RODENT ANIMALS EXPRESSING HUMAN CR1

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority from U.S. Provisional Application No. 63/086,167, filed October 1, 2020, the entire contents of which is incorporated herein by reference.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] The Sequence Listing in the ASCII text file, named as 38224_10747WO01_Sequence Listing of 42 KB, created on September 29, 2021 and submitted to the United States Patent and Trademark Office via EFS-Web, is incorporated herein by reference.

BACKGROUND

[0003] During preclinical drug development stage, candidate agents are typically studied based on their efficacy, toxicity, and other pharmacokinetic and pharmacodynamics properties. Candidate agents, such as antibodies, typically target a human antigen as the end goal of investigation is to develop a human therapy. The ability to sequester the complement pathway provides a significant advantage to candidate therapeutic agents. The complement pathway is part of the innate immune response and assists humoral immune responses in the recruitment of marcrophage and phagocytes to the antigenic site. Activation of the complement pathway results in cytokine release and the opsonization of the antibody-bound antigen by phagocytes. During development of therapeutic agents that are aimed at activation of complement pathway and innate immune response in order to combat human disease, a model non-human animal system that would allow studies into the mechanisms of action and/or therapeutic potential of the agent would be invaluable.

SUMMARY

[0004] In one aspect, disclosed herein is a genetically modified rodent animal comprising in its genome a nucleic acid which comprises a nucleotide sequence encoding a human CR1 polypeptide, wherein the rodent animal displays a human-like expression of the human CR1 polypeptide.

[0005] In some embodiments, the nucleotide sequence encoding the human CR1 polypeptide is a genomic DNA sequence. In some embodiments, the nucleotide sequence encoding the human CR1 polypeptide is a cDNA sequence.

[0006] In some embodiments, the nucleic acid is inserted between the rodent *Cr2* gene locus and the rodent *Cr1like* (*Cr1l*) gene locus in the rodent genome. In some embodiments, the nucleic acid is inserted into the rodent genome via random integration, e.g., into an X chromosome of the rodent (such as a locus other than the rodent *Gata-1* gene locus).

[0007] In some embodiments, the nucleic acid comprises a 5' regulatory region of a human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide. In some embodiments, the 5' regulatory region comprises the promoter region of the human *CR1* gene. In some embodiments, the 5' regulatory region comprises the 5' untranslated region (5' UTR) of the human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide. In some embodiments, the 5' regulatory region comprises the promoter and the 5' untranslated region (5' UTR) of the human *CR1* gene.

[0008] In some embodiments, the nucleic acid comprises a 3' regulatory region of a human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide. In some embodiments, the 3' regulatory region comprises the 3' UTR of the human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide. In some embodiments, the 3' regulatory region comprises the 3' UTR and an additional sequence downsteam of the 3' UTR of the human *CR1* gene.

[0009] In some embodiments, the nucleic acid comprises a human genomic DNA sequence, which comprises the human *CR1* coding sequence from ATG to STOP, with the 5' and 3' untranslated regions (UTRs) and intervening introns, as well as a 5' upstream sequence of at least 4000 bp directly upstream of the 5' UTR and a sequence of at least 150 bp directly downstream of the 3' UTR; and in some such embodiments, the nucleic acid is inserted between the rodent *Cr2* gene locus and the rodent *Cr1l* gene locus.

[0010] In some embodiments, the nucleic acid comprises a 5' regulatory region of a heterologous gene (i.e., a gene that is not human *CR1*), operably linked to the nucleotide sequence encoding the human CR1 polypeptide. In some embodiments, the 5' regulatory region is a 5' regulatory region of a rodent *Gata-1* gene. In some embodiments, the 5' regulatory region comprises the promoter region of a rodent *Gata-1* gene. In some

embodiments, the 5' regulatory region comprises a genomic sequence of at least 14 Kb immediately upstream of the ATG codon of a rodent *Gata-1* gene.

[0011] In some embodiments, the nucleic acid comprises a 3' regulatory region of a heterologous gene (i.e., a gene that is not human *CR1*), operably linked to the nucleotide sequence encoding the human CR1 polypeptide. In some embodiments, the 3' regulatory region comprises the 3' UTR of a heterologous gene, e.g., the 3' UTR (including the polyadenylation sequence) of a human beta-1 globin gene.

[0012] In some embodiments, the nucleic acid comprises a human *CR1* coding sequence (ATG to STOP) (e.g., in the form of cDNA), operably linked to, at the 5', a nucleotide sequence of at least 14 Kb directly upstream of ATG of a rodent *Gata-1* gene (including the promoter of the rodent *Gata1* gene), and at the 3', a 3' UTR sequence containing the poly(A) signal from a human beta globin gene followed by a nucleotide sequence of at least 1.5 Kb directly downstream of the STOP codon of the rodent *Gata-1* gene; and in some such embodiments, the nucleic acid is integrated into an X-chromosome of the rodent (e.g., at a locus other than the rodent *Gata-1* gene locus).

[0013] In some embodiments, a genetically modified rodent animal may comprise multiple nucleic acids in its genome, each comprising a nucleotide sequence encoding a human CR1 polypeptide. In some embodiments, a genetically modified rodent animal comprises in its genome a first nucleic acid comprising a first nucleotide sequence encoding a human CR1 polypeptide, wherein the first nucleic acid is inserted between the rodent *Cr2* gene locus and the *Crll* gene locus in the rodent genome; and a second nucleic acid comprising a second nucleotide sequence encoding a human CR1 polypeptide in operable linkage to a 5' regulatory region of a rodent *Gata-1* gene, and a 3' regulatory region comprising a polyA signal of a human beta-1 globin gene, wherein the second nucleic acid is integrated into an X chromosome of the rodent genome.

[0014] In some embodiments, a genetically modified rodent animal further comprises in its genome a replacement of a rodent C3 gene sequence at an endogenous rodent C3 locus with a human C3 gene sequence to form a modified C3 gene, wherein the rodent C3 gene sequence comprises an exon of the endogenous rodent C3 gene and the human C3 gene sequence comprises exon 2 through exon 41, or exon 1 through exon 41, of the human C3 gene. In some embodiments, expression of the modified C3 gene is under control of a human C3 promoter, or under control of rodent regulatory elements at the endogenous rodent C3 locus.

[0015] In some embodiments, a rodent animal is a male. In some embodiments, a rodent animal is a female.

[0016] In some embodiments, a rodent animal is heterozygous for a nucleic acid exogenously introduced and integrated in the genome. In some embodiments, a rodent animal is homozygous for a nucleic acid exogenously introduced and integrated in the genome.

[0017] In some embodiments, a rodent animal is a mouse. In some embodiments, a rodent animal is a rat.

[0018] In some embodiments, a rodent animal expresses a human CR1 polypeptide on red blood cells. In some embodiments, a rodent animal expresses e human CR1 polypeptide on neutrophils, e.g., netrophils from the blood, spleen or liver. In some embodiments, a rodent animal expresses a human CR1 polypeptide on red blood cells and neutrophils. In some embodiments, a rodent animal expresses a human CR1 polypeptide on red blood cells and/or neutrophils, and additionally on one or more of macrophages, monocytes, or circulating dendritic cells (cDCs).

[0019] In another aspect, provided herein is a cell or tissue isolated from a rodent animal described herein, wherein the genome of the cell or tissue comprises the nucleic acid comprising a nucleotide sequence encoding a human CR1 polypeptide. In some embodiments, the rodent cell is a rodent egg.

[0020] In a further aspect, provided herein is a rodent (such as mouse or rat) embryonic stem (ES) cell, comprising in its genome a nucleic acid which comprises a nucleotide sequence encoding a human CR1 polypeptide, as described herein. In some embodiments, the nucleic acid is inserted between the rodent *Cr2* gene locus and the rodent *Cr1l* gene locus in the rodent genome of the ES cell, and comprises a human genomic DNA sequence, which includess the human *CR1* coding sequence from ATG to STOP, with the 5' and 3' untranslated regions (UTRs) and intervening introns, as well as a 5' upstream sequence of at least 4000 bp directly upstream of the 5' UTR and a sequence of at least 150 bp directly downstream of the 3' UTR. In some embodiments, the nucleic acid is inserted into the genome (e.g., an X chromosome) of the rodent ES cell and comprises a 5' regulatory region of a rodent *Gata-1* gene, and a 3' regulatory region of a human beta-1 globin gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide.

[0021] In still another aspect, disclosed herein is a method of making a genetically modified rodent animal, comprising inserting a nucleic acid into the genome of a rodent ES

cell, wherein the nucleic acid comprises a nucleotide sequence encoding a human CR1 polypeptide as described herein; and making a genetically modified rodent animal using a rodent ES cell obtained. Also disclosed herein is a method of making a genetically modified rodent ES cell, comprising inserting a nucleic acid into the genome of a rodent ES cell, wherein the nucleic acid comprises a nucleotide sequence encoding a human CR1 polypeptide as described herein.

[0022] In some embodiments, the nucleotide sequence encoding the human CR1 polypeptide is a genomic DNA sequence. In some embodiments, the nucleotide sequence encoding the human CR1 polypeptide is a cDNA sequence.

[0023] In some embodiments, the nucleic acid is inserted between the rodent *Cr2* gene locus and the rodent *Cr1like* (*Cr1l*) gene locus in the rodent genome. In some embodiments, the nucleic acid is inserted into the rodent genome via random integration, e.g., into an X chromosome of the rodent (such as a locus other than the rodent *Gata-1* gene locus).

[0024] In some embodiments, the nucleic acid comprises a 5' regulatory region of a human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide. In some embodiments, the 5' regulatory region comprises the promoter region of the human *CR1* gene. In some embodiments, the 5' regulatory region comprises the 5' untranslated region (5' UTR) of the human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide. In some embodiments, the 5' regulatory region comprises the promoter and the 5' untranslated region (5' UTR) of the human *CR1* gene.

[0025] In some embodiments, the nucleic acid comprises a 3' regulatory region of a human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide. In some embodiments, the 3' regulatory region comprises the 3' UTR of the human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide. In some embodiments, the 3' regulatory region comprises the 3' UTR and an additional sequence downsteam of the 3' UTR of the human *CR1* gene.

[0026] In some embodiments, the nucleic acid comprises a human genomic DNA sequence, which comprises the human *CR1* coding sequence from ATG to STOP, with the 5' and 3' untranslated regions (UTRs) and intervening introns, as well as a 5' upstream sequence of at least 4000 bp directly upstream of the 5' UTR and a sequence of at least 150

bp directly downstream of the 3' UTR; and in some such embodiments, the nucleic acid is inserted between the rodent *Cr2* gene locus and the rodent *Crll* gene locus.

[0027] In some embodiments, the nucleic acid comprises a 5' regulatory region of a heterologous gene (i.e., a gene that is not human *CR1*), operably linked to the nucleotide sequence encoding the human CR1 polypeptide. In some embodiments, the 5' regulatory region is a 5' regulatory region of a rodent *Gata-1* gene. In some embodiments, the 5' regulatory region comprises the promoter region of a rodent *Gata-1* gene. In some embodiments, the 5' regulatory region comprises a genomic sequence of at least 14 Kb immediately upstream of the ATG codon of a rodent *Gata-1* gene.

[0028] In some embodiments, the nucleic acid comprises a 3' regulatory region of a heterologous gene (i.e., a gene that is not human *CR1*), operably linked to the nucleotide sequence encoding the human CR1 polypeptide. In some embodiments, the 3' regulatory region comprises the 3' UTR of a heterologous gene, e.g., the 3' UTR (including the polyadenylation sequence) of a human beta-1 globin gene.

[0029] In some embodiments, the nucleic acid comprises a human *CR1* coding sequence (ATG to STOP) (e.g., in the form of cDNA), operably linked to, at the 5', a nucleotide sequence of at least 14 Kb directly upstream of ATG of a rodent *Gata-1* gene (including the promoter of the rodent *Gata1* gene), and at the 3', a 3' UTR sequence containing the poly(A) signal from a human beta globin gene followed by a nucleotide sequence of at least 1.5 Kb directly downstream of the STOP codon of the rodent *Gata-1* gene; and in some such embodiments, the nucleic acid is integrated into an X-chromosome of the rodent (e.g., at a locus other than the rodent *Gata-1* gene locus).

