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Description

FIELD OF INVENTION

[0001] The invention relates to the field of genetically modified murine animals.

BACKGROUND

[0002] Mice transgenic for a human IL-6 gene are known in the art. However, random insertion of a human IL-6 transgene into the mouse genome results in poorly regulated expression of the human IL-6 protein, which manifests itself in a variety of pathologies in such transgenic mice, including, but not limited to, plasmacytosis and glomerulonephritis. As a result, these mice have limited usefulness.

[0003] There is a need for non-human animals, e.g., mice and rats, the express human or humanized IL-6 and/or human or humanized IL-6 receptor. There is a need for such humanized mice that do not exhibit one or more pathologies exhibited by transgenic hIL-6 mice.

SUMMARY

[0004] In one aspect, the invention provides a genetically modified murine animal comprising a replacement at an endogenous murine IL-6 locus of a murine gene encoding IL-6 with a human gene encoding human IL-6, wherein the human gene encoding human IL-6 is under control of endogenous murine regulatory elements at the endogenous murine IL-6 locus. In one embodiment, genetically modified murine animals are provided that express a human IL-6 gene under the control of endogenous murine promoter and/or endogenous murine regulatory elements, from an endogenous murine IL-6 locus.

[0005] In one embodiment, the human gene encoding human IL-6 comprises exons 1 through 5 of the human IL-6 gene found in the CTD-2369M23 bacterial artificial chromosome.

[0006] In another embodiment, the murine animal expresses a humanized IL-6Rα wherein the endogenous murine IL-6Rα gene has been replaced with a human sequence comprising a sequence that encodes an ectodomain of a human IL-6Rα.

[0007] In a further embodiment, the murine animal does not exhibit a feature selected from plasmocytosis, glomerulosclerosis, glomerulonephritis, kidney failure, hypergammaglobulinemia, elevated megakaryocytes in spleen, elevated megakaryocytes in bone marrow, splenomegaly, lymph node enlargement, compacted abnormal plasma cells, and a combination thereof. In such embodiments, the humanized IL-6Rα may comprise murine transmembrane and intracellular domains.

[0008] In a second aspect, the invention provides a genetically modified murine animal, comprising a humanization of an endogenous murine IL-6Rα gene, wherein the humanization comprises a replacement of a murine IL-6Rα ectodomain-encoding sequence with a human IL-6Rα ectodomain-encoding sequence at the endogenous murine IL-6Rα locus, and wherein the humanized IL-6Rα gene is under control of endogenous murine regulatory elements.

[0009] In one embodiment of the second aspect of the invention, the genetically modified murine animal further comprises a humanized IL-6 gene comprising a replacement at an endogenous murine IL-6 locus of a murine gene encoding IL-6 with a human gene encoding human IL-6.

[0010] In a third aspect, the invention provides a method for making a humanized murine animal, comprising replacing a murine gene sequence encoding murine IL-6 with a human gene encoding human IL-6 so that the human IL-6 gene is under control of endogenous murine regulatory elements.

[0011] In a fourth aspect, the invention provides a method for making a humanized murine animal, comprising replacing all murine exons encoding ectodomain sequences of murine IL-6Rα with a human genomic fragment encoding human IL-6Rα ectodomain to form a humanized IL-6Rα gene, wherein the humanized IL-6Rα gene is under control of endogenous murine regulatory elements.

[0012] In a fifth aspect, the invention provides a genetically modified murine animal comprising a humanized IL-6Rα gene comprising a replacement of a murine ectodomain-encoding sequence with a human ectodomain sequence, wherein the humanized IL-6Rα gene comprises murine transmembrane and intracellular domain sequences, wherein the murine animal further comprises a gene encoding a human IL-6, and wherein the genes encoding human IL-6 and humanized IL-6Rα are under control of endogenous murine regulatory elements.

DESCRIPTION

[0013] Described herein are genetically modified murine animals are provided that express a human IL-6 receptor gene (or a gene encoding a human ectodomain and mouse transmembrane and intracellular domains) under the control

of endogenous murine promoter and/or endogenous murine regulatory elements, from an endogenous murine IL-6 receptor locus.

