient and comprise one or more human proteins, e.g. hEPO, hSIRPa, hIL-3, hGM-CSF, hM-CSF, and/or hTPO, may be generated using any convenient method for the generation of genetically modified mice, e.g. as known in the art or as described herein. For example, the generation of the genetically modified immunodeficient mice can be achieved by introduction of the nucleic acid encoding the human protein into an oocyte or stem cells comprising a mutant SCID gene allele that, when homozygous, will result in immunodeficiency as described in greater detail above and in the working examples herein. Mice are then generated with the modified oocyte or ES cells using, e.g. methods described herein and known in the art, and mated to produce the immunodeficient mice comprising the desired genetic modification. As another example, genetically modified mice can be generated in an immunocompetent background, and crossed to a mouse comprising a mutant gene allele that, when hemizygous or homozygous, will result in immunodeficiency, and the progeny mated to create an immunodeficient mouse expressing the at least one human protein of interest.

[0074] The genetically modified mouse is treated so as to eliminate endogenous hematopoietic cells that may exist in the mouse. The treatment comprises irradiating the genetically modified mouse. Newborn genetically modified mouse pups are irradated sublethally. Newborn pups are irradiated 2 x 200 cGy with a four hour interval.

[0075] Genetically modified animals that include a human nucleic acid in substantially all of their cells, as well as genetically modified that include a human nucleic acid in some, but not all their cells are disclosed. In some instances, e.g. targeted recombination, one copy of the human nucleic acid will be integrated into the genome of the genetically modified mice. In other instances, e.g. random integration, multiple copies, adjacent or distant to one another, of the human nucleic acid may be integrated into the genome of the genetically modified mice.

[0076] Thus, the subject genetically modified mice may be an immunodeficient animal comprising a genome that includes a nucleic acid encoding a human polypeptide operably linked to the corresponding mouse promoter, wherein the animal expresses the encoded human polypeptide. In other words, the subject genetically modified immunodeficient mouse comprises a genome that comprises a nucleic acid encoding at least one human polypeptide, wherein the nucleic acid is operably linked to the corresponding mouse promoter and a polyadenylation signal, and wherein the animal expresses the encoded human polypeptide.

[0077] As discussed above, the subject genetically modified mouse is engrafted with human hematopoietic cells. Any source of human hematopoietic cells, human hematopoietic stem cells (HSCs) and/or hematopoietic stem progenitor cells (HSPC) as known in the art or described herein may be transplanted into the genetically modified mice of the present disclosure, e.g. umbilical cord blood, fetal liver, bone marrow, iPSCs, etc. The hematopoietic cells are selected from human umbilical cord blood cells and human fetal liver cells. The amount of cells to be transplanted will be well understood by the ordinarily skilled artisan or may be empirically determined. Engraftment is with about 1-2 x 10^5 human CD34+ cells. Cells may be transplanted into the host subject mouse using any convenient technique known in the art, for example, tail vein injection, retro-orbital injection, injection into neonatal liver, and the like. Cells may be transplanted into the host in any convenient buffered solution, e.g. PBS, Dulbecco's modified medium, Iscove's modified medium, and the like. In some instances, the animal may be irradiated before being engrafted, e.g. as described above, to improve immunodeficiency.

[0078] Human hematopoietic cells so engrafted give rise to one or more human cells selected from a CD34+ cell, a hematopoietic stem cell, a hematopoietic cell, a myeloid precursor cell, a myeloid cell, a dendritic cell, a monocyte, a granulocyte, a neutrophil, a mast cell, a thymocyte, a T cell, a B cell, a platelet, an erythrocyte, and a combination thereof. In one embodiment, the human cell is present at 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after engraftment. Any of a number of assays may be performed to confirm that engraftment is successful, including, for example, flow cytometry assays for the various human hematopoietic cells of interest, blood smears, and immunohistochemistry.

[0079] As discussed above, in some examples, the subject genetically modified mouse engrafted with human hematopoietic cells is infected with a human pathogen. Of particular interest are human pathogens that target human cells of the erythroid lineage. Non-limiting examples of such pathogens include protozoans of the genera *Plasmodium* sp., *Babesia* sp., *Theileria* sp., and the like. In some, the strain of pathogen used is a naturally occurring strain. In certain examples, the strain used has been selected *in vivo* for its competence to grow reproducibly in human erythrocyte-engrafted immunodeficient mice, e.g. *P. falciparum* strains

Pf3D7$^{0087/N9}$ or Pf3D7$^{0087/N5}$.

[0080] The subject engrafted immunodeficient mice may be infected using any method known in the art for infecting animals with pathogen that targets cells of the erythroid lineage. For example, the subject engrafted immunodeficient animals may be inoculated intraperitoneally with parasitized red blood cells (PRBCs). See, e.g., Badell et al. (2000) Human malaria in immunocompromised mice: an in vivo model to study defense mechanisms against Plasmodium falciparum. JEM 192(11):1653-1660; and Moreno et al. (2006) The course of infections and pathology in immunomodulated NOD/LtSz-SCID mice inoculated with Plasmodium falciparum laboratory lines and clinical isolates. Int. J. Parasitol. 36:361-369. As another example, the subject engrafted immunodeficient mice may be inoculated intravascularly with parasitized red blood cells. See, e.g., Angulo-Barturen et al. (2008) A murine model of falciparum-malaria by in vivo selection of competent strains in non-myelodepleted mice engrafted with human erythrocytes. PLoS One 3:e2252; and Jimenez-Diaz et al. (2009) Improved murine model of malaria using Plasmodium falciparum competent strains and non-myelodepleted NOD-scid IL2Rgnull mice engrafted with human erythrocytes. Antimicrob Agents Chemother 53:4533-4536. In some examples, the infection is produced by injecting parasite into the mouse, i.e. not in the context of erythroid cells. In some examples, the subject engrafted immunodeficient mice are chemically depleted in vivo of phagocytic cells prior to and/or during infection. In such examples, any chemotherapeutic agent that selectively depletes host phagocytic cells may be administered to the subject mice, including, for example, clodronate, e.g. as described in the working examples herein; dicholoromethylene diphosphonate, e.g. as described in Badell et al. *supra,* and Moreno et al. *supra;* monoclonal antibody specific for polymorphonuclear neutrophils, e.g. NIMP-R14, as described in Badell et al. *supra* and Moreno et al., *supra;* and the like.

[0081] Percent parasitaemia in the infected genetically modified mice of the present disclosure can be estimated by any convenient method in the art, e.g. microscopically from a Giemsa-stained blood smear, e.g. 3 days after injection; or by flow cytometry, e.g. by measurement of the emission of the nucleic acid dye YOYO-1 or of the cell-permeant dye SYTO-16, e.g. in the presence or absence of TER-119 mAb. See, e.g., Jimenez-Diaz et al. Quantitative measurement of Plasmodium-infected erythrocytes in murine models of malaria by flow cytometry using bidimensional assessment of SYTO-16 fluorescence. Cytometry A 2009, 75:225-235.

USE OF THE SUBJECT GENETICALLY MODIFIED MICE

[0082] The ability to study human tissue in an *in vivo* setting in mice has opened a range of possible avenues of research. Major limitations have hindered the application of the approach and of these one of the most important deficiencies has been the inability of mouse factors to support human cells. Indeed, in the immune system, many essential factors required for human immune cell development and function are species-specific and cannot be effectively provided by the mouse. It was therefore decided to follow a strategy of replacing the mouse genes with their human counterparts, enabling the better development and function of human cells and potentially disabling the same of the corresponding mouse cells. By applying this concept to human cytokine EPO, it is shown herein that replacement of nucleic acid sequence encoding the mouse EPO protein with nucleic acid sequence encoding human EPO protein improves the development and function of cells of the erythroid lineage in the engrafted human immune system in mice.

[0083] For example, the working examples at, e.g. FIG. 5, demonstrate that the expression of hEPO from a nucleic acid sequence under the control of an *EPO* promoter at the genome of a mouse increases the number of human cells of the erythroid lineage (CD235a+) that develop in the bone marrow of human HSC-engrafted animals by about 2-fold (i.e., from about 11% to about 22%). The expression of human Sirpa enhances this effect, resulting in a 3-fold total increase (i.e., from about 11% to about 33%) in the representation of human CD235a+ cells in the bone marrow as compared to mice that do not express either human EPO or human Sirpa (Figure 4a). Moreover, as demonstrated in, e.g. FIG. 6, clodronate mice treatment of human FtSC-engrafted mice expressing human EPO increases by a thousand-fold the number of CD235+ erythroid cells (including CD71+ reticulocytes, FIG. 6, Panel B) in the peripheral blood of these animals, i.e. to about 1% of the total red blood cells, over untreated controls. Importantly, as demonstrated in, e.g. FIG. 7, human red blood cells produced at these engraftment levels in the HSC-engrafted mice expressing human EPO are susceptible to infection with *Plasmodium* sp. such as *P. falciparum.* Accordingly, genetically modified mice expressing human EPO as described herein find particular use in the generation of animals that are susceptible to infection with *Plasmodium* sp., or that will better support human erythrocytes that are acutely grafted into the animal prior to infection in current rodent models.

[0084] As such, the genetically modified mice of the present disclosure find many uses in the art. For example, engrafted genetically modified mice of the present disclosure are useful for studying human erythropoiesis and the function of human erythrocytes. As another example, engrafted genetically modified mice of the present disclosure provide a useful system for screening candidate agents for desired activities *in vivo,* for example, to identify agents that are able to modulate (*i.e.,* promote or suppress) human erythropoiesis and/or the function of human erythrocytes, e.g. in a healthy or a diseased state, e.g. as cancerous cells, during pathogen infection, *etc.,* for example to identify novel therapeutics; or as another example, to identify agents that are toxic to human cells of the erythroid lineage, and to identify agents that prevent against, mitigate, or reverse the toxic effects of toxic agents on human cells of the erythroid lineage; etc. As yet another example, engrafted genetically modified mice of the present disclosure provide a useful system for predicting the responsiveness of an individual to a disease therapy, e.g. by providing an *in vivo* platform for screening the responsiveness of an individual's immune system to an agent, *e.g.* a therapeutic agent, to predict the responsiveness of an individual to that agent.

[0085] As one non-limiting example, engrafted genetically modified mice of the present disclosure find use in the generation of mouse models of pathogen infection by parasites that target human erythroid cells, e.g. *Plasmodium, Babesia, Theileria,* and the like. Such mouse models of infection will be useful in both research, e.g. to better understand the progression of infection in humans, and in drug discovery, e.g. to identify candidate agents that protect against or treat infection by such parasites.

[0086] Protozoans of the genus *Plasmodium* are the cause of malaria in humans. Malaria begins with a bite from an infected *Anopheles* mosquito, which introduces the protozoa via its saliva into the circulatory system, and ultimately to the liver where they mature and reproduce. The protozoa then enter the bloodstream and infect cells of the erythroid lineage at various stages of maturation.

[0087] Five species of *Plasmodium* can infect and be transmitted by humans. The vast majority of deaths are caused by *P. falciparum,* while *P. vivax, P. ovale,* and *P. malariae* cause a generally milder form of malaria that is rarely fatal. This believed to be due at least in part to the type(s) of cells targeted by each species: *P. falciparum* grows in red blood cells (RBCs) of all maturities whereas, for example, *P. vivax* is restricted to growth in reticulocytes, which represent only approximately 1% - 2% of total RBCs in the periphery. In addition, P. *falciparum* causes severe malaria via a distinctive property not shared by any other human malaria, namely, that of sequestration. Within the 48-hour asexual blood stage cycle, the mature forms change the surface properties of infected red blood cells, causing them to stick to blood vessels (a process called cytoadherence). This leads to obstruction of the microcirculation and results in dysfunction of multiple organs.