[0030] In another aspect, disclosed herein is a targeting vector, comprising a nucleic acid which comprises a nucleotide sequence encoding a human CR1 polypeptide, flanked by rodent nucleotide sequences for targeted insertion of the nucleic acid between the rodent *Cr2* gene locus and the rodent *Crll* gene locus in the rodent genome.

[0031] In still another aspect, disclosed herein is a nucleic acid vector, comprising a nucleic acid which comprises nucleotide sequence encoding a human CR1 polypeptide in operable linkage to a 5' regulatory region of a rodent *Gata-1* gene.

[0032] In a further aspect, disclosed herein is a method of assessing the pharmacokinetic properties of a compound targeting human CR1 or another component of the human complement system (such as human C3), the method comprising administering a candidate

compound to a genetically modified rodent animal disclosed herein; and performing an assay to determine one or more pharmacokinetic properties of the compound.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] The file of this patent or patent application contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

[0034] **FIG. 1A** depicts a mouse genomic fragment comprising the *Cr2-Crl1* gene loci and a human genomic fragment comprising the *CR1* gene, with exons shown as by vertical bars. The location of the mouse sequence of 251 bp to be replaced by a human *CR1* genomic sequence is indicated. The locations for the primer sets and probes designed for detecting deletion of mouse sequences (loss of allele or LOA) (7238mTU and 7238mTD) and insertion of the human sequences (gain-of-allele or GOA) (7238hTU and 7238hTD) are also indicated.

[0035] **FIGS. 1B-1C** depict the 7238 and 7239 alleles, respectively, having a human *CR1* genomic sequence inserted between mouse *Cr2* and *Crl1* genes by replacing a mouse genomic sequence of 251 bp. The locations for the primer sets and probes designed for detecting insertion of the human sequences (gain-of-allele or GOA) (7238hTU and 7238hTD) are also indicated. The 7239 allele results from delection of the self-deleting hygromycin resistance cassette in the 7238 allele. Following cassette deletion, a scar containing a loxP site remains.

[0036] **FIG. 2A.** Flow cytometry analysis of blood myeloid cell populations of mice heterozygous for targeted insertion of human *CR1* (MAID7239 Het, or "Het"), as compared to WT control mice having 75%B6 25%129 background ("75/25 WT") (having the same genetic background as MAID 7239 but without the targeted insertion of human *CR1*). Human CR1 was detected on neutrophils at a high level and on macrophages at a very low level.

[0037] **FIG. 2B.** Flow cytometry analysis of myeloid splenic cell populations of mice heterozygous for targeted insertion of human *CR1* (MAID 7239, or "Het"), as compared to WT control mice having 75%B6 25%129 background ("75/25 WT") (having the same genetic background as MAID 7239 but without the targeted insertion of human *CR1*). Human CR1 was detected on neutrophils at a high level, and on macrophages and inflammatory monocytes at a very low level.

[0038] **FIG. 2C.** Flow cytometry analysis of myeloid blood cell populations of mice homozygous for targeted insertion of human *CR1* (MAID7239 HO, or "HO"), as compared to WT control mice having 75%B6 25%129 background ("75/25 WT") (having the same genetic background as MAID 7239 but without the targeted insertion of human *CR1*). Human CR1 was detected on neutrophils at a high level, on circulating dendritic cells (cDCs) at a moderate level, and on macrophages at a very low level.

[0039] **FIG. 2D.** Flow cytometry analysis of myeloid splenic cell populations of mice homozygous for targeted insertion of human *CR1* (MAID7239 HO, or "HO"), as compared to WT control mice having 75%B6 25%129 background ("75/25 WT") (having the same genetic background as MAID 7239 but without the targeted insertion of human *CR1*). Human CR1 was detected on splenic neutrophils at a high level, and on circulating dendritic cells (cDCs), macrophages and inflammatory monocytes at a very low level.

[0040] **FIG. 2E.** Flow cytometry analysis of macrophage populations in peritoneal cavity of mice homozygous for targeted insertion of human *CR1* (MAID7239 HO, or "HO"), as compared to wild type control mice having 75%B6 25%129 background ("75/25 WT"). Large Peritoneal Macrophages are derived from fetal liver derived monocytes or yolk-sac. Small Peritoneal Macrophages are derived from bone marrow derived monocytes. hCR1 was detected on a few large (but not small) peritoneal macrophases in 1 of 3 MAID7239 HO mice examined.

[0041] **FIG. 2F.** Flow cytometry analysis of macrophage populations in the liver of mice homozygous for targeted insertion of human *CR1* (MAID7239 HO, or "HO"), as compared to wild type control mice having 75%B6 25%129 background ("75/25 WT"). hCR1 was detected on all neutrophils in the liver, and on approximately 50% motile macrophases (and possibly cDCs), but not on Kupffer cells, from MAID7239 HO mice.

[0042] **FIG. 3A** shows a moderate decrease in serum BUN levels in C3 Humin CR1 Humin (6149HO 7239HO) male mice as compared to C3 Humin (6149HO 7239WT) male mice. Mice were sacrificed unless indicated with * (which indicates that the mouse died).

[0043] **FIG. 3B** shows a moderate decrease in serum BUN levels in C3 HumIn CR1 HumIn (6149HO 7239HO) female mice as compared to C3 HumIn (6149HO 7239WT) female mice. Mice were sacrifice unless indicated with * (which indicates that the mouse died).

[0044] **FIG. 3C** shows a lack of significant improvement in hC3 serum levels in C3 HumIn CR1 HumIn (6149HO 7239HO) mice as compared to C3 HumIn (6149HO

7239WT) mice. Published C3 concentration in normal human serum is about 1200 □g/ml. Horizonal bars represent medians.

[0045] **FIG. 3D** illustrates that C3 HumIn CR1 HumIn (6149HO 7239HO) mice show no change in human iC3b serum levels compared to C3 HumIn (6149HO 7239WT) mice. The ratio of C3: iC3b was found to be similar between Normal Human Serum (NHS) and 6149HO 7239WT serum.

[0046] **FIG. 3E** shows exacerbated liver injury, but ameliorated kidney injury in C3 HumIn CR1 HumIn (6149HO 7239HO) mice as compared to C3 HumIn (6149HO 7239WT) mice.

[0047] **FIG. 3F** shows a minor improvement in weight gain in C3 HumIn CR1 HumIn (6149HO 7239HO) mice as compared to C3 HumIn (6149HO 7239WT) mice. Both C3 HumIn CR1 HumIn and C3 HumIn mice failed to gain weight with age compared to 75/25 WT Controls.

[0048] **FIG. 3G** shows a minor decrease in serum BUN levels in C3 HumIn CR1 HumIn (6149HO 7239HO) mice as compared to C3 HumIn (6149HO 7239WT) mice. Both C3 HumIn CR1 HumIn (6149HO 7239HO) and C3 HumIn (6149HO 7239WT) mice have elevated serum BUN levels as compared to 72/25 WT controls.

[0049] **FIG. 3H** shows improved survival in C3 HumIn CR1 HumIn (6149HO 7239HO) mice as compared to C3 HumIn (6149HO 7239WT) mice.

[0050] **FIG. 4A** shows the mouse *Gata 1* gene locus, with exons being represented by vertical boxes. The location of the mouse genomic sequence of 672 bp to be deleted and replaced by a human CR1-coding sequence is indicated. The locations for the primer sets and probes designed for detecting deletion of mouse sequences (loss of allele or LOA) (7502mTU and 7502mTD), for confirming the presence of mouse *Gata1* promoter (7502mPU and 7502mPU2), and for retention of mouse *Gata1* sequences (7502mretU and 7502mretD) are also indicated.

[0051] **FIG. 4B** shows the 7502 allele, with a mouse *Gata1 promoter* -human CR1 coding sequence randomly integrated and containing a self-deleting neomycin resistance cassette. Full-length human CR1 coding sequence (ATG to STOP, 6117bp) was inserted into a mouse bacterial artificial chromosome (BAC) containing *Gata1* genomic sequence such that 672 bp of *Gata1*, encompassing mouse coding exon 1 sequence just after the ATG start codon and the following mouse intron, was replaced. The mouse BAC was chosen to include >7Kb sequence upstream of the *Gata1* ATG and >1.5Kb sequence downstream of

the *Gata1* stop codon. A 3' UTR sequence containing the poly(A) signal (135 bp, SEQ ID NO: 4) from a human beta-1 globin gene was inserted just after the human *CR1* stop, followed by a self-deleting neomycin resistance cassette (4810bp). The locations for the primer sets and probes designed for detecting gain of human sequences (7502hTU and 7502hTD), for confirming the presence of mouse *Gata1* promoter (7502mPU and 7502mPU2), and for retention of mouse *Gata1* sequences (7502mretU and 7502mretD)are also indicated.

[0052] **FIG. 4C** shows the 7503 allele, resulting from cassette deletion from the 7502 allele shown in FIG. 4B. Following cassette deletion, a 78bp scar containing a loxP site remains.

[0053] **FIG. 5A** shows the levels of human CR1 on peripheral blood RBCs from 75/25 WT, 7503HET females, 7503HET males, and 7503HO females. MAID7503 HET male and HO female mice show a similar level of hCR1 expression (higher than found in 7503HET females) in comparison to CR1 FMO control (in lieu of an isotype control). All plots gated on Ter119+ mouse RBCs.

[0054] **FIG. 5B** shows the levels of human CR1 on cell populations from lysed blood of 75/25 WT, 7503HET females, 7503HET males, and 7503HO females in comparison to CR1 FMO control (in lieu of an isotype control). MAID7503 HET male and HO female mice show some hCR1 expression (higher than found in 7503HET females).

[0055] **FIG. 5C** shows the levels of human CR1 on cell populations from the spleen of 75/25 WT, 7503HET females, 7503HET males, and 7503HO females in comparison to CR1 FMO control (in lieu of an isotype control). MAID7503 HET male and HO female mice show some hCR1 expression (higher than found in 7503HET females).

[0056] **FIG. 5D** demonstrates human CR1 expression on human peripheral blood RBCs, neutrophils and monocytes express CR1, in comparison to CR1 FMO control (in lieu of an isotype control).

[0057] **FIG. 5E** shows the levels of human CR1 on peripheral blood RBCs from 75/25 WT, B6.Cg-Tg(Gata1-CR1)1Rwf/J females, 7503HO females, in comparison to CR1 FMO control (in lieu of an isotype control). RBCs show a similar level of hCR1 expression compared to human RBCs (and similar to MAID7503 HO RBCs).

[0058] **FIG. 5F** shows the levels of human CR1 on cell populations in peripheral blood from 75/25 WT, B6.Cg-Tg(Gata1-CR1)1Rwf/J females, 7503HO females, in comparison to CR1 FMO control (in lieu of an isotype control). B6.Cg-Tg(Gata1-CR1)1Rwf/J blood

neutrophils express very low levels of hCR1, in contrast to human blood neutrophils and monocytes.

DETAILED DESCRIPTION

[0059] Disclosed herein are rodents (such as mice and rats) genetically modified to comprise a nucleic acid comprising a human CR1 coding sequence integrated in the genome and to display a human-like expression of the human CR1 protein. Also disclosed herein are isolated rodent tissues and cells whose genome comprises a nucleic acid encoding a human CR1 protein. Further disclosed herein are vectors and methods for making a genetically modified rodent animal which displays a human-like expression of the human CR1 protein, as well as methods of using the genetically modified rodent animal for screening candidate compounds that target human CR1 or another component of the human complement system (such as hman C3). The various aspects and embodiments are further described below.

Human CR1/CD35

[0060] Human CR1 (also known as CD35) is encoded by human *CR1* gene and is known to recognize complement coated microbial surface and functions in particle adherence. CR1 functions in immune complex (IC) trafficking/immune-adherence clearance by binding to C3b/C4b opsonized IC to human erythrocytes and transports them to the liver and spleen for uptake and degradation by phagocytes.