[0014] Also described herein is a genetically modified animal (e.g., a murine animal, e.g., a mouse or rat) that expresses a human IL-6 protein, wherein the non-human animal does not exhibit a pathology selected from plasmacytosis, glomerulonephritis, glomerulosclerosis, mesangio-proliferative glomerulonephritis, intestinal lymphoma, kidney lymphoma, splenomegaly, lymph node enlargement, liver enlargement, megakaryocytes in bone marrow, compacted abnormal plasma cells, infiltration of plasma cells into lung or liver or kidney, mesangial cell proliferation in kidney, cerebral overexpression of IL-6, ramified microglial cells in white matter, reactive astrocytosis in brain, kidney failure, elevated megakaryocytes in spleen, muscle wasting (e.g., gastrocnemius muscle wasting), elevated muscle cathepsins B and B+L (e.g., around 20-fold and 6-fold), and a combination thereof.

[0015] Also described herein are non-human animals comprising a normal B cell population. The normal B cell population may be approximately the same in number and immunophenotype as a wild-type animal, e.g., a wild-type mouse.

[0016] Also described herein is a non-human murine animal (e.g., a mouse or rat) which expresses human IL-6 (hIL-6) in serum at a level below about 800 pg/mL, below about 700, 600, 500, 400, 300, or 200 pg/mL. The murine animal may express hIL-6 in serum at a level of about 50 to about no more than 200 pg/mL, about 75-125 pg/mL, or at about 100 pg/mL.

[0017] Also described herein is a non-human animal that expresses hIL-6 and/or hIL-6R, wherein the non-human animal expresses hIL-6 and/or hIL-6R from an endogenous non-human IL-6 locus and/or an endogenous non-human hIL-6R locus. Also described herein is a genetically modified mouse that expresses hIL-6 from an endogenous mouse IL-6 locus, wherein the endogenous mouse IL-6 gene has been replaced with a hIL-6 gene.

[0018] Also described herein is a mouse comprising a cell that expresses an IL-6 receptor (IL-6R) that comprises a human ectodomain on the surface of the cell. The cell may be a lymphocyte, for example a B cell.

[0019] Also described herein are genetically modified murine animals in which about 6.8 kb at the endogenous mouse IL-6 locus, including exons 1 through 5 and a 3' untranslated sequence, is deleted and replaced with about 4.8 kb of human IL-6 gene sequence comprising exons 1 through 5 of the human IL-6 gene. The human IL-6 gene may comprise exons 1 through 5 of the human IL-6 gene of human BAC CTD-2369M23.

[0020] Also described herein is a genetically modified mouse that expresses IL-6 from a human IL-6 gene, wherein the mouse expresses human IL-6 in its serum. The mouse serum may exhibit a serum concentration of human IL-6 of about 25 to about 300 pg/mL, 50 to about 250 pg/mL, 75 to about 200 pg/mL, or 100 to about 150 pg/mL. The level of human IL-6 in the serum of the mouse may be about 100 pg/mL. The level of a pan B cell-specific marker in bone marrow of the mouse may be about the same as that of a wild-type mouse. The level of a pan B cell-specific marker in spleen may be about the same as that of a wild-type mouse. The pan B cell-specific marker may be selected from B220, CD19, CD20, CD22, CD79a, CD79b, L26, and Pax-5 (BSAP).

[0021] Also described herein is a genetically modified mouse that expresses hIL6, wherein the mouse does not exhibit a feature selected from plasmacytosis, splenomegaly, lymph node enlargement, compacted abnormal plasma cells, and a combination thereof. The mouse may comprise a spleen that is about the same weight (per body weight) as a wild-type mouse. The lymph nodes of the mouse may be about the same weight (per body weight) as a wild-type mouse. The plasma cells of the mouse may not exhibit plasmocytosis characteristic of mice that overexpress human IL-6. The mouse may not exhibit glomerulonephritis. The mouse may exhibit a mesangial cell level comparable to a wild-type mouse.