[0088] Symptoms of malaria include fever, chills, headache, sweats, fatigue, anemia, nausea, dry (nonproductive) cough, muscle and/or back pain, and an enlarged spleen. Other symptoms and complications associated with malaria include brain infection (cerebritis), hemolytic anemia, kidney failure, liver failure, meningitis, pulmonary edema, and hemorrhaging from the spleen. Generally, an individual at risk for developing malaria will begin to show symptoms 7 days or more after infection, e.g., 9 to 14 days after the initial infection by *P. falciparum,* 12 to 18 days after the initial infection by *P. vivax* or *P. ovale,* 18 to 40 days after the initial infection by *P. malariae,* or 11 to 12 days after the initial infection by *P. knowlesi.* Anti-malaria agents used in the art to treat or prevent malaria include chloroquine, quinidine, doxycycline, tetracycline, clindamycin, atovaquone plus proguanil (Malarone), Mefloquine, artesunate, and pyrimethamine plus sulfadoxine (Fansidar).

[0089] Methods for determining if a subject has been infected with *Plasmodium* are well known in the art, and include, for example, microscopic examination of blood using blood films, with antigen-based Rapid Diagnostic Tests (RDT), e.g., immunochromatography-based RDTs, by detection of parasite DNA by polymerase chain reaction (PCR), etc. Any convenient method may be used to determine if the human red blood cells of the subject have been infected with the pathogen.

[0090] Another example of pathogens of interest are protozoans of the genus *Babesia. Babesia* infection results in a malaria-like disease called babesiosis. Babesiosis is a vector-borne illness usually transmitted by *Ixodes scapularis* ticks. The disease is typically caused by

B. microti in humans, *B. canis rossi* and *B. canis canis* in dogs, *B. bovis* in cows, and *B. bigemina* in cattle. *Babesia microti,* which infects humans, uses the same tick vector as Lyme disease and ehrlichiosis, and may occur in conjunction with these other diseases. The protozoa can also be transmitted by blood transfusion.

[0091] In humans, babesiosis may be asymptomatic, or characterized by symptoms ranging from mild fever and diarrhea to high fever, shaking chills, and severe anemia. In severe cases, organ failure, including respiratory distress syndrome, may occur. Severe cases occur mostly in people who have had a splenectomy, or persons with an immunodeficiency, such as HIV/AIDS patients. Treatment typically comprises a two-drug regimen of quinine and clindamycin, or of atovaquone and azithromycin. In instances where babesiosis appears life-threatening, a blood exchange transfusion is performed, in which infected red blood cells are removed and replaced with uninfected ones.

[0092] Definitive diagnosis of infection by *Babesia* is by the identification of the parasite on a Giemsa-stained thin blood smear. The parasite appears in erythrocytes as paired merozoites forming the "Maltese cross formation" in humans or "two pears hanging together" in animals. Other diagnostic methods include PCR of peripheral blood, and serologic testing for antibodies (IgG, IgM) against Babesia.

[0093] Yet another malaria-like disease, theileriosis, is caused by protozoans of the genus *Theileria.* In humans, theileriosis is caused by *T. microti;* in horses, by *T. equi* ("Equine Piroplasmosis"); in sheep and goats, by *T. lestoquardi;* and in cattle, African buffalo, water buffalo, and water bucks, by *T. annulata* ("Tropical Theileriosis", also known as "Mediterranean theileriosis") or *T. parva* ("East Coast fever", also known as "Corridor disease"). Theirleriosis is transmitted to the host by various tick species including *Ixodes scapularis, Rhipicephalus, Dermacentor, Haemaphysalis,* and *Hyalomma.* The organism reproduces in the tick as it progresses through its life stage, and matures and enters the saliva after the tick attaches to a host. Usually, the tick must be attached for a few days before it becomes infective. However, if environmental temperatures are high, infective sporozoites can develop in ticks on the ground, and may enter the host within hours of attachment.

[0094] Theirleriosis in humans typically presents as fever and hemolysis. Definitive diagnosis of infection by *Theileria* is by the identification of the parasite on a Giemsa-stained thin blood smear.

[0095] Engrafted genetically modified mice of the present disclosure find use in screening candidate agents to identify those that will prevent (e.g. vaccines) or treat infections by *Plasmodium, Babesia, Theileria,* and other parasites that target human erythrocytes. The terms "treatment", "treating" and the like are used herein to generally include obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein include any treatment of a disease in a mammal, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; or (c) relieving the disease, i.e., causing regression of the disease. Candidate agents of interest as anti-parasitic therapeutics include those that may be administered before, during or after the infection with the parasite, and which, when administered in an effective amount, inhibit the effects of a parasite on an individual (i.e. the host), for example, by killing the parasite or the cell infected by the parasite, by preventing the propagation of the parasite, by preventing the production or action of an agent produced by the parasite that is toxic to the individual (i.e. a toxin), etc. The terms "individual," "subject," "host," and "patient," are used interchangeably herein and include any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans.

[0096] In screening assays for biologically active agents, a human hematopoietic cell-engrafted genetically modified mouse of the present disclosure, e.g. an engrafted Rag2$^{-/-}$Il2rg^{null}Epo$^{h/m}$ mouse, an engrafted Rag2$^{-/-}$Il2rg^{null}Tpo$^{h/h}$Il3/Gmcsf$^{h/h}$Epo$^{h/m}$SIRPα$^{h/h}$ ("TIES") mouse, an engrafted Rag2$^{-/-}$IL2rg$^{y/-}$Tpo$^{h/h}$Mcsf$^{h/h}$Il3$^{h/h}$Gmcsf$^{h/h}$Epo$^{h/h}$hSIRPα+("MISTER-G") mouse, an engrafted Rag2$^{-/-}$Il2rg^{null}Tpo$^{h/h}$Mcsf$^{h/h}$Il3/Gmcsf$^{h/h}$Epo$^{h/h}$SIRPα-tg+ ("SupER-G") mouse, etc. is contacted with a candidate agent of interest and the effect of the candidate agent is assessed by monitoring one or more output parameters. These output parameters may be reflective of the viability of the cells, *e.g.* the total number of hematopoietic cells or the number of cells of a particular hematopoietic cell type, or of the apoptotic state of the cells, *e.g.* the amount of DNA fragmentation, the amount of cell blebbing, the amount of phosphatidylserine on the cell surface, and the like by methods that are well known in the art. Alternatively or additionally, the output parameters may be reflective of the differentiation capacity of the cells, *e.g.* the proportions of differentiated cells and differentiated cell types. Alternatively or additionally, the output parameters may be reflective of the function of the cells, *e.g.* the cytokines and chemokines produced by the cells, the antibodies (e.g. amount or type) produced by the cells, the ability of the cells to home to and extravasate to a site of challenge, the ability of the cells to modulate, *i.e.* promote or suppress, the activity of other cells in vitro or in vivo, the ability to take up hemoglobin, *etc.* Yet other parameters may be reflective of the effect of the agent on infection, e.g. pathogen infection in the animal, e.g. the titer of pathogen in the mouse, *etc.,* as relevant to the studies being performed.

[0097] Parameters are quantifiable components of cells, particularly components that can be accurately measured, desirably in a high throughput system. A parameter can be any cell component or cell product including cell surface determinant, receptor, protein or conformational or posttranslational modification thereof, lipid, carbohydrate, organic or inorganic molecule, nucleic acid, *e.g.* mRNA, DNA, *etc.* or a portion derived from such a cell component or combinations thereof. While most parameters will provide a quantitative readout, in some instances a semi-quantitative or qualitative result will be acceptable. Readouts may include a single determined value, or may include mean, median value or the variance, *etc.* Characteristically a range of parameter readout values will be obtained for each parameter from a multiplicity of the same assays. Variability is expected and a range of values for each of the set of test parameters will be obtained using standard statistical methods with a common statistical method used to provide single values.

[0098] Candidate agents of interest for screening include known and unknown compounds that encompass numerous chemical classes, primarily organic molecules, which may include organometallic molecules, inorganic molecules, genetic sequences, vaccines, antibiotics or other agents suspected of having antibiotic properties, peptides, polypeptides, antibodies, agents that have been approved pharmaceutical for use in a human, *etc.* An important aspect of the invention is to evaluate candidate drugs, including toxicity testing; and the like.

[0099] Candidate agents include organic molecules comprising functional groups necessary for structural interactions, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, frequently at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules, including peptides, polynucleotides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Included are pharmacologically active drugs, genetically active molecules, *etc.* Compounds of interest include chemotherapeutic agents, hormones or hormone antagonists, *etc.* Exemplary of pharmaceutical agents suitable for this invention are those described in, "The Pharmacological Basis of Therapeutics," Goodman and Gilman, McGraw-Hill, New York, N.Y., (1996), Ninth edition. Also included are toxins, and biological and chemical warfare agents, for example see Somani, S. M. (Ed.), "Chemical Warfare Agents," Academic Press, New York, 1992).

[0100] Candidate agents of interest for screening also include nucleic acids, for example, nucleic acids that encode siRNA, shRNA, antisense molecules, or miRNA, or nucleic acids that encode polypeptides. Many vectors useful for transferring nucleic acids into target cells are available. The vectors may be maintained episomally, *e.g.* as plasmids, minicircle DNAs, virus-derived vectors such cytomegalovirus, adenovirus, *etc.,* or they may be integrated into the target cell genome, through homologous recombination or random integration, *e.g.* retrovirus derived vectors such as MMLV, HIV-1, ALV, *etc.* Vectors may be provided directly to the subject cells. In other words, the pluripotent cells are contacted with vectors comprising the nucleic acid of interest such that the vectors are taken up by the cells.

[0101] Methods for contacting cells, *e.g.* cells in culture or cells in a mouse, with nucleic acid vectors, such as electroporation, calcium chloride transfection, and lipofection, are well known in the art. Alternatively, the nucleic acid of interest may be provided to the cells via a virus. In other words, the cells are contacted with viral particles comprising the nucleic acid of interest. Retroviruses, for example, lentiviruses, are particularly suitable to the method of the invention. Commonly used retroviral vectors are "defective", *i.e.* unable to produce viral proteins required for productive infection. Rather, replication of the vector requires growth in a packaging cell line. To generate viral particles comprising nucleic acids of interest, the retroviral nucleic acids comprising the nucleic acid are packaged into viral capsids by a packaging cell line. Different packaging cell lines provide a different envelope protein to be incorporated into the capsid, this envelope protein determining the specificity of the viral particle for the cells. Envelope proteins are of at least three types, ecotropic, amphotropic and xenotropic. Retroviruses packaged with ecotropic envelope protein, *e.g.* MMLV, are capable of infecting most murine and rat cell types, and are generated by using ecotropic packaging cell lines such as BOSC23 (Pear et al. (1993) P.N.A.S. 90:8392-8396). Retroviruses bearing amphotropic envelope protein, *e.g.* 4070A (Danos *et al, supra.),* are capable of infecting most mammalian cell types, including human, dog and mouse, and are generated by using amphotropic packaging cell lines such as PA12 (Miller et al. (1985) Mol. Cell. Biol. 5:431-437); PA317 (Miller et al. (1986) Mol. Cell. Biol. 6:2895-2902); GRIP (Danos et al. (1988) PNAS 85:6460-6464). Retroviruses packaged with xenotropic envelope protein, e.g. AKR env, are capable of infecting most mammalian cell types, except murine cells. The appropriate packaging cell line may be used to ensure that the cells of interest-in some instance, the engrafted cells, in some instance, the cells of the host, *i.e.* the genetically modified animal--are targeted by the packaged viral particles.