[0061] Human CR1 is a type 1 transmembrane protein of about 200 kDa, which in addition to its presence on erythrocytes, is found on neutrophils, monocytes/macrophages, B cells, some T cells, follicular DC, glomerular podocytes, eosinophils, mast cells, and NK cells. The number of CR1 molecules decreases with aging of erythrocytes.

[0062] In addition to *CR1*, a *CR2* gene also exisits in human and encodes CR2/CD21 protein.

[0063] Mice do not have a functional or structural homolog of human *CR1*, but do have *CR1*-like genes; for example, a *Crry* gene (aka *Cr1like* or *Cr1l*) which encodes a Cr1l protein which shares 10% sequence identity with human CR1; and a *Cr2* gene which encodes a Cr2 protein which shares 17% sequence identity with human CR1. Mouse Cr2 and Cr1like proteins are expressed on B cells, follicular DCs, peritoneal macrophages, activated granulocytes, and platelets, but not on erythrocytes. Similar to mice, rat also has *Cr1l* and *Cr2* genes.

[0064] Human *CR1* gene is located on human chromosome 1, and an exemplary genomic sequence can be found under NCBI Gene ID number 1378. Mouse *Cr1l* and *Cr2* genes are located on mouse chromosome 1, and exemplary genomic sequences of these genes can be found under NCBI Gene ID number 12946 and 12902, respectively. Rat *Cr1l* and *Cr2* genes are located on rat chromosome 13, and exemplary genomic sequences of these genes can be found under NCBI Gene ID number 54243 and 289395, respectively. Examples of RefSeq mRNA IDs and Protein IDs are listed below in Table 1.

Table 1

Gene Name	RefSeq mRNA IDs	UniProt ID or NCBI Protein ID	NCBI Gene ID
Human *CR1*	NM_000573.4 (SEQ ID NO: 1)	P17927 (SEQ ID NO: 2)	1378
Mouse *Cr2*	NM_007758.3	Q9DC83	12902
Mouse *Cr1l*	NM_013499.2	Q64735	12946
Rat *Cr2*	NM_001105989.2	NP_001099459.2	289395
Rat *Cr1l*	NM_001005265.1	NP_001005265.1	54243

Genetically Modified Animals Expressing Human CR1

[0065] In one aspect, provided herein is a genetically modified rodent animal (e.g., mouse or rat) comprising an exogenously introduced nucleic acid, integrated in the rodent genome (i.e., rodent germline) and directing expression of a human CR1 polypeptide in the rodent in a human-like manner.

[0066] The exogenously introduced nucleic acid comprises a nucleotide sequence encoding a human CR1 polypeptide. In some embodiments, the nucleic acid also comprises a 5' regulatory region, and/or and a 3' regulatory region, operably linked to the nucleotide sequence encoding a human CR1 polypeptide. In some embodiments, the transgene includes additional elements, such as a reporter gene and a selectable marker gene, among others.

[0067] The term "operably linkage" includes a linkage of nucleic acid elements in a functional relationship. A nucleic acid sequence is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter, or a 5' regulatory region containing a promoter, is considered as operably linked to a coding

sequence if the promoter or the 5' regulatory region effects the transcription of the coding sequence.

[0068] The terms "5' regulatory region" and "3' regulatory region" as used herein include regulatory elements found in the 5' upstream region and the 3' downstream region of a gene. The term "regulatory elements" includes transcriptional regulatory sequences, which include both 5' transcriptional regulatory sequences such as promoter, enhancer, and suppressor elements, and 3' transcriptional regulatory sequences such as a transcriptional termination sequence. The term "regulatory elements" also includes regulatory sequences in the 5' untranslated region (5' UTR) and the 3' UTR that may affect the efficiency of transcription and the stability of transcript, as well the initiation of translation.

Nucleotide sequence encoding a human CR1 polypeptide

[0069] In some embodiments, a nucleic acid that is integrated in the rodent genome comprises a nucleotide sequence encoding a human CR1 polypeptide which comprises an amino acid sequence substantially identical to SEQ ID NO: 2.

[0070] In referring to a given amino acid sequence as being "substantially identical" to a reference sequence, it includes embodiments where the given amino acid sequence is at last 90% identical, at least 95% identical, at least 98% identical, at least 98.5% identical, at least 99% identical, or at least 99.5% identical, to a reference sequence; for example, a given amino acid sequence differs from a reference sequence by 1, 2, 3, 4, or 5 amino acids, or differs by not more than 5, 4, 3, 2, or 1 amino acid(s). The differences may represent polymorphism that naturally exists for a given molecule.

[0071] In some embodiments, the human CR1 polypeptide comprises an amino acid sequence that is at least 90%, at least 95%, at least 98%, or at least 99% identical to SEQ ID NO: 2. In some embodiments, the human CR1 polypeptide comprises the amino acid sequence of SEQ ID NO: 2.

[0072] In some embodiments, a nucleotide sequence encoding a human CR1 polypeptide is a genomic DNA sequence (i.e., having intronic sequences). In some embodiments, a nucleotide sequence encoding a human CR1 polypeptide is a cDNA sequence (i.e., without intronic sequences). Examples of nucleotide sequencse encoding a human CR1 polypeptide suitable for use herein include the genomic DNA sequence as set forth in GenBank under NCBI Gene ID 1378 and the cDNA sequence as set forth in GenBank under Accession No. NM_000573.4 (SEQ ID NO: 1).

[0073] In some embodiments, a nucleotide sequence encoding a human CR1 polypeptide, either a genomic DNA or a cDNA sequence, comprises a coding sequence beginning from the ATG start codon and ending at the Stop codon of a human *CR1* gene.

5' and 3' regulatory regions

[0074] In some embodiments, a nucleic acid that is exogenously introduced and integrated in the rodent geome can additionally include a 5' regulatory region, operably linked to the nucleotide sequence encoding a human CR1 polypeptide that is included in the nucleic acid.

[0075] In some embodiments, a nucleic acid that is exogenously introduced and integrated in the rodent geome includes a 5' regulatory region, operably linked to the human CR1 coding nucleotide sequence.

[0076] In some embodiments, a 5' regulatory region includes a 5' untranslated region (5' UTR). In some embodiments, the 5' regulatory region includes a 5' UTR of a human *CR1* gene, such as the 5' UTR of the human *CR1* gene as set forth in GenBank under NCBI Gene ID 1378 or Accession No. NM_000573.4. In some embodiments, the 5' regulatory region includes a 5' UTR of a heterologous gene, i.e., a gene that is not a human *CR1* gene, such as, e.g., a rodent *Gata-1* gene.

[0077] In some embodiments, a 5' regulatory region comprises transcription regulatory elements such as promoter and/or enhancer. In some embodiments, a 5' regulatory region comprises a nucleotide sequence (including the promoter region) upstream of the 5' UTR of a human *CR1* gene, such as the human *CR1* gene as set forth in GenBank under NCBI Gene ID 1378. In some embodiments, the 5' regulatory region comprises comprises a nucleotide sequence that is immediately upstream of the 5' UTR of a human *CR1* gene and is of at least 1000 bp, at least 1500 bp, at least 2000 bp, at least 2500 bp, at least 3000 bp, at least 3500 bp, at least 4000 bp, at least 4500 bp, at least 5000 bp, at least 6000 bp, at least 7000 bp, at least 8000 bp, at least 9000 bp, at least 10,000 bp, or longer (e.g., up to 15 Kb-20 Kb), in length. In some embodiments, the 5' regulatory region comprises a nucleotide sequence that is immediately upstream of the 5' UTR of a human *CR1* gene and is of at least 4000 bp in length, such as the 4233 bp sequence (SEQ ID NO: 3) upstream of the 5' UTR of the human *CR1* gene as set forth in GenBank under NCBI Gene ID 1378.

[0078] In some embodiments, a 5' regulatory region comprises a nucleotide sequence (including the promoter region) upstream of the 5' UTR of a heterologous gene, i.e., a gene different from a human *CR1* gene. In some embodiments, the heterologous gene is a rodent

gene that displays expression in red blood cells, e.g., a rodent *Gata-1* gene. In some embodiments, a 5' regulatory region comprises a sequence upstream of the 5' UTR of a rodent (e.g., mouse) *Gata-1* gene that includes the promoter of the rodent *Gata-1* gene. In some embodiments, for example, a 5' regulatory region comprises a sequence immediately upstream of the 5' UTR of a rodent (e.g., mouse) *Gata-1* gene that is at least 7000 bp, at least 8000 bp, at least 9000 bp, at least 10,000 bp, at least 11,000 bp, at least 12,000 bp, at least 13,000 bp, at least 14,000 bp, or longer, in length. In some embodiments, a 5' regulatory region comprises a sequence upstream of the 5' UTR of a mouse *Gata-1* gene as set forth in GenBank under NCBI Gene ID 14460. In some embodiments, a 5' regulatory region comprises a sequence that is immediately upstream of the 5' UTR of the mouse *Gata-1* gene as set forth in GenBank under NCBI Gene ID 14460, and that is at least 7000 bp, at least 8000 bp, at least 9000 bp, at least 10,000 bp, at least 11,000 bp, at least 12,000 bp, at least 13,000 bp, at least 14,000 bp, at least 15,000 bp, at least 16,000 bp, at least 17,000 bp, at least 18,000 bp, at least 19,000 bp, at least 20,000 bp or longer (e.g., up to 25-35 Kb), in length.

[0079] In some embodiments, a nucleic acid that is exogenously introduced and integrated in the rodent geome can additionally include a 3' regulatory region, operably linked to the nucleotide sequence encoding a human CR1 polypeptide that is included in the nucleic acid.

[0080] In some embodiments, a 3' regulatory region includes a 3' UTR. In some embodiments, a 3' regulatory region includes the 3' UTR of a human *CR1* gene, such as the 3' UTR of the human *CR1* gene as set forth in GenBank under NCBI Gene ID 1378 or Accession No. NM_000573.4. In some embodiments, a 3' regulatory region includes a 3' UTR of a heterologous gene, i.e., a gene that is not a human *CR1* gene, such as, e.g., a human beta-1 globin gene. In some embodiments, a 3' regulatory region includes a 3' UTR sequence (containing the ploy adenylation signal) of a human beta-1 globin gene as set forth in SEQ ID NO: 4.

[0081] In some embodiments, a 3' regulatory region comprises a sequence downstream of the 3' UTR of a human *CR1* gene, such as the human *CR1* gene as set forth in GenBank under NCBI Gene ID 1378. In some embodiments, the 3' regulatory region comprises a nucleotide sequence that is immediately upstream of the 3' UTR of a human *CR1* gene and is of at least 50 bp, at least 100 bp, at least 150 bp, at least 200 bp, at least 300 bp, at least 400 bp, at least 500 bp, at least 750 bp, at least 1000 bp, or longer (e.g., up to 2500 – 4000

bp), in length. In some embodiments, the 3' regulatory region comprises a nucleotide sequence that is immediately downstream of the 3' UTR of a human *CR1* gene and is of at least 150 bp in length, such as the 159 bp (SEQ ID NO: 41) immediately downstream of the 3' UTR of a human *CR1* gene as set forth in GenBank under NCBI Gene ID 1378.

[0082] In some embodiments, a 3' regulatory region comprises a sequence downstream of the 3' UTR of a heterologous gene. In some embodiments, the heterologous gene is a rodent gene that displays expression in red blood cells, e.g., a rodent *Gata-1* gene. In some embodiments, a 3' regulatory region comprises a sequence downstream of the 3' UTR of a rodent (e.g., mouse) *Gata-1* gene. In some embodiments, a 3' regulatory region comprises a sequence immediately downstream of the 3' UTR of a rodent (e.g., mouse) *Gata-1* gene that is at least 250 bp, at least 500 bp, at least 1000 bp, at least 1500 bp, at least 2000 bp, at least 3000 bp, or longer, in length. In some embodiments, a 3' regulatory region comprises a sequence downstream of the 3' UTR of a mouse *Gata-1* gene as set forth in GenBank under NCBI Gene ID 14460. In some embodiments, a 3' regulatory region comprises a sequence that is immediately downstream of the 3' UTR of a mouse *Gata-1* gene as set forth in GenBank under NCBI Gene ID 14460, and that is at least 250 bp, at least 500 bp, at least 1000 bp, at least 1500 bp, at least 2000 bp, at least 3000 bp, or longer (e.g., up to 4000 – 6000 bp), in length.