[0022] Also described herein is a genetically modified mouse that expresses hIL6 from an endogenous mouse IL-6 locus, wherein the endogenous mouse IL-6 gene has been replaced with a hIL-6 gene, wherein the mouse does not exhibit a feature selected from a morphologically detectable neuropathology, a reactive astrocytosis, and a combination thereof. The mouse may comprises a brain that is morphologically indistinct from a wild-type mouse brain. The mouse may comprise brain tissue that exhibits a level of reactive astrocytosis that is no higher than that of a wild-type mouse. The mouse may not express human IL-6 in neurons. The mouse may comprise activated astrocyte levels that are comparable to activated astrocyte levels in a wild-type mouse.

[0023] The mouse may comprise ramified microglial cells in its white matter, wherein the ramified microglial cells are present in an amount equivalent to an amount of ramified microglial cells in a wild-type mouse.

[0024] The mouse may not exhibit a reactive atrocytosis. The white matter of the mouse may be morphologically indistinct from the white matter of a wild-type mouse. For example, the white matter of the mouse may be histologically indistinct from a wild-type mouse white matter with respect to histochemical staining of reactive astrocytes.

[0025] The mouse may comprise a brain that is morphologically indistinct from a wild-type mouse brain. The mouse may comprise brain tissue that exhibits a level of reactive astrocytosis that is no higher than that of a wild-type mouse.

[0026] Also described herein is a genetically modified mouse that expresses hIL6 from an endogenous mouse IL-6 locus, wherein the endogenous mouse IL-6 gene has been replaced with a hIL-6 gene, wherein the mouse does not exhibit a feature selected from a life span shortened by about 50% or more, kidney failure, hypergammaglobulinemia, elevated megakaryocytes in spleen, elevated megakaryocytes in bone marrow, plasmacytosis of spleen, plasmacytosis of thymus, plasmacytosis of lymph nodes, glomerulonephritis, glomerulosclerosis, and a combination thereof.

[0027] The mice may have a life span that exceeds 20 weeks, for example having a life span that exceeds 30 weeks, 40 weeks, or 50 weeks. The mice may exhibit a life span about equal to that of a wild-type mouse of the same strain.

[0028] The mice may exhibit a level of megakaryocytes in spleen that is no more than about the splenic megakaryocyte level of a wild-type mouse. The mice may comprise lymphoid organs that are essentially devoid of abnormal and compactly arranged plasmacytoid cells.

[0029] The mice may exhibit gamma globulin serum levels equivalent to gamma globulin serum levels in wild-type mice. The levels of α1- and β-globulin in serum of the mice may be equivalent to α1- and β-globulin serum levels of wild-type mice of the same strain.

[0030] Also described herein is a genetically modified mouse that expresses human IL-6 from an endogenous mouse IL-6 locus, wherein the endogenous mouse IL-6 gene has been replaced with a hIL-6 gene, wherein the mouse does not exhibit a feature selected from muscle wasting, an elevated cathepsin B level as compared with a wild-type mouse of the same strain, an elevated cathepsin A+B level as compared with a wild-type mouse of the same strain, an increased liver weight as compared with a wild-type mouse of the same strain, and a combination thereof.

[0031] The weight of the liver of the mouse may be about 800-900 mg at 12 weeks.

[0032] The mouse may exhibit a cathepsin B level throughout its life span that is no more than about the level observed in a wild-type mouse. The mouse may exhibit a cathepsin A+B level throughout its life span that is no more than about the level observed in a wild-type mouse.

[0033] The mouse may, as an adult, exhibit a gastrocnemus muscle weight that is within about 10% of the weight of a wild-type mouse of the same strain. The mouse, as an adult, may exhibit a gastrocnemus muscle weight that is about the same as that of a wild-type mouse.

[0034] Also described herein is a mouse that comprises a nucleotide sequence encoding a human IL-6 protein, wherein the nucleotide sequence encoding the human IL-6 protein replaces in whole or in part an endogenous nucleotide sequence encoding and endogenous mouse IL-6 protein.