[0102] Vectors used for providing nucleic acid of interest to the subject cells will typically comprise suitable promoters for driving the expression, that is, transcriptional activation, of the nucleic acid of interest. This may include ubiquitously acting promoters, for example, the CMV-β-actin promoter, or inducible promoters, such as promoters that are active in particular cell populations or that respond to the presence of drugs such as tetracycline. By transcriptional activation, it is intended that transcription will be increased above basal levels in the target cell by at least about 10 fold, by at least about 100 fold, more usually by at least about 1000 fold. In addition, vectors used for providing reprogramming factors to the subject cells may include genes that must later be removed, *e.g.* using a recombinase system such as Cre/Lox, or the cells that express them destroyed, *e.g.* by including genes that allow selective toxicity such as herpesvirus TK, bcl-xs, etc.

[0103] Candidate agents of interest for screening also include polypeptides. Such polypeptides may optionally be fused to a polypeptide domain that increases solubility of the product. The domain may be linked to the polypeptide through a defined protease cleavage site, *e.g.* a TEV sequence, which is cleaved by TEV protease. The linker may also include one or more flexible sequences, *e.g.* from 1 to 10 glycine residues. In some embodiments, the cleavage of the fusion protein is performed in a buffer that maintains solubility of the product, *e.g.* in the presence of from 0.5 to 2 M urea, in the presence of polypeptides and/or polynucleotides that increase solubility, and the like. Domains of interest include endosomolytic domains, *e.g.* influenza HA domain; and other polypeptides that aid in production, *e.g.* IF2 domain, GST domain, GRPE domain, and the like. Additionally or alternatively, such polypeptides may be formulated for improved stability. For example, the peptides may be PEGylated, where the polyethyleneoxy group provides for enhanced lifetime in the blood stream. The polypeptide may be fused to another polypeptide to provide for added functionality, *e.g.* to increase the *in vivo* stability. Generally such fusion partners are a stable plasma protein, which may, for example, extend the *in vivo* plasma half-life of the polypeptide when present as a fusion, in particular wherein such a stable plasma protein is an immunoglobulin constant domain. In most cases where the stable plasma protein is normally found in a multimeric form, e.g., immunoglobulins or lipoproteins, in which the same or different polypeptide chains are normally disulfide and/or noncovalently bound to form an assembled multichain polypeptide, the fusions herein containing the polypeptide also will be produced and employed as a multimer having substantially the same structure as the stable plasma protein precursor. These multimers will be homogeneous with respect to the polypeptide agent they comprise, or they may contain more than one polypeptide agent.

[0104] The candidate polypeptide agent may be produced from eukaryotic cells, or may be produced by prokaryotic cells. It may be further processed by unfolding, *e.g.* heat denaturation, DTT reduction, *etc.* and may be further refolded, using methods known in the art. Modifications of interest that do not alter primary sequence include chemical derivatization of polypeptides, *e.g.,* acylation, acetylation, carboxylation, amidation, *etc.* Also included are modifications of glycosylation, *e.g.* those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps; *e.g.* by exposing the polypeptide to enzymes which affect glycosylation, such as mammalian glycosylating or deglycosylating enzymes. Also embraced are sequences that have phosphorylated amino acid residues, *e.g.* phosphotyrosine, phosphoserine, or phosphothreonine. The polypeptides may have been modified using ordinary molecular biological techniques and synthetic chemistry so as to improve their resistance to proteolytic degradation or to optimize solubility properties or to render them more suitable as a therapeutic agent. Analogs of such polypeptides include those containing residues other than naturally occurring L-amino acids, *e.g.* D-amino acids or non-naturally occurring synthetic amino acids. D-amino acids may be substituted for some or all of the amino acid residues.

[0105] The candidate polypeptide agent may be prepared by *in vitro* synthesis, using conventional methods as known in the art. Various commercial synthetic apparatuses are available, for example, automated synthesizers by Applied Biosystems, Inc., Beckman, *etc.* By using synthesizers, naturally occurring amino acids may be substituted with unnatural amino acids. The particular sequence and the manner of preparation will be determined by convenience, economics, purity required, and the like. Alternatively, the candidate polypeptide agent may be isolated and purified in accordance with conventional methods of recombinant synthesis. A lysate may be prepared of the expression host and the lysate purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. For the most part, the compositions which are used will comprise at least 20% by weight of the desired product, more usually at least about 75% by weight, preferably at least about 95% by weight, and for therapeutic purposes, usually at least about 99.5% by weight, in relation to contaminants related to the method of preparation of the product and its purification. Usually, the percentages will be based upon total protein.

[0106] In some cases, the candidate polypeptide agents to be screened are antibodies. The term "antibody" or "antibody moiety" is intended to include any polypeptide chain-containing molecular structure with a specific shape that fits to and recognizes an epitope, where one or more non-covalent binding interactions stabilize the complex between the molecular structure and the epitope. The specific or selective fit of a given structure and its specific epitope is sometimes referred to as a "lock and key" fit. The archetypal antibody molecule is the immunoglobulin, and all types of immunoglobulins, IgG, IgM, IgA, IgE, IgD, *etc.,* from all sources, *e.g.* human, rodent, rabbit, cow, sheep, pig, dog, other mammal, chicken, other avians, *etc.,* are considered to be "antibodies." Antibodies utilized in the present invention may be either polyclonal antibodies or monoclonal antibodies. Antibodies are typically provided in the media in which the cells are cultured. Antibody production and screen is discussed in greater detail below.

[0107] Candidate agents may be obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds, including biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, *etc.* to produce structural analogs.

[0108] Candidate agents are screened for biological activity by administering the agent to at least one and usually a plurality of samples, sometimes in conjunction with samples lacking the agent. The change in parameters in response to the agent is measured, and the result evaluated by comparison to reference samples, *e.g.* in the presence and absence of the agent, obtained with other agents, *etc.* In instances in which a screen is being performed to identify candidate agents that will prevent, mitigate or reverse the effects of a pathogen, the screen is typically performed in the presence of the pathogenic agent, where the pathogenic agent is added at the time most appropriate to the results to be determined. For example, in cases in which the protective/preventative ability of the candidate agent is tested, the candidate agent may be added before the pathogen, simultaneously with the pathogen, or subsequent to infection by the pathogen. As another example, in cases in which the ability of the candidate agent to reverse the effects of a pathogen is tested, the candidate agent may be added subsequent to infection with the pathogen. As mentioned above, in some instances, the "sample" is a genetically modified mouse that has been engrafted with cells, *e.g.* the candidate agent is provided to an immunodeficient mouse, comprising a nucleic acid encoding human EPO operably linked to an EPO promoter that has been engrafted with human hematopoietic cells. In some instances, the sample is the human hematopoietic cells to be engrafted, *i.e.* the candidate agent is provided to cells, e.g. reticulocytes, erythrocytes, etc., prior to engraftment into the immunodeficient genetically modified mouse.

[0109] If the candidate agent is to be administered directly to the engrafted genetically modified mouse, the agent may be administered by any of a number of well-known methods in the art for the administration of peptides, small molecules and nucleic acids to mice. For example, the agent may be administered orally, mucosally, topically, intradermally, or by injection, *e.g.* intraperitoneal, subcutaneous, intramuscular, intravenous, or intracranial injection, and the like. The agent may be administered in a buffer, or it may be incorporated into any of a variety of formulations, *e.g.* by combination with appropriate pharmaceutically acceptable vehicle. "Pharmaceutically acceptable vehicles" may be vehicles approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, such as humans. The term "vehicle" refers to a diluent, adjuvant, excipient, or carrier with which a compound of the invention is formulated for administration to a mammal. Such pharmaceutical vehicles can be lipids, *e.g.* liposomes, *e.g.* liposome dendrimers; liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like, saline; gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents may be used. Pharmaceutical compositions may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. The agent may be systemic after administration or may be localized by the use of regional administration, intramural administration, or use of an implant that acts to retain the active dose at the site of implantation. The active agent may be formulated for immediate activity or it may be formulated for sustained release. For some conditions, particularly central nervous system conditions, it may be necessary to formulate agents to cross the blood-brain barrier (BBB). One strategy for drug delivery through the blood-brain barrier (BBB) entails disruption of the BBB, either by osmotic means such as mannitol or leukotrienes, or biochemically by the use of vasoactive substances such as bradykinin. A BBB disrupting agent can be co-administered with the agent when the compositions are administered by intravascular injection. Other strategies to go through the BBB may entail the use of endogenous transport systems, including Caveolin-1 mediated transcytosis, carrier-mediated transporters such as glucose and amino acid carriers, receptor-mediated transcytosis for insulin or transferrin, and active efflux transporters such as p-glycoprotein. Active transport moieties may also be conjugated to the therapeutic compounds for use in the invention to facilitate transport across the endothelial wall of the blood vessel. Alternatively, drug delivery of agents behind the BBB may be by local delivery, for example by intrathecal delivery, *e.g.* through an Ommaya reservoir (see *e.g.* US Patent Nos. 5,222,982 and 5,385,582); by bolus injection, *e.g.* by a syringe, *e.g.* intravitreally or intracranially; by continuous infusion, *e.g.* by cannulation, *e.g.* with convection (see *e.g.* US Application No. 20070254842); or by implanting a device upon which the agent has been reversably affixed (see *e.g.* US Application Nos. 20080081064 and 20090196903).

[0110] If the agent(s) are provided to cells prior to engraftment, the agents are conveniently added in solution, or readily soluble form, to the medium of cells in culture. The agents may be added in a flow-through system, as a stream, intermittent or continuous, or alternatively, adding a bolus of the compound, singly or incrementally, to an otherwise static solution. In a flow-through system, two fluids are used, where one is a physiologically neutral solution, and the other is the same solution with the test compound added. The first fluid is passed over the cells, followed by the second. In a single solution method, a bolus of the test compound is added to the volume of medium surrounding the cells. The overall concentrations of the components of the culture medium should not change significantly with the addition of the bolus, or between the two solutions in a flow through method.

[0111] A plurality of assays may be run in parallel with different agent concentrations to obtain a differential response to the various concentrations. As known in the art, determining the effective concentration of an agent typically uses a range of concentrations resulting from 1:10, or other log scale, dilutions. The concentrations may be further refined with a second series of dilutions, if necessary. Typically, one of these concentrations serves as a negative control, *i.e.* at zero concentration or below the level of detection of the agent or at or below the concentration of agent that does not give a detectable change in the phenotype.

[0112] An analysis of the response of cells in the engrafted genetically modified animal to the candidate agent may be performed at any time following treatment with the agent. For example, the cells may be analyzed 1, 2, or 3 days, sometimes 4, 5, or 6 days, sometimes 8, 9, or 10 days, sometimes 14 days, sometimes 21 days, sometimes 28 days, sometimes 1 month or more after contact with the candidate agent, *e.g.* 2 months, 4 months, 6 months or more. In some embodiments, the analysis comprises analysis at multiple time points. The selection of the time point(s) for analysis will be based upon the type of analysis to be performed, as will be readily understood by the ordinarily skilled artisan.

[0113] The analysis may comprise measuring any of the parameters described herein or known in the art for measuring cell viability, cell proliferation, cell identity, cell morphology, and cell function, particularly as they may pertain to cells of the immune cells. For example, flow cytometry may be used to determine the total number of hematopoietic cells or the number of cells of a particular hematopoietic cell type. Histochemistry or immunohistochemistry may be performed to determine the apoptotic state of the cells, e.g. terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) to measure DNA fragmentation, or immunohistochemistry to detect Annexin V binding to phosphatidylserine on the cell surface. Flow cytometry may also be employed to assess the proportions of differentiated cells and differentiated cell types, *e.g.* to determine the ability of hematopoietic cells to survive and/or differentiate in the presence of agent. ELISAs, Westerns, and Northern blots may be performed to determine the levels of cytokines, chemokines, immunoglobulins, *etc.* expressed in the engrafted genetically modified mice, *e.g.* to assess the function of the engrafted cells, to assess the survival of erythrocytes, etc. In vivo assays to test the function of immune cells, as well as assays relevant to particular diseases or disorders of interest such as anemia, e.g. sickle cell anemia, *etc.* may also be performed. See, *e.g.* Current Protocols in Immunology (Richard Coico, ed. John Wiley & Sons, Inc. 2012) and Immunology Methods Manual (I. Lefkovits ed., Academic Press 1997).