Embodiments of exogenously introduced nucleic acids

[0083] In some embodiments, a nucleic acid exogenously introduced and integrated in the rodent genome comprises a human genomic DNA which includes the entire human *CR1* coding sequence from ATG to STOP, with the 5' and 3' untranslated regions (UTRs) and intervening introns, as well as a 5' upstream sequence of at least 4000 bp directly upstream of the 5' UTR and a sequence of at least 150 bp directly downstream of the 3' UTR. In some embodiments, the nucleic acid integrated in the rodent genome comprises a human genomic DNA which includes the entire human *CR1* coding sequencec from ATG to STOP, with the 5' and 3' untranslated regions (UTRs) and intervening introns, as well as an additional sequence of 4,233 bp directly upstream of the 5' UTR and a sequence of 159 bp directly downstream of the 3' UTR.

[0084] In some embodiments, a nucleic acid exogenously introduced and integrated in the rodent genome comprises a human *CR1* coding sequence (ATG to STOP) (e.g., in the form of cDNA), operably linked to, at the 5', a nucleotide sequence of at least 14 Kb directly upstream of ATG of a rodent *Gata-1* gene (including the promoter of the rodent *Gata1*

gene), and at the 3', a 3' UTR sequence containing the poly(A) signal from a human beta globin gene followed by a nucleotide sequence of at least 1.5 Kb directly downstream of the STOP codon of the rodent *Gata-1* gene.

[0085] In some embodiments, a genetically modified rodent animal (e.g., mouse or rat) comprises multiple (i.e., two or more) exogenously introduced nucleic acids integrated in the rodent genome, with each nucleic acid being any of the nucleic acids described above and encoding a human CR1 polypeptide. Such rodent animal can be made by crossing (i.e., cross-breeding) rodent animals comprising one nucleic acid integrated in the rodent genome.

Location of an exogenously introduced nucleic acid in rodent genome

[0086] In some embodiments, a nucleic acid is integrated to a selected site in the rodent genome. Integration into a specific site can be accomplished by utilizing a nucleic acid construct specifically designed for targeted insertion into the site. In some embodiments, a nucleic acid comprising a human CR1 coding sequence is integrated in the rodent genome between the rodent *Cr2* gene locus and the rodent *Cr1l* gene locus; namely, at a location 3' (downstream) from the 3' UTR of the rodent *Cr2* gene and 5' (upstream) to the 5' UTR of the rodent *Cr1l* gene. In some embodiments, the nucleic acid is integrated in the rodent genome at a location of not more than 3000 bp, not more than 2500 bp, not more than 2000 bp, not more than 1500 bp, not more than 1250 bp, or not more than 1000 bp downstream from the 3' UTR of the rodent *Cr2* gene. In some embodiments, the nucleic acid is integrated in the rodent genome at about 900 bp downstream from the 3' UTR of the rodent *Cr2* gene.

[0087] In some embodiments, a nucleic acid that is integrated in the rodent genome between the rodent *Cr2* gene locus and the rodent *Cr1l* gene locus comprises a human genomic DNA which includes the entire human *CR1* coding sequence from ATG to STOP, with the 5' and 3' untranslated regions (UTRs) and intervening introns, as well as a 5' upstream sequence of at least 4000 bp directly upstream of the 5' UTR and a sequence of at least 150 bp directly downstream of the 3' UTR. In some embodiments, a nucleic acid integrated in the rodent genome between the rodent *Cr2* gene locus and the rodent *Cr1l* gene locus comprises a human genomic DNA which includes the entire human *CR1* coding sequencec from ATG to STOP, with the 5' and 3' untranslated regions (UTRs) and intervening introns, as well as an additional sequence of 4,233 bp directly upstream of the 5' UTR and a sequence of 159 bp directly downstream of the 3' UTR.

[0088] In some embodiments, a nucleic acid is integrated randomly to one or more sites in the rodent genome. In cases of random integration, multiple copies of a nucleic acid may be integrated at multiple sites in the rodent genome; or alternatively, multiple copies of a nucleic acid may be integrated in tandem into one locus of the genome. In some embodiments, only one copy of a nucleic acid encoding a human CR1 polypeptide is integrated into the rodent genome. In some embodiments, a nucleic acid is integrated through random integration to an X chromosome of the rodent genome. In some embodiments, only one copy of a nucleic acid is integrated through random integration to an X chromosome of the rodent genome. In some embodiments, one copy of a nucleic acid is integrated through random integration to a locus of an X chromosome that is not the rodent *Gata-1* gene locus.

[0089] In some embodiments, a nucleic acid that is randomly integrated in the rodent genome comprises a human *CR1* coding sequence (ATG to STOP) (e.g., in the form of cDNA), operably linked to, at the 5', a nucleotide sequence of at least 14 Kb directly upstream of ATG of a rodent *Gata-1* gene (including the rodent *Gata1* promoter), and at the 3', a 3' UTR sequence containing the poly(A) signal from a human beta globin gene followed by a nucleotide sequence of at least 1.5 Kb directly downstream of the STOP codon of a rodent *Gata-1* gene; in some of such embodiments, one copy of the nucleic acid encoding a human CR1 polypeptide is integrated through random integration to an X chromosome of the rodent genome, e.g., integrated to a locus that is not the rodent *Gata-1* gene locus.

[0090] In some embodiments, a genetically modified rodent animal comprising a first nucleic acid comprising a first nucleotide sequence encoding a human CR1 polypeptide integrated at a specific site in the rodent genome, and a second nucleic acid comprising a second nucleotide sequence encoding a human CR1 polypeptide integrated randomly in the rodent genome. In some embodiments, a genetically modified rodent animal comprises a first nucleic acid integrated between the rodent *Cr2* gene locus and the rodent *Cr1l* gene locus in the rodent genome, and a second nucleic acid integrated into a locus on an X chromosome in the rodent genome; in some such embodiments, the first nucleic acid comprises a human genomic DNA which includes the entire human *CR1* coding sequence from ATG to STOP, with the 5' and 3' untranslated regions (UTRs) and intervening introns, as well as a 5' upstream sequence of at least 4000 bp (e.g., a sequence of 4233 bp) directly upstream of the 5' UTR and a sequence of at least 150 bp (e.g., a sequence of 159

bp) directly downstream of the 3' UTR; and the second nucleic acid comprises a human *CR1* coding sequence (ATG to STOP) (e.g., in the form of cDNA), operably linked to, at the 5', a nucleotide sequence of at least 14 Kb directly upstream of ATG of a rodent *Gata-1* gene (including the rodent *Gata1* promoter), and at the 3', a 3' UTR sequence containing the poly(A) signal from a human beta globin gene followed by a nucleotide sequence of at least 1.5 Kb directly downstream of the STOP codon of a rodent *Gata-1* gene.

Heterozygosity/Homozygosity, Gender and Strain Background

[0091] In some embodiments, a genetically modified rodent animal is heterzogous for a nucleic acid exogenously introduced and integrated in the rodent genome. In some embodiments, a genetically modified rodent animal is homozygous for a nucleic acid exogenously introduced and integrated in the rodent genome. In embodiments where a genetically modified rodent animal comprises multiple nucleic acids each comprising a nucleotide sequence encoding a human CR1 polypeptide, the rodent animal can be heterozygous or homozygous for one nucleic acid, and independently heterozygous or homozygous for another nucleic acid.

[0092] In some embodiments, the genetically modified rodent animal is a male animal. In some embodiments, the genetically modified rodent animal is a female animal.

[0093] In some embodiments, the rodent is a mouse. In some embodiments, the rodent is a mouse of a C57BL strain, for example, a C57BL strain selected from C57BL/A, C57BL/An, C57BL/GrFa, C57BL/KaLwN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, and C57BL/Ola. In other embodiments, the rodent is a mouse of a 129 strain, for example, a 129 strain selected from the group consisting of 129P1, 129P2, 129P3, 129X1, 129S1 (e.g., 129S1/SV, 129S1/SvIm), 129S2, 129S4, 129S5, 129S9/SvEvH, 129/SvJae, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, 129T2 (see, e.g., Festing et al. (1999), *Mammalian Genome* 10:836; Auerbach et al. (2000), *Biotechniques* 29(5):1024-1028, 1030, 1032). In some embodiments, the rodent is a mouse that is a mix of an aforementioned 129 strain and an aforementioned C57BL/6 strain. In certain embodiments, the mouse is a mix (i.e., hybrid) of aforementioned 129 strains, or a mix of aforementioned C57BL strains, or a mix of a C57BL strain and a 129 strain. In certain embodiments, the mouse is a mix of a C57BL/6 strain with a 129 strain. In specific embodiments, the mouse is a VGF1 strain, also known as F1H4, which is a hybrid of C57BL/6 and 129. In other embodiments, the mouse is a

BALB strain, e.g., BALB/c strain. In some embodiments, the mouse is a mix of a BALB strain and another aforementioned strain.

[0094] In some embodiments, the rodent is a rat. In certain embodiments, the rat is selected from a Wistar rat, an LEA strain, a Sprague Dawley strain, a Fischer strain, F344, F6, and Dark Agouti. In other embodiments, the rat is a mix of two or more strains selected from the group consisting of Wistar, LEA, Sprague Dawley, Fischer, F344, F6, and Dark Agouti.

Human like expression of human CR1 in a genetically modified rodent animal

[0095] Genetically modified rodent animals provided herein express human CR1 in a human-like expression pattern. By "human-like" expression pattern it is meant that the human CR1 polypeptide is expressed on cells in a rodent characteristic of the human cells which express human CR1 in human. For example, human CR1 is expressed on erythrocytes and neutrophils in human, among other cells, whereas mouse Cr2 protein is not expressed on neutrophils in mouse, yet mouse Cr1like protein is expressed on all mouse cells including erythrocytes. Thus, in some embodiments, a human-like expression pattern comprises expression of a human CR1 on erythrocytes in the rodent. In some embodiments, a human-like expression pattern comprises expression of a human CR1 on neutrophils in the rodent, e.g., neutrophils found in the blood, the spleen, and/or the liver. In some emebodiments, a human-like expression pattern comprises expression of a human CR1 on erythrocytes and neutrophils e.g., neutrophils found in the blood, the spleen, and/or the liver) in the rodent. In some embodiments, a human-like expression pattern comprises expression of a human CR1 in the rodent on one or more of macrophages (such as macrophages in the blood, large peritoneal macrophages, macrophages in the spleen, and motile macrophages in the liver), monocytes (inflammatory and/or resident monocytes), and circulating dendritic cells (cDCs), in addition to erythrocytes and/or neutrophils.

Other Genetic Modifications In a Genetically Modified Rodent

[0096] In addition to comprising a nucleic acid encoding a human CR1 polypeptide Modified C3 gene integrated in the geneome, a genetically modified rodent may comprise other genetic modifications. In some embodiments, a genetically modified rodent disclosed herein also comprises in its genome, a replacement at an endogenous rodent C3 locus of a rodent gene sequence comprising an exon of a C3 gene with a nucleic acid sequence comprising at least one exon of a human C3 gene to form a modified C3 gene, as described

for example in U.S. Patent 9,795,121 B1 (Regeneron Pharmaceuticals, Inc.), incorporated herein in its entirety.

[0097] In some embodiments, a nucleic acid sequence comprising at least one exon of a human C3 gene comprises coding exon 1 through coding exon 41 of the human C3 gene. In some embodiment, a nucleic acid sequence comprising at least one exon of a human C3 gene comprises 5′ regulatory elements and coding exon 1 through coding exon 41 of the human C3 gene. In some embodiments, a nucleic acid sequence comprising at least one exon of a human C3 gene comprises coding exon 2 through coding exon 41 of the human C3 gene.