[0035] Also described herein is a mouse that comprises a replacement at an endogenous mouse IL-6 receptor locus of mouse IL-6Rα ectodomain with an ectodomain sequence of a human IL-6Rα to form a chimeric human/mouse IL-6Rα gene.

[0036] The chimeric IL-6Rα gene may be under the control of a mouse promoter and/or mouse regulatory elements at the endogenous mouse IL-6Rα locus.

[0037] About 35.4 kb of mouse IL-6Rα ectodomain-encoding sequence may be replaced with about 45.5 kb of human IL-6R ectodomain-encoding sequence. The human IL-6R ectodomain-encoding sequence may encompass the first (ATG) codon in exon 1 through exon 8.

[0038] The mouse IL-6Rα sequence that is replaced may include a contiguous sequence that encompasses exons 1 through 8, for example, exons 1 through 8 and a portion of intron 8 may be deleted.

[0039] Also described herein is a genetically modified mouse, comprising a replacement at an endogenous mouse IL-6 locus of a mouse gene encoding IL-6 with a human gene encoding human IL-6, wherein the human gene encoding human IL-6 is under control of endogenous mouse regulatory elements at the endogenous mouse IL-6 locus.

[0040] The human gene encoding human IL-6 may be a human IL-6 gene of BAC ID CTD-2369M23.

[0041] The mouse may express a mouse IL-6Rα. The mouse may express a human IL-6Rα. The humanized IL-6Rα may comprise a human ectodomain. The humanized IL-6Rα may comprise a mouse transmembrane domain and a mouse cytoplasmic domain. The mouse may express a humanized IL-6Rα that comprises a humanization of ectodomain but not transmembrane and/or cytosolic domain.

[0042] The mouse may not exhibit a feature selected from plasmocytosis, glomerulosclerosis, glomerulonephritis, kidney failure, hypergammaglobulinemia, elevated megakaryocytes in spleen, elevated megakaryocytes in bone marrow, splenomegaly, lymph node enlargement, compacted abnormal plasma cells, and a combination thereof.

[0043] Also described herein is a genetically modified mouse, comprising a humanization of an endogenous mouse IL-6Rα gene, wherein the humanization comprises a replacement of mouse IL-6Rα ectodomain-encoding sequence with human IL-6Rα ectodomain-encoding sequence at the endogenous mouse IL-6Rα locus.

[0044] A contiguous mouse sequence comprising mouse exons 1 through 8 may be replaced with a contiguous genomic fragment of human IL-6Rα sequence encoding a human IL-6Rα ectodomain. The contiguous genomic fragment of human IL-6Rα sequence encoding the ectodomain may be from BAC CTD-2192J23.

[0045] The mouse may further comprise a humanized IL-6 gene. The mouse may comprise a replacement at an endogenous mouse IL-6 locus of a mouse IL-6 gene with a human IL-6 gene. The humanized IL-6 gene may be under control of endogenous mouse regulatory elements.

[0046] Also described herein is a method for making a humanized mouse, comprising replacing a mouse gene sequence encoding mouse IL-6 with a human gene encoding human IL-6.

[0047] The replacement may be at an endogenous mouse IL-6 locus, and the human gene encoding human IL-6 may be operably linked to endogenous mouse regulatory sequences.

[0048] Also described herein is a method for making a humanized mouse, comprising replacing mouse exons encoding

ectodomain sequences of mouse IL-6Rα with a human genomic fragment encoding ectodomain sequences of human IL-6Rα to form a humanized IL-6Rα gene.

[0049] The replacement may be at an endogenous mouse IL-6Rα locus, and the humanized IL-6Rα gene may be operably linked to endogenous mouse regulatory sequences.

[0050] Also described herein is a genetically modified mouse, comprising a humanized IL-6Rα gene comprising a replacement of mouse ectodomain-encoding sequence with human ectodomain sequence, wherein the humanized IL-6Rα gene comprises a mouse transmembrane sequence and a mouse cytoplasmic sequence; wherein the mouse further comprises a gene encoding a human IL-6, wherein the gene encoding a human IL-6 is under control of endogenous mouse IL-6 regulatory elements.