[0114] So, for example, a method is provided for determining the effect of an agent on erythroid cells infectable or infected by pathogen, comprising administering the agent to a human EPO mouse, *e.g.* a $Rag2^{-/-}IL2rg^{-/-}EPO^{m/h}$ mouse, that has been engrafted with human reticulocytes and/or erythrocytes; measuring a parameter of the viability of the engrafted cells over time in the presence of the agent; and comparing that measurement to the measurement from an engrafted human EPO mouse not exposed to the agent. The agent is determined to be anti-pathogenic if it reduces the infection of and/or the death of human erythrocytes in the peripheral blood of the mouse by at least 20%, 30%, 40% or more, in some instances 50%, 60%, 70% or more, e.g. 80%, 90% or 100%, i.e., to undetectable amounts, following a single administration or two or more administrations of the agent over a selected period of time. In a specific embodiment, the administration of the drug or combination of drugs is at least three days, at least one week, at least 10 days after engraftment with human hematopoietic cells, for example, two week, three weeks, or four weeks after engraftment with human hematopoietic cells, e.g. 6 weeks, 8 weeks, 10 weeks or more after engraftment with human hematopoietic cells.

[0115] Other examples of uses for the subject mice are provided elsewhere herein. Additional applications of the genetically modified and engrafted mice described in this disclosure will be apparent to those skilled in the art upon reading this disclosure.

EXAMPLES

[0116] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

[0117] General methods in molecular and cellular biochemistry can be found in such standard textbooks as Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook et al., HaRBor Laboratory Press 2001); Short Protocols in Molecular Biology, 4th Ed. (Ausubel et al. eds., John Wiley & Sons 1999); Protein Methods (Bollag et al., John Wiley & Sons 1996); Nonviral Vectors for Gene Therapy (Wagner et al. eds., Academic Press 1999); Viral Vectors (Kaplift & Loewy eds., Academic Press 1995); Immunology Methods Manual (I. Lefkovits ed., Academic Press 1997); and Cell and Tissue Culture: Laboratory Procedures in Biotechnology (Doyle & Griffiths, John Wiley & Sons 1998).

[0118] Reagents, cloning vectors, and kits for genetic manipulation referred to in this disclosure are available from commercial vendors such as BioRad, Stratagene, Invitrogen, Sigma-Aldrich, and ClonTech.

Example 1

Generation of human EPO knock-in mice.

[0119] Coding sequence at the mouse erythropoietin gene locus (Mouse NCBI Gene ID:13856; MGL95407; RefSeq transcript cDNA at NM_007942.2 (SEQ ID NO:1) and encoded protein at NP_031968 (SEQ ID NO:2)) was replaced with coding sequence from the human erythropoietin gene locus (Human NCBI Gene ID:2056; HGNC: 3415; RefSeq cDNA transcript at NM_000799.2 (SEQ ID NO:3) and encoded protein at NP_000790 (SEQ ID NO:4)).

Table 1

SEQ ID NO:1	1 gatgaagact tgcagcgtgg acactggccc agccccgggt cgctaaggag ctccggcagc 61 taggcgcgga gatgggggtg cccgaacgtc ccaccctgct gcttttactc tccttgctac 121 tgattcctct gggcctccca gtcctctgtg ctcccccacg cctcatctgc gacagtcgag 181 ttctggagag gtacatctta gaggccaagg aggcagaaaa tgtcacgatg ggttgtgcag 241 aaggtcccag actgagtgaa aatattacag tcccagatac caaagtcaac ttctatgctt 301 ggaaaagaat ggaggtggaa gaacaggcca tagaagtttg gcaaggcctg tccctgctct 361 cagaagccat cctgcaggcc caggccctgc tagccaattc ctcccagcca ccagagaccc 421 ttcagcttca tatagacaaa gccatcagtg gtctacgtag cctcacttca ctgcttcggg 481 tactgggagc tcagaaggaa ttgatgtcgc ctccagatac caccccacct gctccactcc 541 gaacactcac agtggatact ttctgcaagc tcttccgggt ctacgccaac ttcctccggg 601 ggaaactgaa gctgtacacg ggagaggtct gcaggagagg ggacaggtga catgctgctg 661 ccaccgtggt ggaccgacga acttgctccc cgtcactgtg tcatgccaac cctcc (SEQ ID NO:1)
SEQ ID NO:2	MGVPERPTLLLLLSLLLIPLGLPVLCAPPRLICDSRVLERYILEAKEAENVTMGCAEGPRLSENITVP DTKVNFYAWKRMEVEEQAIEVWQGLSLLSEAILQAQALLANSSQPPETLQLHIDKAISGLRSLTSL LRVLGAQKELMSPPDTTPPAPLRTLTVDTFCKLFRVYANFLRGKLKLYTGEVCRRGDR (SEQ ID NO:2)

Table 1 Cont.

SEQ ID NO:3	1 cccggagccg gaccggggcc accgcgcccg ctctgctccg acaccgcgcc ccctggacag 61 ccgccctctc ctccaggccc gtggggctgg ccctgcaccg ccgagcttcc cgggatgagg 121 gcccccggtg tggtcacccg gcgcgcccca ggtcgctgag ggaccccggc caggcgcgga 181 gatgggggtg cacgaatgtc ctgcctggct gtggcttctc ctgtccctgc tgtcgctccc 241 tctgggcctc ccagtcctgg gcgccccacc acgcctcatc tgtgacagcc gagtcctgga 301 gaggtacctc ttggaggcca aggaggccga gaatatcacg acgggctgtg ctgaacactg 361 cagcttgaat gagaatatca ctgtcccaga caccaaagtt aatttctatg cctggaagag 421 gatggaggtc gggcagcagg ccgtagaagt ctggcagggc ctggccctgc tgtcggaagc 481 tgtcctgcgg ggccaggccc tgttggtcaa ctcttcccag ccgtgggagc ccctgcagct 541 gcatgtggat aaagccgtca gtggccttcg cagcctcacc actctgcttc gggctctggg 601 agcccagaag gaagccatct cccctccaga tgcggcctca gctgctccac tccgaacaat 661 cactgctgac actttccgca aactcttccg agtctactcc aatttcctcc ggggaaagct 721 gaagctgtac acaggggagg cctgcaggac aggggacaga tgaccaggtg tgtccacctg 781 ggcatatcca ccacctccct caccaacatt gcttgtgcca caccctcccc cgccactcct 841 gaaccccgtc gaggggctct cagctcagcg ccagcctgtc ccatggacac tccagtgcca 901 gcaatgacat ctcaggggcc agaggaactg tccagagagc aactctgaga tctaaggatg 961 tcacagggcc aacttgaggg cccagagcag gaagcattca gagagcagct ttaaactcag 1021 ggacagagcc atgctgggaa gacgcctgag ctcactcggc accctgcaaa atttgatgcc 1081 aggacacgct ttggaggcga tttacctgtt ttcgcaccta ccatcaggga caggatgacc 1141 tggagaactt aggtggcaag ctgtgacttc tccaggtctc acgggcatgg gcactccctt 1201 ggtggcaaga gcccccttga caccggggtg gtgggaacca tgaagacagg atgggggctg 1261 gcctctggct ctcatggggt ccaagttttg tgtattcttc aacctcattg acaagaactg 1321 aaaccaccaa aaaaaaaaaa(SEQ ID NO:3)
SEQ ID NO:4	MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAEHC SLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQPWEP LQLHVDKAVSGLRSLTTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRVYSNFLR

Table 1 Cont.	
	GKLKLYTGEACRTGDR (SEQ ID NO:4)

[0120] Specifically, the mouse genomic region at GRCm38: ch5: 137482017:137485745 (minus strand) was deleted and human genomic sequence from GRCh37: ch7:100318604:100321567 (plus strand) was inserted in its place. This resulted in the replacement of coding exons 1 to 5 -- the entire coding region -- of the mouse Epo gene with exons 1 to 5 plus the 3' human untranslated region of the human EPO gene. In total, 3729 nt of mouse sequence was replaced with 2964 nt human sequence.

[0121] Briefly, a targeting construct for replacing the mouse EPO gene with the human EPO gene in a single targeting step was constructed using VELOCIGENE® genetic engineering technology (see, Valenzuela et al. (2003), *supra,* and U.S. Patent No. 6,586,251). Mouse and human EPO DNA were obtained from bacterial artificial chromosomes bMQ-386K4 and RP1 1-797M3, respectively. A PspXI-linearized targeting construct generated by gap repair cloning containing mouse EPO upstream and downstream homology arms flanking a 2964 nt human EPO sequence extending from the ATG in exon 1 through the stop codon in exon 5 (i.e. including 3' downstream sequence) plus a floxed neo selection cassette was electroporated into Rag2$^{-/-}$ IL2rg $^{Y/-}$ ES cells (FIG. 2 and FIG. 3). The junction between the mouse EPO 5' untranslated region (UTR) and exon 1 of human EPO is provided as SEQ ID NO: 5, wherein the final mouse nucleotide prior to the first nucleotide of the human gene is the "G" (shown in brackets) in the portion of SEQ ID NO:5 which is not bolded in Table 2 below, and the first nucleotide of the human sequence is the "A" (shown in brackets) in the bolded portion of SEQ ID NO:5 in Table 2 below.

Table 2

SEQ ID NO:5	TCTTCCAGGCTAGTGGGGTGATCTGGCCCTACAGAACTTCCAAGGATGAAGACTTGCAG CGTGGACACTGGCCCAGCCCCGGGTCGCTAAGGAGCTCCGGCAGCTAGGCGCGGA[G][*A* ***]TGGGGGTGCACG*GTGAGTACTCGCGGCTGGGCGCTCCCGCCCGCCCGGGTCCCT** **GTTTGAGCGGGGATTTAGCGCCCCGGCTATTGGCCAGGAGGTGGCTGGGTTCAAG** (SEQ ID NO:5)
	(coding sequence of human exon 1 is italicized, bold is human, not bold is mouse)

[0122] The junction between the human 3' UTR and the 5' end of the selection cassette is provided as SEQ ID NO:6, wherein the final nucleotide of the human sequence is the "C" (shown in single brackets) in the bolded portion of SEQ ID NO:6 in Table 3 below and the first nucleotide of the selection cassette sequence is the "C" (shown in double brackets) in the non-bolded portion of SEQ ID NO:6 in Table 3 below; the downstream junction region also contained a loxP site at the 3' end for removal of a floxed ubiquitin promoter-driven neo cassette.