[0098] In some embodiments, a genetically modified rodent is a mouse, whose genome comprises a replacement of a mouse C3 genomic sequence comprising 5′ regulatory elements and all of the coding exons 1 through 41 of the endogenous mouse C3 gene with a human C3 genomic sequence comprising 5′ regulatory elements and all of the coding exons 1 through 41 of the human C3 gene. In some embodiments, a genetically modified rodent is a mouse, whose genome comprises a replacement of a mouse C3 genomic sequence comprising coding exons 2 through 41 of the endogenous mouse C3 gene with a human C3 genomic sequence comprising coding exons 2 through 41 of the human C3 gene.

[0099] In some embodiments, a genetically modified rodent does not express an endogenous rodent C3 protein. In some embodiments, a genetically modified rodent express an endogenous rodent C5 protein.

[0100] Genetically modified rodent animals comprising a modified C3 gene encoding human C3 are prone to high rates of spontaneous death and additionally exhibit physiological, morphological, and histological symptoms which resemble complement-related nephropathies, as well as symptoms consistent with liver fibrosis, as described in U.S. Patent 10,765,762 (Regeneron Pharmaceuticals, Inc.). In accordance with this disclosure, expression of a human CR1 polypeptide can ameliorate the injury to the kidney and symptoms of complement-related nephropathy, which result from human C3 being expressed from a modified C3 gene in the rodent. Complement-related nephropathy may be reflected by one or more symptoms selected from (i) one or more of glomerulonephritis, basophilic tubules, sclerotic glomeruli, dilated tubules with protein casts, mesangial matrix expansion, glomerular hypertrophy, mononuclear interstitial inflammation, (ii) C3 protein deposition in the kidney, (iii) deposition of C5b-9 membrane attack complexes in the kidney, (iv) one or more of elevated blood urea nitrogen (BUN), serum lipase, serum

cystatin C, or serum non-high density lipoproteins, (v) increased urinary albumin or C5a, (vi) spontaneous death, (vii) decreased weight, decreased bone density, and/or decreased body fat, or a combination of any one of (i) to (vii). Those skilled in the art would be able to readily assess a change, if any, in a relevant parameter or symptom, and determine whether the change is statistically significant to constitute amelioration of nephropathy in the rodent as a result of human CR1 expression and in comparison to appropriate control rodents without the human CR1 expression.

Nucleic Acid Vectors and Methods of Making A Rodent Expressing Human CR1

Targeting vector for targeted insertion

[0101] Rodents comprising an exogenously introduced nucleic acid which comprises a nucleotide sequence encoding a human CR1 polypeptide can be made using various methods. In some embodiments, a targeting nucleic acid construct (i.e., a targeting vector) is constructed to carry a desired nucleic acid is constructed. The term "targeting vector" as used herein refers to vectors designed to have a nucleic acid carried on the vector to be inserted into a target locus of the rodent genome.

[0102] The nucleic acid carried on a targeting vector can be any of the nucleic acids described herein above. Depending on size (e.g., whether a genomic DNA or cDNA is used), a nucleic acid can be cloned directly from cDNA sources or synthetically made. Alternatively, bacterial artificial chromosome (BAC) libraries can provide human *CR1* nucleic acid sequences.

[0103] The targeting vector can include, in addition to a nucleic acid to be integrated which comprises a nucleotide sequence encoding human CR1, flanking nucleic acid sequences that are of suitable lengths and homologous to rodent sequences at a selected endogenous rodent locus (e.g., a locus between the rodent *Cr2* gene locus and the rodent *Crl1* gene locus) so as to be capable of mediating homologous recombination and integration of the nucleic acid that comprises a nucleotide sequence encoding human CR1 into the endogenous rodent locus. The flanking nucleic acid sequences can be 5 kb, 10 kb, 15 kb, 20 kb, 25 kb, 30 kb, 35 kb, 40 kb, 45 kb, 50 kb, 55 kb, 60 kb, 65 kb, 70 kb, 75 kb, in length, or any value between the above-recited lengths.

[0104] In some embodiments, a targeting vector also includes a selectable marker gene (e.g., a self deleting cassette containing a selectable marker gene, as described in U.S. Patent Nos. 8,697,851, 8,518,392 and 8,354,389, all of which are incorporated herein by

reference), which can be flanked by or comprises site-specific recombination sites (e.g., *lox*P, Frt, etc.). The selectable marker gene can be placed on the targeting vector adjacent to the nucleic acid that comprises a nucleotide sequence encoding human CR1, to permit easy selection of transfectants.

Transgenic vector for random integration

[0105] In some embodiments, a nucleic acid that comprises a nucleotide sequence encoding human CR1, can be inserted into a rodent genome using a transgenic vector designed for random integration.

[0106] A transgenic vector comprises any of the human CR1 polypeptide-encoding nucleic acids described above. In some embodiments, a transgenic vector comprises a human *CR1* coding sequence (ATG to STOP) (e.g., in the form of cDNA), operably linked to, at the 5', a nucleotide sequence of at least 14 Kb directly upstream of ATG of a rodent *Gata-1* gene (including the rodent *Gata1* promoter), and at the 3', a 3' UTR sequence containing the poly(A) signal from a human beta globin gene followed by a nucleotide sequence of at least 1.5 Kb directly downstream of the STOP codon of a rodent *Gata-1* gene.

[0107] A transgenic vector can include additional elements, such as a selectable marker gene, placed on the transgenic vector adjacent to the human *CR1* nucleic acid. The selectable marker gene can be, e.g., a self deleting cassette containing a selectable marker gene, as described in U.S. Patent Nos. 8,697,851, 8,518,392 and 8,354,389, all of which are incorporated herein by reference, which can be flanked by or comprises site-specific recombination sites (e.g., *lox*P, Frt, etc.).

[0108] The transgenes disclosed herein can be made using known methods. For example, a transgene can be assembled using bacterial homologous recombination and VELOCIGENE® technology (see, e.g., U.S. 6,586,251 and Valenzuela *et al.,* High-throughput engineering of the mouse genome coupled with high-resolution expression analysis, 2003, *Nature Biotech.* 21(6):652-659). An example of a transgenic vector carrying a human CR1 nucleic acid is described in Example 4 hereinbelow.

Introduction of a vector and integration of a human CR1 nucleic acid into a rodent genome

[0109] In some embodiments, a vector carrying a desired nucleic acid to be integrated, either a targeting vector or a transgenic vector, can be introduced into rodent embryonic stem (ES) by, e.g., electroporation. Both mouse ES cells and rat ES cells have been

described in the art. See, e.g., US 7,576,259, US 7,659,442, US 7,294,754, and US 2008-0078000 A1 (all of which are incorporated herein by reference) describe mouse ES cells and the VELOCIMOUSE® method for making a genetically modified mouse; and US 2014/0235933 A1, US 2014/0310828 A1, and US 2014/0309487 A1 (all of which are incorporated herein by reference) describe rat ES cells and methods for making a genetically modified rat.

[0110] ES cells having a desired nucleic acid integrated in the rodent genome can be selected. In embodiments where a targeting vector is used, ES cells having the nucleic acid integrated into a target locus are selected. In embodiments where a transgenic vector is used, ES cells having the nucleic acid integrated into the genome are selected irrespective of the site(s) where the integration occurs; in some such embodiments, one or more copies of the nucleic acid may be integrated at one or more sites; and in some such embodiments, one copy of the nucleic acid may be integrated at one site.

[0111] ES cells having a desired nucleic acid integrated in the genome are then used as donor ES cells for injection into a pre-morula stage embryo (e.g., 8-cell stage embryo) by using the VELOCIMOUSE® method (see, e.g., US 7,576,259, US 7,659,442, US 7,294,754, and US 2008-0078000 A1), or methods described in US 2014/0235933 A1 and US 2014/0310828 A1. The embryo comprising the donor ES cells is incubated until blastocyst stage and then implanted into a surrogate mother to produce an F0 rodent fully derived from the donor ES cells. Rodent pups bearing the exogenous nucleic acid can be identified by genotyping of DNA isolated from tail snips using a modification of allele (MOA) assay (Valenzuela *et al.*, *supra*) that detects the presence of the exogenous nucleic acid sequence.

[0112] In other embodiments, a genetically modified rodent can be made without using ES cells. For example, the genome of a non-ES cell of a rodent (e.g., a fibroblast or an induced pluripotent cell) can be modified based on conventional transformation methods (e.g., electroporation), and the modified genome of such non-ES cell can be transferred to a suitable recipient cell, e.g., an oocyte, by employing the nuclear transfer technique. The modified cell (e.g., the modified oocyte) is then gestated under suitable conditions to form an embryo. See, e.g., Han et al., "*Nuclear Transfer in Mouse Oocytes and Embryos*", *Methods in Enzymology* 476: 171-184 (2010), and Zhou et al., "*Generation of Fertile Cloned Rats by Regulating Oocyte Activation*", *Science* 302: 1179 (2003).

Crossing and backcrossing

[0113] Genetically modified rodent animals comprising an exogenous nucleic acid encoding a human CR1 polypeptide can be crossed with other rodent animals. A manner of preparation is to generate a series of rodent animals, each containing one of the desired nucleic acids or transgenes. Such rodent animals are bred together through a series of crosses, backcrosses and selections, to ultimately generate a single rodent animal containing all desired nucleic acids, where the mammal is otherwise congenic (genetically identical) to the wild type except for the presence of the desired nucleic acids. In one embodiment, a mouse comprising an exogenous nucleic acid a human CR1-coding sequence and comprising a human C3 gene sequence is produced in this manner. In another embodiment, a mouse is prepared in this manner to comprise (i) a nucleic acid comprising a nucleotide sequence encoding a human CR1 polypeptide and integrated between the mouse *Cr2* gene locus and the mouse *Cr1l* gene locus, and (ii) a nucleic acid comprising a nucleotide sequence encoding a human CR1 polypeptide, operably linked to a mouse *Gata1* promoter and integrated in an X chromosome.

[0114] Typically, crossing and backcrossing is accomplished by mating siblings or a parental strain with an offspring, depending on the goal of each particular step in the breeding process. In certain cases, it may be necessary to generate a large number of offspring in order to generate a single offspring that contains each of the desired nucleic acids in the proper chromosomal location. In addition, it may be necessary to cross or backcross over several generations to ultimately obtain the desired genotype.

Use of a Genetically Modified Rodent Expressing Human CR1

[0115] Genentically modified rodent animals expressing a human CR1 can be used to screening and testing candidate drugs targeting human CR1. Because CR1 is a negative regulator of the complement system, expression of human CR1 can also be useful in abrogating complement overactivation which causes unwanted kidney and liver injury in a C3 humanized rodent, thereby facilitating generation of rodent animals with fully functional human complement activity. Genentically modified rodent animals expressing a human CR1 and human C3 can also be used to screen and test candidate drugs targeting human C3 or otherwise modulating the human complement system.

[0116] In one aspect, provided herein are methods for assessing candidate compounds targeting human CR1 or another component of the human complement system (such as human C3). The method utilizes any of the genetically modified rodents (for example, mice

or rats) disclosed herein. Candidate compounds can be, without limitation, small molecule chemical compounds, antibodies, proteins, inhibitory nucleic acids, or any combination thereof.

[0117] In some embodiments, the present invention provides a method of assessing the pharmacokinetic properties of a compound targeting human CR1 or another component of the human complement system (such as human C3), the method comprising the steps of administering a compound to a genetically modified rodent animal disclosed herein; and performing an assay to determine one or more pharmacokinetic properties of the compound. Pharmacokinetic properties include, but are not limited to, how an animal processes the compound into various metabolites (or detection of the presence or absence of one or more metabolites, including, but not limited to, toxic metabolites), half-life, circulating levels of compound after administration (e.g., serum concentration of compound), anti- compound response (e.g., anti- compound antibodies), compound absorption and distribution, route of administration, routes of excretion and/or clearance of the compound. In some embodiments, pharmacokinetic and pharmacodynamic properties of compounds (e.g., human CR1 modulators) are monitored in or through the use of a genetically modified rodent animal disclosed herein.