[0051] The mouse may be incapable of expressing a fully mouse IL-6Rα and incapable of expressing a mouse IL-6.

[0052] The genetically modified mice described herein may comprise the genetic modifications in their germline.

[0053] Also described herein is a tissue, cell, or membrane fragment from a mouse as described herein.

[0054] The tissue or cell may be from a mouse that expresses a human IL-6 protein, but that does not express a mouse IL-6 protein. The tissue or cell may be from a mouse that expresses a humanized IL-6Rα protein, but not a mouse IL-6Rα protein. The humanized IL-6Rα protein may comprise a human ectodomain and a mouse transmembrane domain and a mouse cytosolic domain. The tissue or cell may be from a mouse that expresses a human IL-6, a humanized IL-6Rα, and that does not express a mouse IL-6 and does not express an IL-6Rα that comprises a mouse ectodomain.

[0055] Also described herein is an *ex vivo* complex of a mouse cell bearing a humanized IL-6Rα (human ectodomain and mouse transmembrane and mouse cytoplasmic domain) and a human IL-6.

[0056] Also described herein is a mouse embryo comprising a genetic modification as described herein.

[0057] Also described herein is a mouse host embryo that comprises a donor cell that comprises a genetic modification as described herein.

[0058] Also described herein is a pluripotent or totipotent non-human animal cell comprising a genetic modification as described herein. The cell may be a murine cell. The cell may be an ES cell.

[0059] Also described herein is a mouse egg, wherein the mouse egg comprises an ectopic mouse chromosome, wherein the ectopic mouse chromosome comprises a genetic modification as described herein.

[0060] The mouse, embryo, egg, or cell that is genetically modified to comprise a human IL-6 gene or human or humanized IL-6Rα gene is of a mouse that is of 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 another embodiment, the mouse is a 129 strain selected from the group consisting of a strain that is 129P1, 129P2, 129P3, 129X1, 129S1 (e.g., 129S1/SV, 129S1/Svlm), 129S2, 129S4, 129S5, 129S9/SvEvH, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, 129T2 (see, e.g., Festing et al. (1999) Revised nomenclature for strain 129 mice, Mammalian Genome 10:836, see also, Auerbach et al (2000) Establishment and Chimera Analysis of 129/SvEv- and C57BL/6-Derived Mouse Embryonic Stem Cell Lines). The genetically modified mouse may be a mix of an aforementioned 129 strain and an aforementioned C57BL/6 strain. The mouse may be a mix of aforementioned 129 strains, or a mix of aforementioned BL/6 strains. The 129 strain of the mix may be a 129S6 (129/SvEvTac) strain. The mouse may be a BALB strain, e.g., BALB/c strain. The mouse may be a mix of a BALB strain and another aforementioned strain. The mouse may be a Swiss or Swiss Webster mouse.

[0061] Each of the aspects and embodiments described herein are capable of being used together, unless excluded either explicitly or clearly from the context of the embodiment or aspect.

BRIEF DESCRIPTION OF THE DRAWINGS

[0062]

FIG. 1 provides an illustration, not to scale, of the human (top) and mouse (bottom) IL-6 genomic loci. Exons I, II, III, IV, and V (in both human and mouse) are indicated by closed boxes to the right in the figure. Selected putative regulatory regions are indicated by open boxes to the left in the figure.

FIG. 2 shows acute phase response (mSAA level) in the presence or absence of turpentine in wild-type mice, humanized ectodomain IL-6R mice, and mice with humanized IL-6 and IL-6R genes.

FIG. 3 shows turpentine-dependent acute phase response (SAA) in wild-type mice the absence or presence of anti-mouse IL-6R antibody (left); and turpentine-dependent acute phase response in humanized IL-6/IL-6R mice in the absence or present of anti-human IL-6R antibody (right).