Table 3

SEQ ID NO:6	***...............ACTCCGAACAATCACTGCTGACACTTTCCGCAAACTCTTCCGAGTCTACTCCAATTTCCTCCGGGGAAAGCTGAAGCTGTACACAGGGGAGGCCTGCAGGACAGGGGACAGATGA*****CCAGGTGTGTCCACCTGGGCATATCCACCACCTCCCTCACCAACATTGCTTGTGCCACACCCTCCCCCGCCACTCCTGAACCCCGTCGAGGGGCTCTCAGCTCAGCGCCAGCCTGTCCCATGGACACTCCAGTGCCAGCAATGACATCTCAGGGGCCAGAGGAACTGTCCAGAGAGCAACTCTGAGATCTAAGGATGTCACAGGGCCAACTTGAGGGCCCAGAGCAGGAAGCATTCAGAGAGCAGCTTTAAACTCAGGGACAGAGCCATGCTGGGAAGACGCCTGAGCTCACTCGGCACCCTGCAAAATTTGATGCCAGGACACGCTTTGGAGGCGATTTACCTGTTTTCGCACCTACCATCAGGGACAGGATGACCTGGAGAACTTAGGTGGCAAGCTGTGACTTCTCCAGGTCTCACGGGCATGGGCACTCCCTTGGTGGCAAGAGCCCCCTTGACACCGGGGTGGTGGGAACCATGAAGACAGGATGGGGGCTGGCCTCTGGCTCTCATGGGGTCCAAGTTTTGTGTATTCTTCAACCTCATTGACAAGAACTGAAACCACCAATATGACTCTTGGCTTTTCTGTTTTCTGGGAACCTCCAAATCCCCTGGCTCTGTCCCACTCCTGGCAGCAGTGCAGCAGGTCCAGGTCCGGGAAACGAGGGGTGGAGGGGGCTGGGCCCTACGTGCTGTCTCACACAGCCTGTCTGACCTCTCGACCCTACCGGGCCTGAGGCCACAAGCTCTGCCTACGCTGGTCAATAAGGTGTCTCCATTCAAGGCCTCACCGCAGTAAGGCAGCTGCCAA**[C][[C]]TCGAGATAACTTCGTATAATGTATGCTATACGAAGTTATATGCATGGCCTCCGCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCTCCTCACGGCGAGCGCTGCCACGTCAGACGAAGGGCGCAG... .... (bold is human, italicized is coding sequence of human exon 5; not bold is 5' end of selection cassette)

[0123] The junction between the 3' end of the selection cassette and the mouse genome is provided as SEQ ID NO:7, where the "C" shown in single brackets is the final nucleotide of the neo cassette and the first nucleotide of the mouse genome following the cassette is the "G" shown in double brackets, as shown in Table 4 below.

Table 4

SEQ ID NO: 7	*GCCTCTGTTCCACATACACTTCATTCTCAGTATTGTTTTGCCAAGTTCTAATTCCATCAGACCTCGACCTGCAGCCCCTAGATAACTTCGTATAATGTATGCTATACGAAGTTATGCTAG[C]*[[G]]CCAACCCGCTAGGACAAGTGCTGAGTGAGCTGGGGCCACCGTTTGAGGAAACAGGAGCCAGTACAGAGGGGTTCCCCTTTAGGGGTTGGTGGCAATGGGCGACCCTGGTTAATGGATCATT...............
	(3' end of selection cassette shown in italics, mouse sequence is shown not italicized.)

[0124] Correctly targeted hEPO ES cell clones were identified by a loss-of-native-allele (LONA) assay (Valenzuela et al. (2003), *supra)* in which the number of copies of the native, unmodified EPO gene were determined by two TaqMan™ quantitative polymerase chain reactions (qPCRs) specific for sequences in the mouse EPO gene that were targeted for deletion. The qPCR assays comprised the following primer-probe sets (written 5' to 3'): upstream forward primer, CATCTGCGACAGTCGAGTTC (SEQ ID NO:8); upstream reverse primer, CCAGGGAGCTTACCGTGAC (SEQ ID NO:9); upstream probe, FAM-AGGTACATCTTAGAGGCCAAGGAGGCA-BHQ (SEQ ID NO:10); downstream forward primer, ACAGCCGAGTCCTGGAGAG (SEQ ID NO:11); downstream reverse primer, AAGCCCTGAGCGTGAGTTC (SEQ ID NO:12); downstream probe, FAM-AGGCCAAGGAGGCCGAGAATATCACG-BHQ (SEQ ID NO:13); in which FAM refers to the 5-

carboxyfluorescein fluorescent probe and BHQ refers to the fluorescence quencher of the black hole quencher type (Biosearch Technologies). DNA purified from ES cell clones that have taken up the targeting vector and incorporated in their genomes was combined with TaqMan™ Gene Expression Master Mix (Life Technologies) according to the manufacturer's suggestions in a 384-well PCR plate (MicroAmp™ Optical 384-Well Reaction Plate, Life Technologies) and cycled in an Applied Biosystems Prism 7900HT, which collects fluorescence data during the course of the PCRs and determines a threshold cycle (Ct), the fractional PCR cycle at which the accumulated fluorescence reaches a pre-set threshold. The upstream and downstream EPO-specific qPCRs and two qPCRs for non-targeted reference genes were run for each DNA sample. The differences in the Ct values (ΔCt) between each EPO-specific qPCR and each reference gene qPCR were calculated, and then the difference between each ΔCt and the median ΔCt for all samples assayed was calculated to obtain ΔΔCt values for each sample. The copy number of the EPO gene in each sample was calculated from the following formula: copy number = $2 \cdot 2^{-\Delta\Delta Ct}$. A correctly targeted clone, having lost one of its native copies, will have an EPO gene copy number equal to one. Confirmation that the human EPO gene sequence replaced the deleted mouse EPO gene sequence in the humanized allele was confirmed by a TaqMan™ qPCR assay that comprises the following primer-probe sets (written 5' to 3'): the human forward primer, GAGCCCTGCACTGGACAAC (SEQ ID NO:14); the human reverse primer, TCCCATGAACGCTGAGAGTC (SEQ ID NO:15); and the human probe, AGGGTCAAGGAGCCATAGACAGAATGGC (SEQ ID NO:16).

[0125] Correctly targeted ES cells were electroporated with a transient Cre-expressing vector to remove the drug selection cassette. To generate a mouse comprising human EPO and deficient for *Rag2* and *Il2rg,* correctly targeted ES cells were identified as described above and introduced into preimplantation embryo using techniques known in the art. Human EPO knock-in (KI) mice were then backcrossed to generate mice deficient for *Rag2* and *Il2rg* and expressing human EPO.

Example 2

Generation of human SIRPα-mice.

[0126] In connection with some of the examples described herein, a genetically modified mouse including a nucleic acid sequence encoding human SIRPα randomly integrated into the genome of the genetically modified mouse was prepared as described in U.S. Patent Application Publication No. 2013-0340105.

[0127] In connection with some of the examples described herein, a human SIRPα knock-in mouse was prepared as described below. Human SIRPα is known to exist in at least 10 allelic forms. In this particular example, human SIRPα variant 1 is employed for humanizing an endogenous SIRPα gene of a mouse.

[0128] A targeting vector for humanization of an extracellular region of a SIRP (e.g., SIRPα) gene was constructed using VELOCIGENE® technology (see, e.g., U.S. Pat. No. 6,586,251 and Valenzuela et al. (2003), *supra).*

[0129] Briefly, mouse bacterial artificial chromosome (BAC) clone bMQ-261H14 was modified to delete the sequence containing exons 2 to 4 of an endogenous SIRPα gene and insert exons 2 to 4 of a human SIRPα gene using human BAC clone CTD-3035H21. The genomic DNA corresponding to exons 2 to 4 of an endogenous SIRPα gene (-8555 bp) was replaced in BAC clone bMQ-261H14 with a -8581 bp DNA fragment containing exons 2 to 4 of a human SIRPα gene from BAC clone CTD-3035H21. Sequence analysis of the human SIRPα allele contained in BAC clone CTD-3035H21 revealed the allele to correspond to human variant 1. A neomycin cassette flanked by *lox*P sites was added to the end of the -8581 bp human DNA fragment containing exons 2 to 4 of the human SIRPα gene (FIG. 4).

[0130] Upstream and downstream homology arms were obtained from mouse BAC DNA at positions 5' and 3' of exons 2 and 4, respectively, and added to the -8581 bp human fragment-neomycin cassette to create the final targeting vector for humanization of an endogenous SIRPα gene, which contained from 5' to 3' a 5' homology arm containing 19 kb of mouse DNA 5' of exon 2 of the endogenous SIRPα gene, a -8581 bp DNA fragment containing exons 2 to 4 of a human SIRPα gene, a neomycin cassette flanked by *lox*P sites, and a 3' homology arm containing 21 kb of mouse DNA 3' of exon 4 of an endogenous SIRPα gene. Targeted insertion of the targeting vector positioned the neomycin cassette in the fifth intron of a mouse SIRPα gene between exons 4 and 5. The targeting vector was linearized by digesting with SwaI and then used in homologous recombination in bacterial cells to achieve a targeted replacement of exons 2 to 4 in a mouse SIRPα gene with exons 2 to 4 of a human SIRPα gene (FIG. 4).

[0131] The targeted BAC DNA (described above) was used to electroporate mouse ES cells to created modified ES cells comprising a replacement of exons 2 to 4 in an endogenous mouse SIRPα gene with a genomic fragment comprising exons 2 to 4 of a human SIRPα gene. Positive ES cells containing a genomic fragment comprising exons 2 to 4 of a human SIRPα gene were identified by quantitative PCR using TAQMAN™ probes (Lie and Petropoulos, 1998. Curr. Opin. Biotechnology 9:43-48). The nucleotide sequence across the upstream insertion point included the following, which indicates endogenous mouse sequence upstream of the insertion point (contained within the parentheses below) linked contiguously to a human SIRPα genomic sequence present at the insertion point:
(AGCTCTCCTACCACTAGACTGCTGAGACCCGCTGCTCTGCTCAGGACTCGATTTC
CAGTACACAATCTCCCTCTTTGAAAAGTACCACACATCCTGGGGT)GCTCTTGCAT
TTGTGTGACACTTTGCTAGCCAGGCTCAGTCCTGGGTTCCAGGTGGGGACTCAAA
CACACTGGCACGAGTCTACATTGGATATTCTTGGT (SEQ ID NO: 17). The nucleotide sequence across the downstream insertion point at the 5' end of the neomycin cassette included the following, which indicates human SIRPα genomic sequence contiguous with cassette sequence downstream of the insertion point (contained within the parentheses below

with *lox*P sequence italicized):
GCTCCCCATTCCTCACTGGCCCAGCCCCTCTTCCCTACTCTTTCTAGCCCCTGCCTC
ATCTCCCTGGCTGCCATTGGGAGCCTGCCCCACTGGAAGCCAG(TCGAG*ATAACTT*
*CGTATAATGTATGCTATACGAAGTTAT*ATGCATGGCCTCCGCGCCGGGTTTTGGCGCC
TCCCGCGGGCGCCCCCCTCCTCACGGCGA) (SEQ ID NO: 18). The nucleotide sequence across the downstream insertion point at the 3' end of the neomycin cassette included the following, which indicates cassette sequence contiguous with mouse genomic sequence 3' of exon 4 of an endogenous SIRPα gene (contained within the parentheses below):
CATTCTCAGTATTGTTTTGCCAAGTTCTAATTCCATCAGACCTCGACCTGCAGCCC
CTAGATAACTTCGTATAATGTATGCTATACGAAGTTATGCTAGC(TGTCTCATAGA
GGCTGGCGATCTGGCTCAGGGACAGCCAGTACTGCAAAGAGTATCCTTGTTCATA
CCTTCTCCTAGTGGCCATCTCCCTGGGACAGTCA) (SEQ ID NO: 19). Positive ES cell clones were then used to implant female mice using the VELOCIMOUSE® method (see, e.g., U.S. Pat. No. 7,294,754 and Poueymirou et al. 2007, F0 generation mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analyses Nature Biotech. 25(1):91-99, *supra)* to generate a litter of pups containing an insertion of exons 2 to 4 of a human SIRPα gene into an endogenous SIRPα gene of a mouse.