[0118] Genetically modified rodent animals disclosed herein provide an *in vivo* system for assessing the on-target toxicity of a compound (e.g., a compound targeting human CR1 or human C3). In some embodiments, a compound may be delivered or administered to one or more genetically modified rodent animals disclosed herein, followed by monitoring of or performing one or more assays on the animals (or cells isolated therefrom) to determine the on-target toxic effect of the compound on the animals. Exemplary on-target effects include too high of a dose, chronic activation/inactivation, and correct action in an incorrect tissue.

[0119] Genetically modified rodent animals disclosed herein provide an *in vivo* system for assessing the off-target toxicity of a compound (e.g., a compound targeting human CR1 or human C3). In some embodiments, a compound may be delivered or administered to one or more genetically modified rodent animals disclosed herein, followed by monitoring of or performing one or more assays on the animals (or cells isolated therefrom) to determine the off-target toxic effect of the compound on the animals. Off-target effects can occur when a compound interacts with an unintended target (e.g., cross-reactivity to a common epitope). Exemplary off-target effects include incorrect activation/inhibition of an

incorrect target regardless of the tissue in which the incorrect target is found. In some embodiments, off-target effects of a compound are determined by comparing the effects of administering the compound to non-human animals of the present invention to one or more reference non-human animals.

[0120] Exemplary parameters that may be measured in rodent animals (or in and/or using cells isolated therefrom) for assessing the pharmacokinetic properties, on-target toxicity, and/or off-target toxicity of a compound include, but are not limited to, agglutination, autophagy, cell division, cell death, complement-mediated hemolysis, DNA integrity, compound-specific antibody titer, compound metabolism, gene expression arrays, metabolic activity, mitochondrial activity, oxidative stress, phagocytosis, protein biosynthesis, protein degradation, protein secretion, stress response, target tissue compound concentration, non-target tissue compound concentration, transcriptional activity and the like.

[0121] In some embodiments, rodent animals disclosed herein are used to identify a compound capable of modulating complement activation comprising administering the compound to any of the rodent animals described herein; and assaying if complement activation in the rodent is modulated, thereby identifying a compound capable of modulating complement activation. In some embodiments, a compound modulates complement activation by increasing complement activity. In some embodiments, a compound modulates complement activation by decreasing complement activity.

[0122] In some embodiments, a candidate compound is administered directly to a rodent following experimental induction of complement activation (for example, in a kidney ischemia/reperfusion model) and the effects of the compound with respect to their ability to bind human and modulate human CR1, and/or to modulate the complement system are assessed. In other embodiments, a candidate compound is contacted with serum obtained from a rodent and complement activity is assessed using any commonly used *in vitro* assessment technique (such as, but not limited to CH_{50} assays).

[0123] The invention can be further understood by reference to the following examples, which are provided by way of illustration and are not meant to be limiting.

EXAMPLES

[0124] 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 the compositions and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the disclosure.

Example 1. Generation of Mouse Comprising Targeted Insertion of Human *CR1*

[0125] *Generation of mourse having targeted insertion of human CR1* - A large targeting vector (LTVEC) was generated using human and mouse bacterial artificial chromosomes (BAC) DNA and VELOCIGENE® technology (see, e.g., US Patent No. 6,586,251 and Valenzuela et al. (2003) High-throughput engineering of the mouse genome couple with high-resolution expression analysis. Nat. Biotech. 21(6): 652-659, both incorporated herein by reference). The LTVEC was constructed by replacing a genomic sequence of 251 bp between mouse *Cr2* and *Crll* loci with a human genomic sequence of 131,456 bp which includes the entire *CR1* locus from ATG to STOP, with the 5' and 3' untranslated regions (UTRs) and intervening introns, as well as an additional sequence of 4,233 bp (SEQ ID NO: 3) directly upstream of the 5' UTR and a sequence of 159 bp (SEQ ID NO: 41) directly downstream of the 3' UTR. The mouse sequence of 251bp being replaced is downstream of the *Cr1* 3' UTR and upstream of the *Crll* 5' UTR. A self-deleting hygromycin resistance cassette (which contains a protamine-promoter driven Cre that deletes in the male germ cells) was placed directly downstream of the 131,456 bp human insert. See FIG. 1A.

[0126] The resultant vector was used to electroporate hybrid 129S6/SvEvTac:C57Bl/6Ntac F1 mouse embryonic stem (ES) cells and positive selection was accomplished based on hygromycin resistance. Correctly targeted ES cells were identified by a modification of allele assay (MOA). Specific primer sets and probes were designed for detecting deletion of mouse sequences (loss of allele or LOA) and insertion of the human sequences (gain-of-allele or GOA). The locations of the primers and probes are depicted in FIG. 1A.

Table 2: Primer and Probes Used to Confirm Loss of Mouse Sequence (LOA) or Gain of Human Sequence (GOA)

Description	**Sequence**	**SEQ ID NO**
7238mTU	Fwd: TGAGCCTACTCAACCTTAACAGT	5
	Probe (BHQ): TCTGTCTGGTGGCATAGTTCACTTGC	6
	Rev: TGGCCTGTTTGAAGGAATTGTTG	7
7238mTD	Fwd: GCATGCAAACAAACCATTGGAA	8
	Probe (BHQ):AAAGGAAATGAGAAGACAGTAAAACCTGCA	9 10
	Rev: CCCGTCTAAGAAACACTGAGGTA	

7238hTU	Fwd: GCAGTGGAAGGCGCAGATG	11
	Probe (BHQ): AGCGGGTGCCGCACGAAATTC	12
	Rev: CAGCCGAGGCTGTGAATACAC	13
7238hTD	Fwd: TGGGCAAAGGACATACAGCTA	14
	Probe (BHQ): TCACCAAGAAAGAAGGGCAAATAGGTGG	15
	Rev: GGCCAATTCCCAATCACTTAGTTTC	16

[0127] Correctly targeted ES clones were used as donor ES cells and microinjected into 8-cell stage Swiss Webster embryos, resulting in F0 VelociMice® fully derived from the injected modified ESC (Poueymirou 2007; Nature Biotech 25(1):91-99). These F0 mice, designated MAID 7238, were subsequently bred to 100% C57Bl/6NTac mice. The resistance cassette in MAID 7238 was removed by self-deleting technology, resulting in MAID 7239.

Example 2 - Phenotyping of Mouse Comprising Targeted Insertion of Human *CR1*

[0128] *Phenotyping of MAID 7239 Het (F1 mice heterozygous for targeted insertion of human CR1 with SDC deleted)* – Flow cytometry was performed on cells from the whole blood, lysed blood, and spleen of MAID 7239 Het mice (F1: 10 wk old, males, n=3, 75%/25% B6/129 background) and MAID 7238 wild type mice (10 wk old, males, n=3, 75%/25% B6/129 background). MAID 7239 Het mice had normal myeloid cell populations in the blood and in the spleen as compared to MAID 7238 wild type mice. As shown in FIG. 2A for cells from the lysed blood, a high level of hCR1 was detected on neutrophils (at approximately the same expression level seen in human blood), and a very low level of hCR1 was detected on macrophages. However, hCR1 was not detectable on red blood cells (RBCs) from the whole blood. As shown in FIG. 2B for cells isolated from the spleen, a high level of hCR1 was detected on neutrophils, and a very low level of hCR1 was detected on macrophages and inflammatory monocytes.

[0129] *Phenotyping of MAID 7239 HO (F2 mice homozygous for targeted insertion of human CR1 with SDC deleted)* – Flow cytometry was performed on cells from the whole blood, lysed blood, and spleen of MAID 7239 HO mice (F2: 6-7 wk old, males, n=3 (data not shown); 12 week old, females, n=3; 75%B6 25%129 background) and MAID 7239 wild type mice (6-7 wk old, males, n=3 (data not shown); 12 wk old, females, n=3; 75%B6 25%129 background). MAID 7239 HO mice had normal myeloid cell populations in the

blood and in the spleen as compared to MAID 7239 wild type mice. As shown in FIG. 2C for cells from the lysed blood, a high level of hCR1 was detected on neutrophils (at approximately the same expression level seen in human blood); a moderate level of hCR1 was detected on cDCs, and a low level of hCR1 was detected on macrophages. However, hCR1 was not detectable on red blood cells (RBCs) from the whole blood. As shown in FIG. 2D for cells isolated from the spleen, a high level of hCR1 was detected on neutrophils, and a very low level of hCR1 was detected on macrophages, inflammatory monocytes and cDCs. Flow cytometry was performed on cells from the peritoneal cavity and digested liver of MAID 7239 HO mice (75% C57BL/6NTac 25% 129S6/SvEvTac background, Males n=2, Female n=1, 15 weeks old, F6) and MAID 7239 wild type mice (75/25WT (50500): 75% C57BL/6NTac 25% 129S6/SvEvTac background, Males n=2, Female n=1, 15 weeks old, F1). hCR1 was also detected on a few large (but not small) peritoneal macrophases in 1 of 3 mice examined (FIG. 2E). hCR1 was detected on all neutrophils in the liver, and on approximately 50% motile macrophages (and possibly cDCs) (FIG. 2F).

Materials and Methods

[0130] All mice were housed and bred in the specific pathogen-free conditions at Regeneron Pharmaceuticals. Mice were sacrificed, and spleens and blood were harvested. Blood was collected into BD microtainer tubes with EDTA (Cat # 365973). Red blood cells from spleen and blood preparations were lysed with ACK lysis buffer, followed by washing with complete RPMI medium. In some instances, liver and peritoneal cells were harvested. Liver was collected, chopped into small pieces and digested in a mix of Liberase TH (Cat# 5401151001, Roche, used at a final concentration of 0.7 U/ml) and DNase (cat# 10104159001, Roche, used at a final concentration of 20 ug/ml) in HBSS for 20 min at 37°C, after which reaction was stopped with EDTA at a final concentration of 10 mM, followed by washing with complete RPMI medium and red cell lysis with ACK lysis buffer. Peritoneal macrophages were collected via peritoneal lavage by flushing peritoneal cavity with 5-6 ml of ice-cold PBS with a 27g needed using a 10 ml syringe. Red cells were lysed with ACK, followed by washing with complete RPMI medium.

[0131] *Flow cytometry on unlysed blood, lysed blood and spleen*: $1x10^6$ cells were incubated with anti-mouse CD16/CD32 (2.4G2, BD) on ice for 10 minutes, followed by labeling with the following antibody panels for 30 min on ice. Panel used on unlysed blood: anti-mouse PeCy7- TER119 (TER119, BD), anti-human PE-CR1 (E11, BD) or PE-

IgG1, K isotype control (MOPC-21, BD). Panel used on lysed blood, spleen, liver, peritoneal lavage: anti-mouse FITC-Ly6C (HK1.4, Biolegend), PeCy7-F4/80 (BM8, Biolegend), PerCP-Cy5.5-Ly6G (1A8, BD), Pacific Blue-CD3 (17A2, BioLegend), APC-CD11c (N418, Biolegend), APC-eFlour780-CD11b (M1/70, eBioscience), A700-CD19 (1D3, BD) and anti-human PE-CR1 (E11, BD) or PE-IgG1, K isotype control (MOPC-21, BD).

[0132] *Flow cytometry on liver and peritoneal lavage*: $1x10^6$ cells were first washed in PBS then incubated with LIVE/DEAD™ Fixable Yellow Dead Cell Stain (Cat# L34959, Invitrogen) for 30 min on ice, followed by washing in PBS, then incubation with anti-mouse CD16/CD32 (2.4G2, BD) on ice for 10 minutes, followed by labeling with the following antibody panels for 30 min on ice.

[0133] Following staining, cells were washed and fixed in 2% formaldehyde. Data acquisition was performed on a BD LSRFortessa™ Flow Cytometer and analyzed with FlowJo software.

[0134] Macrophages (F4/80+ CD11b- and F4/80+ CD11b+), Inflammatory Monocytes (CD11b+ Ly6C+ Ly6G-), Resident Monocytes & NK cells (CD11b+ Ly6C- Ly6G-), Neutrophils CD11b+ Ly6C- Ly6G+), cDCs (CD11b+ CD11c). In the peritoneal cavity, small Peritoneal Macrophages (F4/80 lowCD11b low), Large Peritoneal Macrophages (F4/80 high CD11b high), cDCs (CD11b+CD11c+). In the liver, Motile Macrophages (F4/80+ CD11b high), Kupffer Cell Macrophages (F4/80 high CD11b low), Neutrophils (CD11b+ Ly6C- Ly6G+).