[0132] Targeted ES cells described above were used as donor ES cells and introduced into an 8-cell stage mouse embryo by the VELOCIMOUSE® method *(supra).* Mice bearing the humanization of exons 2 to 4 of an endogenous SIRPα gene were identified by genotyping using a modification of allele assay (Valenzuela et al. (2003), *supra)* that detected the presence of the human SIRPα gene sequences.

[0133] Mice bearing the humanized SIRPα gene construct (i.e., containing human SIRPα exons 2 to 4 in a mouse SIRPα gene) can be bred to a Cre deletor mouse strain (see, e.g., International Patent Application Publication No. WO 2009/114400) in order to remove any loxed neomycin cassette introduced by the targeting vector that is not removed, e.g., at the ES cell stage or in the embryo. Optionally, the neomycin cassette is retained in the mice.

Example 3

Generation of compound knock-in mice.

[0134] Human EPO knock-in mice were crossed to mice expressing other human genes of interest either as random integrants into the mouse genome or as knock-ins, i.e. from the corresponding mouse locus. For example, *Rag2*$^{-/-}$, *IL-2rg*$^{Y/-}$, *hEPO* KI mice were crossed with mice expressing human TPO from the mouse TPO locus (Rongvaux et al., 2011, Proc Natl Acad Sci USA, 108(6): 2378-2383), human IL-3 and human GM-CSF from the mouse IL-3/GM-CSF locus (Willinger et al, 2011, Proc Natl Acad Sci USA, 108(6): 2390-2395), human M-CSF from the mouse M-CSF locus (Rathinam et al, 2011, Blood, 118(11): 3119-3128), and/or human SIRPa expressed as a random integrant (Strowig et al., 2011, Proc Natl Acad Sci USA, 108(32): 13218-13223) or from the mouse locus as described above to generate mice expressing a combination of these human proteins ($Rag2^{-/-}Il2rg^{null}hSIRP\alpha^{h/h}\ Tpo^{h/h}\ Mcsf^{h/h}\ Il3/Gmcsf^{h/h}\ EPO^{h/h}$). Genetically modified mice expressing one or more of human TPO, human IL-3, human GM-CSF, human M-CSF, and human SIRPa are described in greater detail in U.S. Patent Nos. 8,541,646 and 8,847,004; U.S. Patent Application Publication No. 2014/0134662; and PCT International Publication No. WO/2014/039782.

Example 4

Development of a humanized mouse model for the blood stages of *P. falciparum* and P. *vivax*

[0135] It is demonstrated here that genetic humanization of the murine host by providing growth factors with limited cross-reactivity from mouse to man can successfully boost human cell engraftment in general and erythropoiesis in particular in human hematopoietic stem cell (HSC)-engrafted mice.

[0136] MITERG mice expressing human EPO from their genome (to enhance terminal erythropoiesis) as well as human TPO, MCSF, and IL-3 (to enhance HSC maintenance and early erythropoiesis) feature higher levels of human erythropoiesis in the bone marrow of human HSC-engrafted mice than mice not expressing hEPO (FIG. 5A, compare "hSIRPa-, hEPO+" (i.e. "MITERG mice"), to "hSIRPa-, hEPO-" (i.e. "MITRG mice")), in fact equivalent to mouse erythropoiesis in the same animal. However, these mice lack significant levels of circulating human erythroid cells (data not shown).

[0137] It was hypothesized that low levels of circulating human erythroid cells in the periphery results from destruction (erythrophagocytosis) of the peripheral human RBCs by mouse macrophages. The role of SIRPa in this process was evaluated by generating mice in which hSIRPa was expressed from a locus in the mouse genome other than the mSIRPa locus, i.e., as a randomly integrated transgene, i.e. as an hSIRPa-tg (Rag2$^{-/-}$ Il2rg^{null}Tpo$^{h/h}$Mcsf$^{h/h}$Il3$^{h/h}$Gmcsf$^{h/h}$Epo$^{h/h}$SIRPα-tg+, i.e. "MISTER-G mice") and mice in which hSIRPa was expressed as a knock-in (KI) from the mSIRPa locus, i.e. as an hSIRPa KI (Rag2$^{-/-}$ Il2rg^{null}Tpo$^{h/h}$Mcsf$^{h/h}$Il3$^{h/h}$Gmcsf$^{h/h}$Epo$^{h/h}$SIRPα$^{h/h}$, i.e. "SupER-G mice").

[0138] Expression of human SIRPa, e.g. as a randomly-integrated transgene in MISTER-G mice, promoted a further increase in the number of human erythroid cells in the bone marrow of human HSC-engrafted mice expressing human EPO (FIG. 5, Panel A, compare "hSIRPa+, hEPO+" to "hSIRPa-, hEPO+"). Introduction of human SIRPa significantly improved the survival of most hematopoietic cells in the periphery, with the frequency of human $CD45^+$ cells including lymphoid and myeloid cells in peripheral blood increasing by at least 10 fold over that observed in mice that did not express hSIRPa. However, hSIRPa KI has little effect on the engraftment levels of human RBCs in peripheral blood (FIG. 5, Panel B).

[0139] Since human SIRPα knock-in is not sufficient to increase human erythroid cells in the peripheral blood, clodronate liposomes were used for the depletion of macrophages, specifically red pulp macrophages in the spleen and Kupffer cells in liver. A dramatic increase of circulating human cells of the erythroid lineage was observed in SuPER-G mice to 1% of the total circulating erythroid cells after the depletion of tissue resident macrophages by clodronate liposome (FIG. 6, Panel A). In addition, most human erythroid cells in the periphery after clodronate treatment were reticulocytes ($CD71^+$) (FIG. 6, Panel B) This is an exciting observation because it indicates that these mice would be good candidates to support *P. vivax* infection, which preferentially infects reticulocytes.

[0140] Infection of erythroid cells is an essential part of the life cycle of *Plasmodium* sp. However, the required frequency of human RBC necessary for successful *in vivo* infection with different Plasmodium species has not been established. The only in vivo models for *P. falciparum* presently available are based on the transfer of human RBCs into immunodeficient strains such as NOD/scid or NSG. In these mice, infection with merozoites can be achieved by i.v. injection of infected erythrocytes only after daily injection of large numbers of human erythrocytes. At the time of infection, human RBCs comprise roughly half of total erythrocyte number in these animals.

[0141] As demonstrated above, SupER-G mice engrafted with human hematopoietic stem cells (HSC) developed human erythroid cells in the *bone marrow* (FIG. 5B), and ii) clodronate treatment increased the frequency of human erythroid cells in the *periphery* of engrafted SupER-G mice (FIG. 6). To determine if engrafted, clodronate-treated SupER-G mice comprised a sufficient number of human erythroid cells in the periphery to sustain a successful *Plasmodium* infection in vivo, peripheral blood was collected from SupER-G mice engrafted with fetal liver or adult HSCs, and the blood cultured in vitro with *P. falciparum* strain 3D7-infected blood. To facilitate multiple rounds of parasite replication, fresh human red blood cells were added into the infection culture 48 hours later, the expectation being that amplification by reinfection of the subsequently added human RBCs will only occur if the human RBCs harvested from the HSC-engrafted SupER-G mice had produced the full infectious cycle of *P. falciparum.* Twelve days after infection, advanced stage of parasite infection and amplification of merozoites from schizont was observed in all blood samples from engrafted SuPER-G mice ("engrafted mouse RBCs, adult 1", "engrafted mouse RBCs, adult 2", and "engrafted mouse RBCs, fetal liver") but not from control mice that are either unengrafted or acutely engrafted with 0.1% hRBCs by injection ("spiked control") (FIG. 7A and data not shown). Exponential increase of parasitemia was observed by Giemsa staining and quantitative PCR (FIG. 7B). This indicates that engrafted, clodronate-treated SupER-G produce a sufficient number of human red blood cells to sustain an infection with *P. falciparum.* From this, it is expected that in vivo infection of clodronate-dosed engrafted mice with *P. falciparum* and *P. vivax* merozoites will be successful.

Example 5

The TIES mice ($Rag2^{-/-}Il2rg^{null}Tpo^{h/h}Il3/Gmcsf^{h/h}Epo^{h/m}SIRP\alpha^{h/h}$)

[0142] Due to low fertility, developmental incompetency and high mortality, mice with $Epo^{h/h}$ are not ideal for infection study. Instead, mice heterozygous for the hEPO gene (i.e. $Epo^{h/m}$, e.g., the TIES mice) are fully capable of producing erythropoietin (EPO) and support all stages of erythropoiesis. Further improvement in viability from 8-10 weeks to 4 months was achieved by retaining the mouse M-CSF gene at the mouse locus rather than replacing it with human M-CSF, because high level of human myeloid cell engraftment supported by human Macrophage colony stimulating factor (M-CSF) knock-in cause destruction of mouse red blood cells which leads to anemia and death of engrafted mice.

[0143] Like SupER-G mice, TIES mice support human erythropoiesis and maintain a frequency of 1% human erythroid cells in the peripheral blood if dosed with clodronate. In addition, most human erythroid cells in the periphery after clodronate treatment were reticulocytes (CD71+). This suggests that these mice will be good candidates to support P. vivax infection.

[0144] It was demonstrated that engrafted TIES mice can maintain a frequency of 1% human erythroid cells in the peripheral blood if dosed with clodronate. In addition, the ability of TIES mice to support a transfusion of human RBCs was determined. As shown in FIG. 8, clodronate treatment stabilized the transfused population at more than 20% of the total population of cells in the periphery. These levels, realized at about 4 hours after transfusion, were sustained at least 12 hours after transfusion. It is expected that these RBC frequencies will be sufficient to support an in vivo infection of Plasmodium of different species.

REFERENCES CITED IN THE DESCRIPTION

Cited references

Patent documents cited in the description

- US6586251B [0067] [0070] [0121] [0128]
- US7576259B [0069]
- US7659442B [0069]
- US7294754B [0069] [0070] [0131]
- US5222982A [0109]
- US5385582A [0109]
- US20070254842A [0109]
- US20080081064A [0109]
- US20090196903A [0109]
- US20130340105 [0126]
- WO2009114400A [0133]
- US8541646B [0134]
- US8847004B [0134]
- US20140134662 [0134]
- WO2014039782A [0134]

Non-patent literature cited in the description

- **ANGULO-BARTUREN et al.**A murine model of falciparum-malaria by in vivo selection of competent strains in non-myelodepleted mice engrafted with human erythrocytesPLoS One, 2008, vol. 3, e2252- [0004] [0080]
- **JIMENEZ-DIAZ et al.**Improved murine model of malaria using Plasmodium falciparum competent strains and non-myelodepleted NOD-scid IL2Rgnull mice engrafted with human erythrocytesAntimicrob Agents Chemother, 2009, vol. 53, 4533-4536 [0004] [0080]
- **BADELL et al.**Human malaria in immunocompromised mice: an in vivo model to study defense mechanisms against Plasmodium falciparumJEM, 2000, vol. 192, 111653-1660 [0004] [0080]
- **MORENO et al.**The course of infections and pathology in immunomodulated NOD/LtSz-SCID mice inoculated with Plasmodium falciparum laboratory lines and clinical isolatesInt. J. Parasitol., 2006, vol. 36, 361-369 [0004] [0080]
- **FESTING et al.**Mammalian Genome, 1999, vol. 10, 836- [0050]
- **AUERBACH et al.**Establishment and Chimera Analysis of 129/SvEv- and C57BL/6-Derived Mouse Embryonic Stem Cell Lines, 2000, [0050]
- **SUNDBERGICHIKI**Genetically Engineered Mice HandbookCRC Press20060000 [0067]
- **HOFKERVAN DEURSEN**Genetically modified Mouse Methods and ProtocolsHumana

Press20020000 [0067]

- **JOYNER**Gene Targeting: A Practical ApproachOxford University Press20000000 [0067]
- **TURKSEN**Embryonic stem cells: Methods and Protocols in Methods Mol Biol.Humana Press20020000 [0067]
- **MEYER et al.**Proc. Nat. Acad. Sci. USA20100000vol. 107, 15022-15026 [0067]
- **GIBSON**A Primer Of Genome ScienceSinauer20040000 [0067]
- **RATHINAM et al.**Blood, 2011, vol. 118, 3119-28 [0067]
- **WILLINGER et al.**Proc Natl Acad Sci USA, 2011, vol. 108, 2390-2395 [0067]
- **RONGVAUX et al.**Proc Natl Acad Sci USA, 2011, vol. 108, 2378-83 [0067]
- **VALENZUELA et al.**Nat Biot, 2003, vol. 21, 652-659 [0067]
- Manipulating the Mouse EmbryoA Laboratory ManualCold Spring Harbor Laboratory Press20020000 [0068]
- **NAGY et al.**Manipulating the Mouse Embryo: A Laboratory ManualCold Spring Harbor Laboratory Press20020000 [0069]
- **NAGY et al.**Development, 1990, vol. 110, 815-821 [0069]
- **KRAUS et al.**Genesis, 2010, vol. 48, 394-399 [0069]
- **VALENZUELA et al.**High throughput engineering of the mouse genome coupled with high-resolution expression analysisNature Biotech., 2003, vol. 21, 6652-59 [0070]
- **POUEYMIROU et al.**F0 generation mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analysesNature Biotech., 2007, vol. 25, 191-99 [0070]
- **JIMENEZ-DIAZ et al.**Quantitative measurement of Plasmodium-infected erythrocytes in murine models of malaria by flow cytometry using bidimensional assessment of SYTO-16 fluorescenceCytometry A, 2009, vol. 75, 225-235 [0081]
- **GOODMAN AND GILMAN**The Pharmacological Basis of TherapeuticsMcGraw-Hill19960000 [0099]
- Chemical Warfare AgentsAcademic Press19920000 [0099]
- **PEAR et al.**P.N.A.S., 1993, vol. 90, 8392-8396 [0101]
- **MILLER et al.**Mol. Cell. Biol., 1985, vol. 5, 431-437 [0101]
- **MILLER et al.**Mol. Cell. Biol., 1986, vol. 6, 2895-2902 [0101]
- **DANOS et al.**PNAS, 1988, vol. 85, 6460-6464 [0101]
- Current Protocols in ImmunologyJohn Wiley & Sons, Inc.20120000 [0113]
- Immunology Methods ManualAcademic Press19970000 [0113] [0117]
- **SAMBROOK et al.**Molecular Cloning: A Laboratory ManualHaRBor Laboratory Press20010000 [0117]
- Short Protocols in Molecular BiologyJohn Wiley & Sons19990000 [0117]
- **BOLLAG et al.**Protein MethodsJohn Wiley & Sons19960000 [0117]
- Nonviral Vectors for Gene TherapyAcademic Press19990000 [0117]
- Viral VectorsAcademic Press19950000 [0117]
- **DOYLEGRIFFITHS**Cell and Tissue Culture: Laboratory Procedures in BiotechnologyJohn Wiley & Sons19980000 [0117]
- **LIEPETROPOULOS**Curr. Opin. Biotechnology, 1998, vol. 9, 43-48 [0131]
- Nature Biotech., vol. 25, 191-99 [0131]
- **RONGVAUX et al.**Proc Natl Acad Sci USA, 2011, vol. 108, 62378-2383 [0134]

- **WILLINGER et al.**Proc Natl Acad Sci USA, 2011, vol. 108, 62390-2395 [0134]
- **RATHINAM et al.**Blood, 2011, vol. 118, 113119-3128 [0134]
- **STROWIG et al.**Proc Natl Acad Sci USA, 2011, vol. 108, 3213218-13223 [0134]

Patentkrav

1. Genetisk modificeret mus, der omfatter:

en nukleinsyresekvens, der koder for et humant EPO-protein (hEPO), der er operabelt forbundet med en endogen muse-*EPO*-genpromoter ved muse-EPO-locuset, hvor den operable kobling resulterer i en nulmutation i muse-*EPO*-genet ved muse-*EPO*-genlocuset,

en nukleinsyresekvens, der koder for et humant M-CSF (hM-CSF)-protein, der er operabelt forbundet med en endogen muse-*M-csf*-promoter ved muse-*M-csf*-genlocuset, og hvor musen er heterozygot nul eller homozygot nul for muse-*M-csf*-genet,

en nukleinsyresekvens, der koder for et humant IL-3 (hIL-3)-protein, der er operabelt forbundet med en endogen muse-*IL-3*-promoter ved muse-*IL-3*-genlocuset, og hvor musen er heterozygot nul eller homozygot nul for muse-*IL-3*-genet,

en nukleinsyresekvens, der koder for et humant GM-CSF (hGM-CSF)-protein, der er operabelt forbundet med en endogen muse-*Gm-csf*-promoter ved muse-*Gm*-csf-genlocuset, og hvor musen er heterozygot nul eller homozygot nul for muse-*Gm-csf*-genet,

en nukleinsyresekvens, der koder for et humant TPO (hTPO)-protein, der er operabelt forbundet med en endogen muse-*TPO*-promoter ved muse-*TPO*-genlocuset, og hvor musen er heterozygot nul eller homozygot nul for muse-*TPO*-genet, og

et SIRPa-transgen, der koder for et humant Sirpa (hSirpa)-protein, der er operabelt forbundet med en *Sirpa*-promoter, hvor musen eksprimerer hSirpa-proteinet,

hvor den genetisk modificerede mus er immundeficient.

2. Mus ifølge krav 1, hvor musen er homozygot for allelen omfattende nukleinsyresekvensen, der koder for hEPO'et.

3. Mus ifølge krav 1 eller 2, hvor nukleinsyresekvensen, der koder for hEPO'et, omfatter en human *EPO*-genomisk-kodende og -ikke-kodende sekvens.

4. Mus ifølge et hvilket som helst af kravene 1-3, hvor nukleinsyresekvensen, der koder for hEPO'et, omfatter en human *EPO*-cDNA-sekvens.

5. Mus ifølge et hvilket som helst af kravene 1-4, hvor musen er homozygot for: nukleinsyren, der koder for hTPO, nukleinsyren, der koder for hMcsf, nukleinsyren, der koder for hIL-3, og nukleinsyren, der koder for hGmcsf.

6. Mus ifølge et hvilket som helst af kravene 1-5, hvor musen yderligere omfatter en indpodning af humane hæmatopoietiske celler, hvor de humane hæmatopoietiske celler eventuelt omfatter en eller flere celler udvalgt fra gruppen bestående af en human CD34-positiv celle, en human hæmatopoietisk stamcelle, en human myeloid precursorcelle, en human erythroid precursorcelle, en human myeloid celle, en human dendritisk celle, en human monocyt, en human granulocyt, en human erythrocyt, en human neutrofil, en human mastcelle, en human thymocyt og en human B-lymfocyt.

7. Mus ifølge krav 6, hvor musen omfatter clodronat.

8. Mus ifølge krav 7, hvor musen yderligere omfatter en infektion med et patogen, der er rettet mod humane celler af erythroid-afstamningen, hvor patogenet eventuelt er udvalgt blandt en *Plasmodium* sp., *Babesia* sp. og en *Theileri* sp.

9. Mus ifølge et hvilket som helst af kravene 1-8, hvor immundefekten er forårsaget af en deficiens med hensyn til både Rag2 og Il2rg.

DRAWINGS

```
mEpo    1  MGVPERPT-LLLLLSLLLIPLGLPVLCAPPRLICDSRVLERYILEAKEAENVTMGCAEGPRLSENITVPDTKVNFYAWKRMEVEEQAIEVWQGLSLLSEA  99
hEPO    1  MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEA 100

mEpoo  100 ILQAQALLANSSQPPETLQLHIDKAISGLRSLTSLLRVLGAQKELMSPPDTTPPAPLRTLTVDTFCKLFRVYANFLRGKLKLYTGEVCRRGDR 192
hEPO   101 VLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKLKLYTGEACRTGDR 193

mEpo (SEQ ID NO:2)
hEPO (SEQ ID NO:4)
```

FIG. 1

EPO humanization Schematic

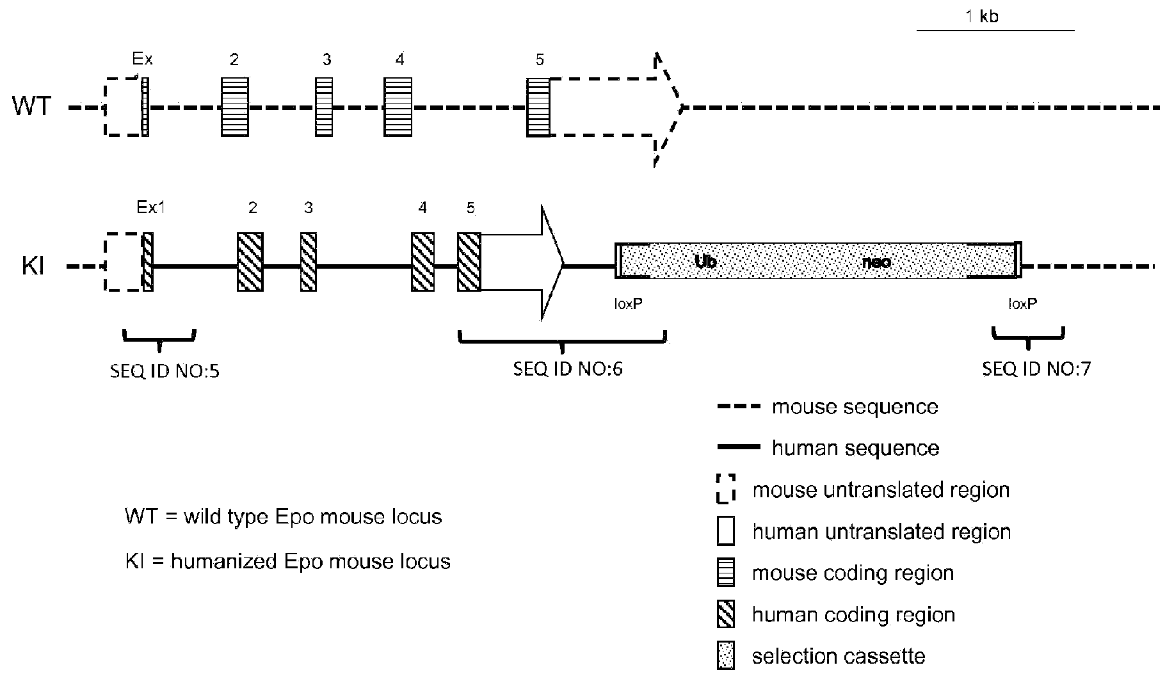
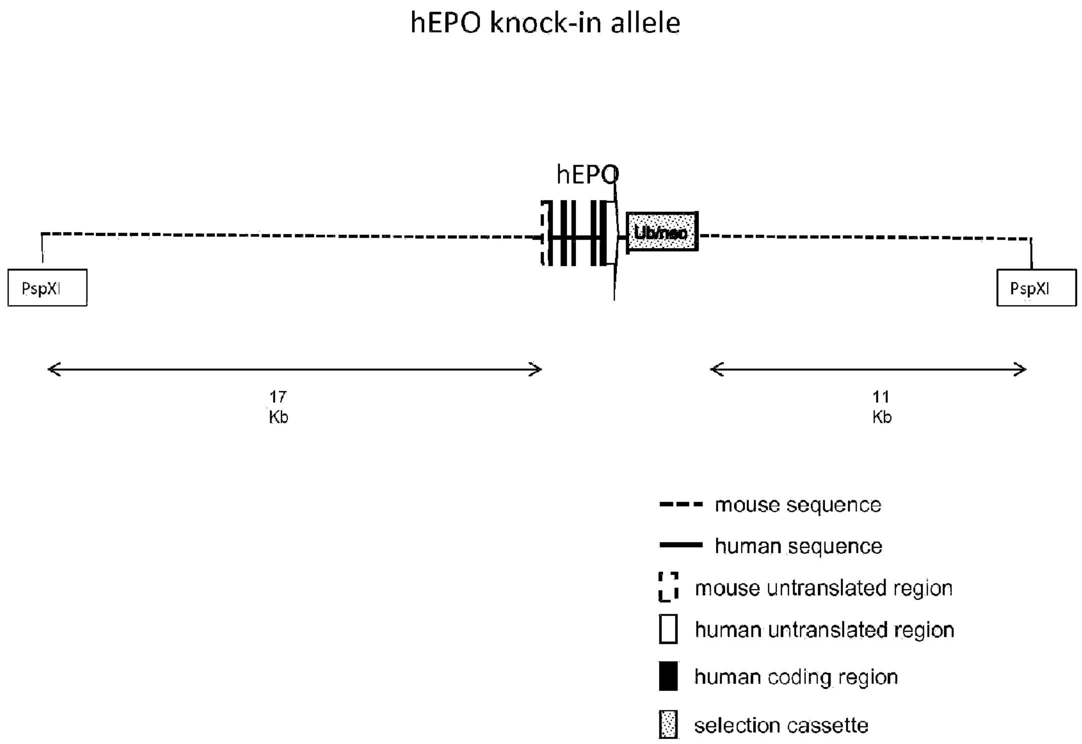