Example 3. Generation and Phenotyping of Mouse Comprising Targeted Insertion of Human *CR1* and Human *C3*

[0135] MAID 6149 mice were generated by replacing the mouse C3 gene locus spanning 5′ regulatory elements and all of the coding exons 1 through 41 with a human genomic fragment comprising 5′ regulatory elements and all of the coding exons 1 through 41 of the human C3 gene (see, e.g., U.S. Patent 9795121 B1, incorporated herein by reference). MAID 6149 mice were shown to be prone to high rates of spontaneous death and exhibit physiological, morphological, and histological symptoms which closely resemble complement-related nephropathies and liver fibrosis. MAID7239 HO mice described in Example 1 were crossed with MAID6149 mice to produce doubly homozygous mice in order to assess the effect of CR1 humanization on disease phenotypes in MAID6149 mice.

[0136] In the experiments described in this Example, the following mice were used: C3 HumIn CR1 HumIn (6149HO 7239HO) mice: 75% C57BL/6NTac 25% 129S6/SvEvTac background, Male, n=9, F2 and Female, n=6, F2; C3 HumIn (6149HO 7239WT) - 75% C57BL/6NTac 25% 129S6/SvEvTac background, Male, n=5, F2 and Female, n=2, F2; 75/25 WT (50500): 75% C57BL/6NTac 25% 129S6/SvEvTac background, Male, n=10, F1 and Female, n=4, F1.

Materials and Methods

[0137] Mice were sacrificed and serum was collected in BD tube # 365967.

[0138] Mouse C3 was measured with Complement C3 Mouse ELISA Kit (Cat# ab157711, Abcam) as per manufacturer's instructions. The absorbance at 450nm was determined on the Molecular Devices SpectraMax M5. Data was analyzed in Prism software.

[0139] Human C3 was measured with Complement C3 Human ELISA Kit (Cat# ab108822, Abcam) as per manufacturer's instructions. The absorbance at 450nm was determined on the Molecular Devices SpectraMax M5. Data was analyzed in Prism software.

[0140] Human iC3b was measured with MicroVue Complement iC3b Human EIA Kit (Cat# ab108822, Quidel) as per manufacturer's instructions. The absorbance at 450nm was determined on the Molecular Devices SpectraMax M5. Data was analyzed in Prism software.

[0141] Blood urea nitrogen was measured with a QuantiChrom Urea Assay Kit (Cat # DIUR-100, Bioassay Systems) as per manufactur's instructions.

Cohort #1

[0142] Visual inspection and serum BUN levels (serum biomarker of kidney injury) were used as indicator of disease progression in C3 HumIn (MAID6149HO) mice and C3 HumIn CR1 HumIn (MAID 6149HO 7239HO) mice, as compared to WT controls. Upon finding sick mice with elevated BUN levels, the mice were sacrificed to collect serum and tissues. The analysis shows moderate improvement in BUN levels in C3 HumIn CR1 HumIn (6149HO 7239HO) mice compared to C3 HumIn (6149HO) mice, despite no improvement in hC3 levels; and possible indication of exacerbated liver injury, with an improvement in kidney injury, in C3 HumIn CR1 HumIn (6149HO 7239HO) mice compared to C3 HumIn (6149HO) mice. See FIGS. 3A-3E.

Cohort #2

[0143] Weight gain, BUN levels (serum biomarker of kidney injury), and survival were examined in C3 HumIn (MAID6149HO) mice and C3 HumIn CR1 HumIn (MAID 6149HO 7239HO) mice, as compared to WT controls. As shown in FIGS. 3F-3H, minor improvement in weight gain/BUN levels and significant improvement in survival were observed in C3 HumIn CR1 HumIn (6149HO 7239HO) mice compared to C3 HumIn (6149HO) mice.

Example 4. Generation of Mouse Comprising Transgenic Human *CR1* Driven by a mouse *GATA-1* Promoter

[0144] Mouse *Gata1* gene is located at X chromosome, and its genomic sequence can be found under NCBI Gene ID number 14460. Examples of RefSeq mRNA ID and UniProt ID can be found under GenBank Accession No. NM_008089.2 and UniProt ID No. P17679, respectively.

[0145] The genetically engineered $TG^{Gata1\text{-}CR1}$ mouse strain containing a randomly inserted copy of mouse *Gata1* promoter-human *CR1* cDNA-human beta globin polyA sequence was created using Regeneron's VelociGene® technology (Valenzuela 2003, supra; Poueymirou 2007, supra). Hybrid 129S6/SvEvTac:C57Bl/6NTac F1 embryonic stems cells (ESC) were targeted for random insertion of a mouse bacterial artificial chromosome (BAC) containing *Gata1* genomic sequence (including 33 Kb upstream of ATG), modified so that a full-length human *CR1* coding sequence (ATG to stop) replaced coding exon 1 and the following intron of *Gata1*. A 3' UTR sequence containing the poly(A) signal from a human beta globin gene, as set forth in SEQ ID NO: 4, was placed 3' to the human *CR1* stop codon, followed by a neomycin resistance cassette for selection in ESCs. See FIG. 4A-4B.

[0146] To ensure that human CR1 protein would express in a *Gata1* dependent manner, ESC clones were screened to ensure that at least 14 Kb (and possibly entire 33 Kb) upstream of ATG (including *Gata1* promoter) (determined by TaqMan assay) and 1.5 Kb of 3' UTR (determined by Targeted Locus Amplification ("TLA") analysis) were included in transgene insertions. Clones were also screened by TaqMan to ensure that the transgene did not target endogenous *Gata1* (located on the X chromosome). The sequences of the primer sets and probes are set forth in Table 3 below, with their locations depicted in FIG. 4A.

Table 3: Primer and Probes in Mouse Taqman LOA Assays (7502mTU and 7502mTD), Human Taqman GOA Assays (7502hTU and 7502hTD), Mouse Promoter Assays (7502mPU and 7502mPU2), and Mouse Retention Assays (7502mretU and 7502retD)

Description	Sequence	SEQ ID NO
7502mTU	Fwd: CATCAGCACTGGCCTACTACA	17
	Probe (BHQ): AAGCTGAGGCCTACAGACACTCCC	18
	Rev: AGGCAGCCACCCAACAGTTAC	19
7502mTD	Fwd: TGACCAGAGGGACATAGAACTCC	20
	Probe (BHQ):	21
	TCACCCAAGCAGCAAGAGACTATTGTA	22
	Rev: TCCCAACATGGTGGCTAGTTT	
7502hTU	Fwd: GCCAGGCCTACCAACCTA	23
	Probe (BHQ):	24
	TGATGAGTTTGAGTTTCCCATTGGGACA	25
	Rev: CAGGGCGGCATTCATAGTTCAG	
7502hTD	Fwd: TCTCGTGCACATGATGCTC	26
	Probe (BHQ):	27
	TCATAGTTGGCACTTTATCTGGTACGATC	28
	Rev: ACGCTGCTGCCTCCTTGAG	
7502mPU	Fwd: AGCTGGGTGGGTTAGTGGAGAA	29
	Probe (BHQ):AGTGCTAGCTGTTGGTCCAGCA	30
	Rev: TGCCGCTTGCCTTTGTAAG	31
7502mPU2	Fwd: TCTGCGCCATGTTTGACTTTG	32
	Probe (BHQ):TGGCTTCTACTAGGCACACGACGG	33
	Rev: GGTGCTGCATACTTCCTCTCTA	34
7502mretU	Fwd: GGAAGGGAAGAGCAACAACAC	35
	Probe (BHQ):TCTTGGACACCTTGAAGACGGAGC	36
	Rev: CCAGCGTCAGGAGGTCTG	37
7502mretD	Fwd: GGCCTGTCAGCCATCTTATGC	38
	Probe (BHQ):TTTCCTGGACCTCTGCTGGGATCG	39
	Rev: TGGTGCTGCTGGTGGTAG	40

[0147] Transgenic ESC clones were microinjected into 8-cell Swiss Webster embryos, resulting in F0 VelociMice® fully derived from the injected modified ESC (Poueymirou 2007). These F0 mice were subsequently bred to homozygosity on a 100% C57Bl/6NTac background, which were designated as MAID7502. The resistance cassette was removed by self-deleting technology, thereby generating strain MAID7503. Subsequent analysis using targeted locus amplification (TLA; Cergentis) determined that the transgene was present as a single copy on the X chromosome, but not targeted into the endogenous *Gata1* locus. All animals were maintained in the Regeneron Animal Facility during the entire study period.

Example 5. Phenotyping of Mouse Comprising Transgenic Human *CR1* Driven by a mouse *GATA-1* Promoter

[0148] Flow cytometry was performed on cells from the blood and spleen of MAID 7503 mice and the results are shown in FIGS. 5A-5C. RBCs from MAID7503 HET male and HO female mice showed a similar level of hCR1 expression (higher than found in 7503HET females (FIG. 5A). MAID7503 HET male and HO female mice showed some hCR1 expression (higher than found in 7503HET females) for both blood and splenic cell populations (FIGS. 5B-5C). In summary, hCR1 expression was best observed on surface of RBCs in MAID7503 HET males and 7503HO female mice. Unlike human blood leukocytes, hCR1 was weakly expressed in mouse blood and splenic monocytes/neutrophils (large variability) in MAID7503 HET male/female and HO mice.

[0149] hCR1 expression on RBCs and blood/spleen leukocytes was also examined in B6.Cg-Tg(Gata1-CR1)1Rwf/J mice (Jackson Labs, generated by Robert W. Finberg, University of Massachusetts, as described in Repik et al., *Clinical and Experimental Immunology* 140: 230–240, 2005) and human blood. hCR1 expression was observed on surface of RBCs in both B6.Cg-Tg(Gata1-CR1)1Rwf/J (Jackson Labs) and MAID7503 HO mice (FIG. 5E). Unlike in human blood leukocytes (FIG. 5D), hCR1 was very poorly expressed in mouse blood monocytes/neutrophils in B6.Cg-Tg(Gata1-CR1)1Rwf/J mice (FIG. 5F). hCR1 expression in blood monocytes/neutrophils was previously observed in MAID7503 HO mice (see FIG. 5B). No hCR1 expression was observed in splenic monocytes/neutrophils in either mouse strain.

WHAT IS CLAIMED IS:

1. A genetically modified rodent animal comprising in its genome a nucleic acid which comprises a nucleotide sequence encoding a human CR1 polypeptide, wherein the rodent animal displays a human-like expression of the human CR1 polypeptide.

2. The rodent animal of claim 1, wherein the nucleotide sequence encoding a human CR1 polypeptide is a human genomic DNA sequence.

3. The rodent animal of claim 1, wherein the nucleotide sequence encoding a human CR1 polypeptide is a cDNA sequence.

4. The rodent animal according to any one of claims 1-3, wherein the nucleic acid is inserted between the rodent *Cr2* gene locus and the rodent *Cr1like* (*Cr1l*) gene locus in the rodent genome.

5. The rodent animal according to any one of claims 1-3, wherein the nucleic acid is inserted into an X-chromosome of the rodent genome.

6. The rodent animal according to any one of claims 1-5, wherein the nucleic acid comprises a promoter of a human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide.

7. The rodent animal according to any one of claims 1-6, wherein the nucleic acid comprises the 5' untranslated region (5' UTR) of a human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide.

8. The rodent animal according to any one of claims 1-7, wherein the nucleic acid comprises the 3' UTR of a human *CR1* gene.

9. The rodent animal according to any one of claims 1-5, wherein the nucleic acid comprises a 5' regulatory region of a rodent *Gata-1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide.

10. The rodent animal of claim 9, wherein the 5' regulatory region comprises the promoter region of said rodent *Gata-1* gene.