FIG. 2



hEPO knock-in allele

FIG. 3

Sirpa humanization Schematic

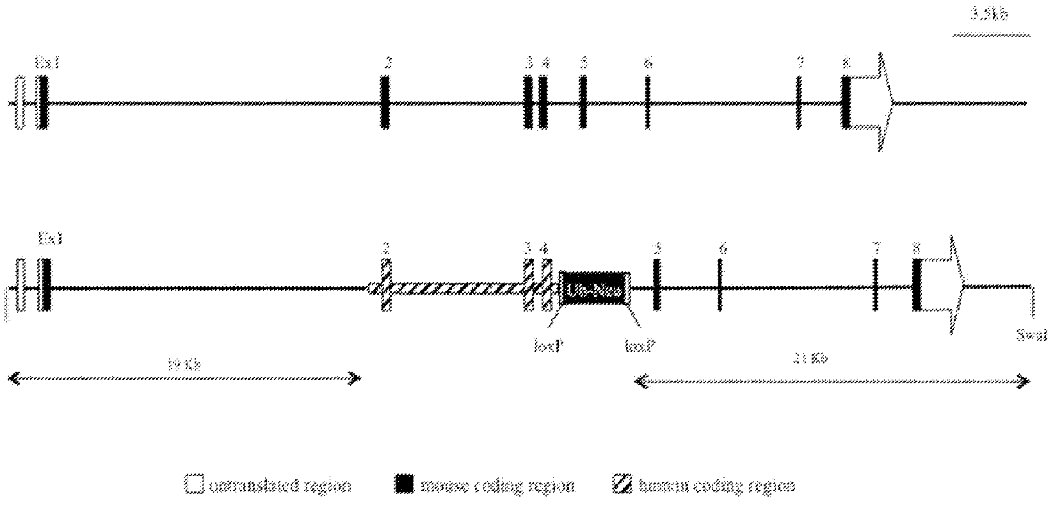


FIG. 4

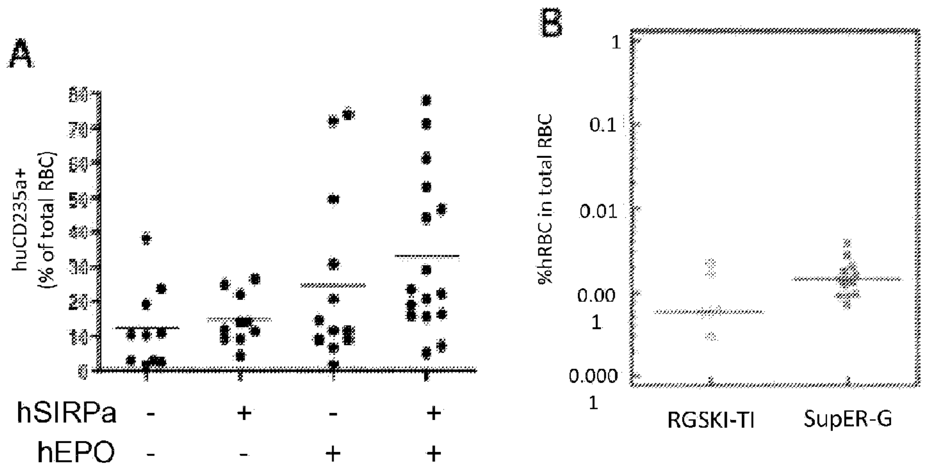

A
huCD235a+
(% of total RBC)
80
70
60
50
40
30
20
10
0
hSIRPa - + - +
hEPO - - + +
B
%hRBC in total RBC
1
0.1
0.01
0.00
1
0.000
1
RGSKI-TI
SupER-G

FIG. 5

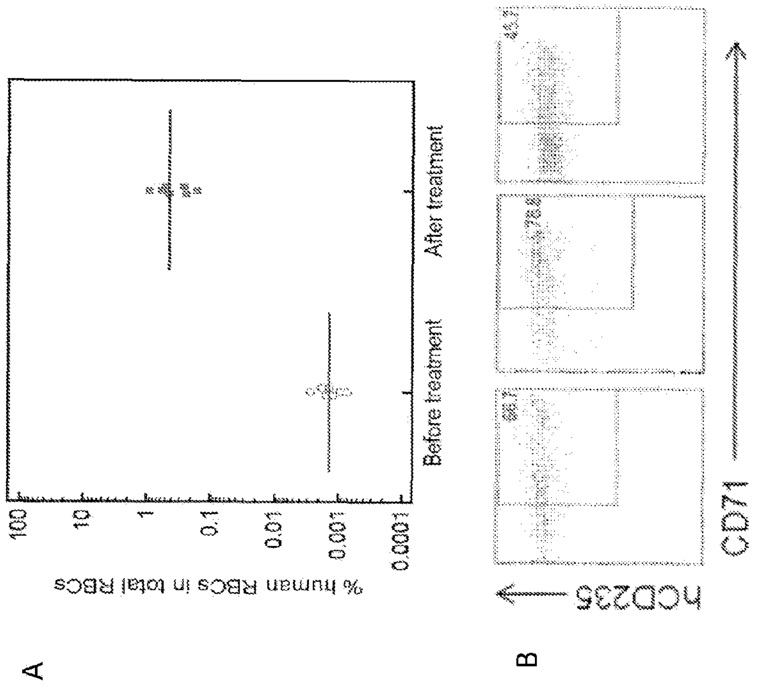

A
% human RBCs in total RBCs
100
10
1
0.1
0.01
0.001
0.0001
Before treatment
After treatment
B
hCD235
CD71
88.7
78.8
45.7

FIG. 6

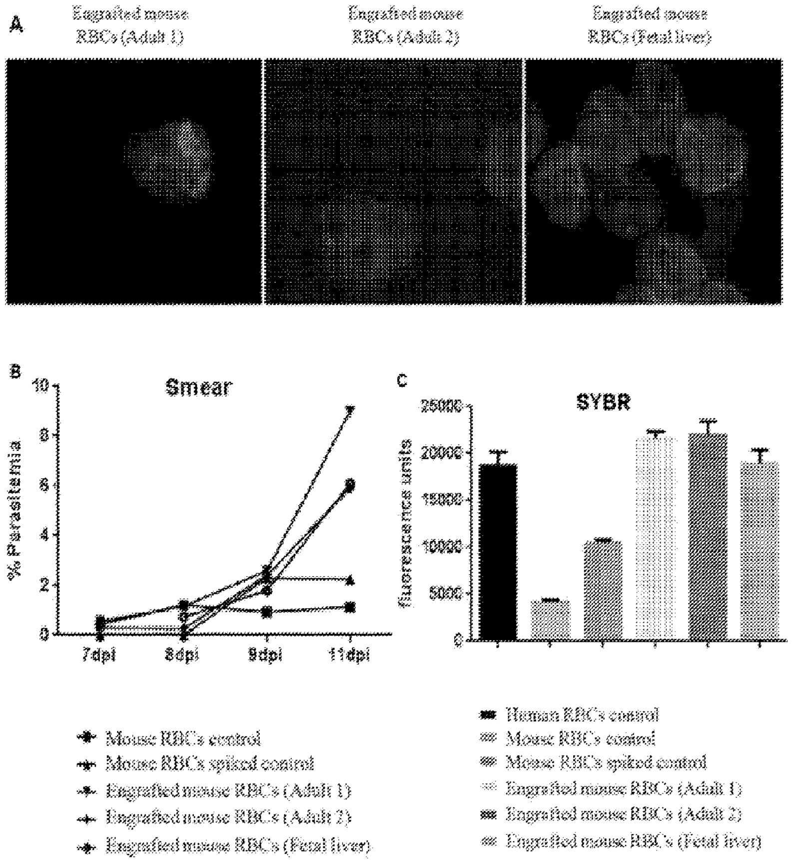

A
Engrafted mouse RBCs (Adult 1)
Engrafted mouse RBCs (Adult 2)
Engrafted mouse RBCs (Fetal liver)
B
Smear
% Parasitemia
0
2
4
6
8
10
7dpi
8dpi
9dpi
11dpi
Mouse RBCs control
Mouse RBCs spiked control
Engrafted mouse RBCs (Adult 1)
Engrafted mouse RBCs (Adult 2)
Engrafted mouse RBCs (Fetal liver)
C
SYBR
fluorescence units
0
5000
10000
15000
20000
25000
Human RBCs control
Mouse RBCs control
Mouse RBCs spiked control
Engrafted mouse RBCs (Adult 1)
Engrafted mouse RBCs (Adult 2)
Engrafted mouse RBCs (Fetal liver)

FIG. 7

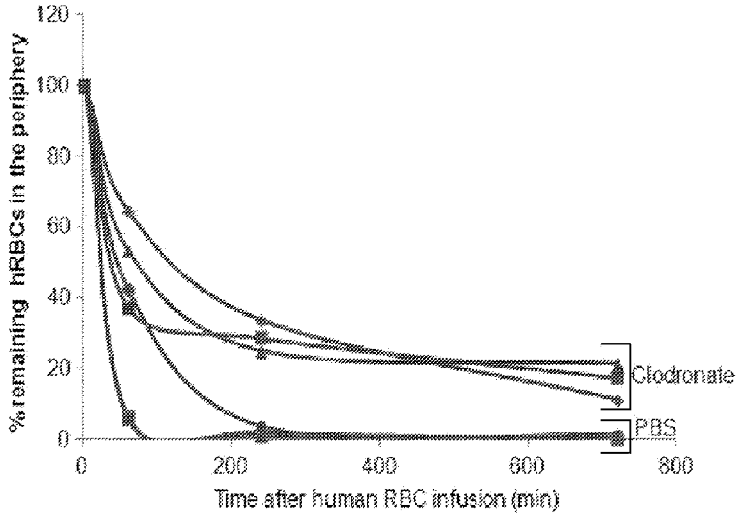

120
100
80
60
40
20
0
%remaining hRBCs in the periphery
0
200
400
600
800
Time after human RBC infusion (min)
Clodronate
PBS

FIG. 8

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.