11. The rodent of claim 10, wherein the 5' regulatory region comprises a genomic sequence of at least 14 Kb immediately upstream of the ATG codon of said rodent *Gata-1* gene.

12. The rodent animal of any one of claims 1-5 or 9-11, wherein the nucleic acid comprises a 3' UTR comprising the polyadenylation sequence from a human beta-1 globin gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide.

13. The rodent animal of claim 1, wherein the nucleic acid comprises a human genomic DNA sequence, which comprises the human *CR1* coding sequence from ATG to STOP, with the 5' and 3' untranslated regions (UTRs) and intervening introns, as well as a 5' upstream sequence of at least 4000 bp directly upstream of the 5' UTR and a sequence of at least 150 bp directly downstream of the 3' UTR; and wherein the nucleic acid is inserted between the rodent *Cr2* gene locus and the rodent *Cr1l* gene locus.

14. The rodent animal of claim 1, wherein the nucleic acid comprises a human *CR1* coding cDNA sequence from ATG to STOP, operably linked to, at the 5', a nucleotide sequence of at least 14 Kb directly upstream of ATG of a rodent *Gata-1* gene, and at the 3', a 3' UTR sequence ofa human beta globin gene, and wherein the nucleic acid is integrated into an X-chromosome of the rodent.

15. The rodent animal according to any one of claims 1-14, wherein the rodent animal is a male.

16. The rodent animal according to any one of claims 1-14, wherein the rodent animal is a female.

17. The rodent animal according to any one of claims 1-14, wherein the rodent animal is heterozygous for the nucleic acid.

18. The rodent animal according to any one of claims 1-14, wherein the rodent animal is homozygous for the nucleic acid.

19. The rodent animal according to any one of claims 1-18, wherein the human CR1 polypeptide is expressed on red blood cells and/or neutrophils in the rodent animal.

20. The rodent animal according to any one of claims 1-19, wherein the rodent animal is a mouse or a rat.

21. A genetically modified rodent animal, comprising in its genome

a first nucleic acid comprising a first nucleotide sequence encoding a human CR1 polypeptide, wherein the first nucleic acid is inserted between the rodent *Cr2* gene locus and the *Cr1l* gene locus in the rodent genome, and

a second nucleic acid comprising a second nucleotide sequence encoding a human CR1 polypeptide in operable linkage to a 5' regulatory region of a rodent *Gata-1* gene, wherein the second nucleic acid is integrated into an X chromosome of the rodent genome.

22. The rodent animal of claim 21, wherein the first nucleic acid comprises a promoter of a human *CR1* gene, operably linked to the first nucleotide sequence.

23. The rodent animal of claim 21 or 22, wherein the first nucleic acid comprises a 5' UTR, and/or a 3' UTR of a human *CR1* gene, operably linked to the first nucleotide sequence.

24. The rodent animal according to any one of claims 21-23, wherein the first nucleotide sequence is a human genomic DNA sequence.

25. The rodent animal according to any one of claims 21-24, wherein the rodent animal is heterzygous for the first nucleic acid.

26. The rodent animal according to any one of claims 21-24, wherein the rodent animal is homozygous for the first nucleic acid.

27. The rodent animal according to any one of claims 21-26, wherein the second nucleic acid comprises a 3' UTR comprising the polyadenylation sequence from a human beta-1 globin gene, operably linked to the second nucleotide sequence.

28. The rodent animal according to any one of claims 21-27, wherein the second nucleotide sequence is a cDNA sequence.

29. The rodent animal according to any one of claims 21-28, wherein the rodent animal is a male.

30. The rodent animal according to any one of claims 21-28, wherein the rodent animal is a female.

31. The rodent animal of claim 30, wherein the rodent animal is heterozygous for the second nucleic acid.

32. The rodent animal of claim 30, wherein the rodent animal is homozygous for the second nucleic acid.

33. The rodent animal according to any one of claims 21-32, wherein the human CR1 polypeptide is expressed on red blood cells, neutrophils, macrophages, monocytes, or cDCs in the rodent.

34. The rodent animal according to any one of claims 21-33, wherein the rodent animal is a mouse or a rat.

35. The rodent animal according to any one of claims 1-34, further comprising in its genome a replacement of a rodent C3 gene sequence at an endogenous rodent C3 locus with a human C3 gene sequence to form a modified C3 gene, wherein the rodent C3 gene sequence comprises an exon of the endogenous rodent C3 gene and the human C3 gene sequence comprises exon 2 through exon 41, or exon 1 through exon 41, of the human C3 gene.

36. The rodent animal of claim 35, wherein expression of the modified C3 gene is under control of a human C3 promoter, or under control of rodent regulatory elements at the endogenous rodent C3 locus.

37. The rodent animal of claim 35 or 36, wherein the rodent animal exhibits improved survival and/or ameliorated kidney injury as compared to a rodent animal expressing human C3 without human CR1.

38. A cell or tissue isolated from a rodent according to any one of claims 1-37, whose genome comprises the nucleic acid comprising a nucleotide sequence encoding a human CR1 polypeptide, wherein optionally the cell is an egg.

39. A rodent embryonic stem (ES) cell, comprising in its genome a nucleic acid which comprises a nucleotide sequence encoding a human CR1 polypeptide.

40. The rodent ES cell of claim 39, wherein the nucleic acid comprises a nucleotide sequence encoding a human CR1 polypeptide, and wherein the nucleic acid is inserted between the rodent *Cr2* gene locus and the rodent *Crl1* gene locus in the rodent genome.

41. The rodent ES cell of claim 39, wherein the nucleic acid comprises a nucleic acid comprising a nucleotide sequence encoding a human CR1 polypeptide in operable

linkage to a 5' transcriptional regulatory region of a rodent *Gata-1* gene, and wherein the nucleic acid is integrated into an X chromosome of the rodent genome.

42. A method of making a genetically modified rodent animal, comprising:

    a) inserting a nucleic acid into the genome of a rodent ES cell, wherein the nucleic acid comprises a nucleotide sequence encoding a human CR1 polypeptide; and

    b) making a genetically modified rodent animal using a rodent ES cell obtained from step a).

43. The method of claim 42, wherein the nucleotide sequence encoding a human CR1 polypeptide is a human genomic DNA sequence.

44. The method claim 42, wherein the nucleotide sequence encoding a human CR1 polypeptide is a cDNA sequence.

45. The method of any one of claims 42-44, wherein the nucleic acid is inserted between the rodent *Cr2* gene locus and the rodent *Cr1like* (*Cr1l*) gene locus in the rodent genome.

46. The method of any one of claims 42-44, wherein the nucleic acid is inserted into an X-chromosome of the rodent genome.

47. The method according to any of claims 42-46, wherein the nucleic acid comprises a promoter of a human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide.

48. The method according to any of claims 42-47, wherein the nucleic acid comprises the 5' untranslated region (5' UTR) of a human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide.

49. The method according to any of claims 42-48, wherein the nucleic acid comprises the 3' UTR of a human *CR1* gene.

50. The method according to any of claims 42-47, wherein the nucleic acid comprises a 5' regulatory region of a rodent *Gata-1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide.

51. The method of claim 50, wherein the 5' regulatory region comprises the promoter region of said rodent *Gata-1* gene.

52. The method of claim 51, wherein the 5' regulatory region comprises a genomic sequence of at least 14 Kb immediately upstream of the ATG codon of said rodent *Gata-1* gene.

53. The method of any of claims 42-46 or 50-52, wherein the nucleic acid comprises a 3' UTR comprising the polyadenylation sequence from a human beta-1 globin gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide.

54. The method of claim 42, wherein the nucleic acid comprises a human genomic DNA sequence, which comprises the human *CR1* coding sequence from ATG to STOP, with the 5' and 3' untranslated regions (UTRs) and intervening introns, as well as a 5' upstream sequence of at least 4000 bp directly upstream of the 5' UTR and a sequence of at least 150 bp directly downstream of the 3' UTR; and wherein the nucleic acid is inserted between the rodent *Cr2* gene locus and the rodent *Cr1l* gene locus.

55. The method of claim 42, wherein the nucleic acid comprises a human *CR1* coding cDNA sequence from ATG to STOP, operably linked to, at the 5', a nucleotide sequence of at least 14 Kb directly upstream of ATG of a rodent *Gata-1* gene, and at the 3', a 3' UTR sequence of a human beta globin gene, and wherein the nucleic acid is integrated into an X-chromosome of the rodent.

56. The method according to any one of claims 42-55, wherein the rodent animal is a mouse or a rat.

57. A method of assessing the pharmacokinetic properties of a compound targeting human CR1, the method comprising administering a candidate compound to a genetically modified rodent animal according to any one of claims 1-37; and performing an assay to determine one or more pharmacokinetic properties of the compound.

58. A method of assessing the pharmacokinetic properties of a compound targeting human C3, the method comprising administering a candidate compound to a genetically modified rodent animal according to any one of claims 35-37; and

performing an assay to determine one or more pharmacokinetic properties of the compound.

59. A targeting vector, comprising a nucleic acid which comprises a nucleotide sequence encoding a human CR1 polypeptide, flanked by rodent nucleotide sequences for targeted insertion of the nucleic acid between the rodent *Cr2* gene locus and the rodent *Cr1l* gene locus in the rodent genome.

60. A nucleic acid vector, comprising a nucleic acid which comprises nucleotide sequence encoding a human CR1 polypeptide in operable linkage to a 5' regulatory region of a rodent *Gata-1* gene.

61. A method of making a genetically modified rodent ES cell, comprising:

inserting a nucleic acid into the genome of a rodent ES cell, wherein the nucleic acid comprises a nucleotide sequence encoding a human CR1 polypeptide.

62. The method of claim 61, wherein the nucleotide sequence encoding a human CR1 polypeptide is a human genomic DNA sequence.

63. The method claim 61, wherein the nucleotide sequence encoding a human CR1 polypeptide is a cDNA sequence.

64. The method of any one of claims 61-63, wherein the nucleic acid is inserted between the rodent *Cr2* gene locus and the rodent *Cr1like* (*Cr1l*) gene locus in the rodent genome.

65. The method of any one of claims 61-63, wherein the nucleic acid is inserted into an X-chromosome of the rodent genome.

66. The method according to any of claims 61-65, wherein the nucleic acid comprises a promoter of a human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide.

67. The method according to any of claims 61-66, wherein the nucleic acid comprises the 5' untranslated region (5' UTR) of a human *CR1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide.

68. The method according to any of claims 61-67, wherein the nucleic acid comprises the 3' UTR of a human *CR1* gene.

69. The method according to any of claims 61-65, wherein the nucleic acid comprises a 5' regulatory region of a rodent *Gata-1* gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide.

70. The method of claim 69, wherein the 5' regulatory region comprises the promoter region of said rodent *Gata-1* gene.

71. The method of claim 70, wherein the 5' regulatory region comprises a genomic sequence of at least 14 Kb immediately upstream of the ATG codon of said rodent *Gata-1* gene.

72. The method of any of claims 61-65 or 69-71, wherein the nucleic acid comprises a 3' UTR comprising the polyadenylation sequence from a human beta-1 globin gene, operably linked to the nucleotide sequence encoding the human CR1 polypeptide.

73. The method of claim 61, wherein the nucleic acid comprises a human genomic DNA sequence, which comprises the human *CR1* coding sequence from ATG to STOP, with the 5' and 3' untranslated regions (UTRs) and intervening introns, as well as a 5' upstream sequence of at least 4000 bp directly upstream of the 5' UTR and a sequence of at least 150 bp directly downstream of the 3' UTR; and wherein the nucleic acid is inserted between the rodent *Cr2* gene locus and the rodent *Cr1l* gene locus.

74. The method of claim 61, wherein the nucleic acid comprises a human *CR1* coding cDNA sequence from ATG to STOP, operably linked to, at the 5', a nucleotide sequence of at least 14 Kb directly upstream of ATG of a rodent *Gata-1* gene, and at the 3', a 3' UTR sequence of a human beta globin gene, and wherein the nucleic acid is integrated into an X-chromosome of the rodent.

75. The method according to any one of claims 61-74, wherein the rodent ES cell is a mouse ES cell or a rat ES cell.