FIG. 4 shows FACS analysis for splenic B cells of wild-type and humanized IL-6 mice; pan B cell marker.

FIG. 5 shows FACS analysis for splenic T cells of wild-type an humanized IL-6 mice; T helper cells and cytotoxic T cells.

FIG. 6 shows FACS analysis for splenic cells of wild-type and humanized IL-6 mice; Ly6G/C(Gr1).

FIG. 7 shows FACS analysis for splenic cells of wild-type and humanized IL-6 mice; NK cells and granulocytes

($Ly6G^{hi+}/CD11b^{hi+}$).

FIG. 8 shows FACS analysis for blood B cells of wild-type and humanized IL-6 mice; pan B cell marker.

FIG. 9 shows FACS analysis for blood T cells of wild-type and humanized IL-6 mice; T helper cells and cytotoxic T cells.

FIG. 10 shows FACS analysis for blood myeloid cells of wild-type and humanized IL-6 mice; $Gr1^+$ cells.

FIG. 11 shows FACS analysis for blood myeloid cells of wild-type and humanized IL-6 mice; CD11b vs. Ly6G/C(Gr1).

FIG. 12 shows FACS analysis for blood myeloid cells of wild-type and humanized IL-6 mice; DX5 vs CD11b cells.

FIG. 13 shows FACS analysis of bone marrow IgM/CD24/B220 for wild-type and humanized IL-6 mice. Top: normal progression in bone marrow. Bottom: FACS analysis for wild-type, hIL-6 heterozygotes, and hIL-6 homozygotes (IgM staining).

FIG. 14 shows FACS analysis of bone marrow IgM/CD24/B220 for wild-type and humanized IL-6 mice. Top: normal progression in bone marrow. Bottom: FACS analysis for wild-type, hIL-6 heterozygotes, and hIL-6 homozygotes (CD24 staining).

FIG. 15 shows FACS analysis of bone marrow CD43 and B220 for wild-type and humanized IL-6 mice. Top: normal progression in bone marrow. Bottom: FACS analysis for wild-type, hIL-6 heterozygotes, and hIL-6 homozygotes (CD43 staining).

DETAILED DESCRIPTION

IL-6 and IL-6R

[0063] The IL-6 receptor (IL-6R) was long characterized as a receptor for a B cell stimulatory factor (BSF-2, or B cell Stimulatory Factor 2; also, BCDF, or B Cell Differentiation Factor) responsible for inducing B cells to synthesize immunoglobulin (Yamasaki et al. (1988) Cloning and Expression of the Human Interleukin-6(BSF-2/IFNβ 2) Receptor, Science 241:825-828). IL-6 was first described as interferon-β2 as the result of its discovery during a search for a virally-induced protein termed interferon-β, by treating human fibroblasts with dsRNA poly(I)poly(C) to induce an anti-viral response (Weissenbach et al. (1980) Two interferon mRNAs in human fibroblasts: In vitro translation and Escherichia coli cloning studies, Proc. Natl Acad. Sci. USA 77(12):7152-7156; Keller et al. (1996) Molecular and Cellular Biology of Interleukin-6 and Its Receptor, Frontiers in Bioscience 1:d340-357).

[0064] The human cDNA encodes a 468 amino acid protein having a 19-mer signal sequence and a cytoplasmic domain of about 82 amino acids that lacks a tyrosine kinase domain (see, *Id.*). The N-terminal (ectodomain) of the protein has an Ig superfamily domain of about 90 amino acids, a 250-amino acid domain between the Ig superfamily domain and the membrane, a transmembrane span of about 28 amino acids (see, *Id.*). The ectodomain of the receptor binds its ligand IL-6, which triggers association with gp130 in the membrane and it is this complex that conducts signal transduction; the cytoplasmic domain reportedly does not transduce signal (Taga et al. (1989) Interleukin-6 Triggers the Association of Its Receptor with a Possible Signal Transducer, gp130, Cell 58:573-581). Indeed, a soluble form of IL-6R lacking a cytoplasmic domain can associate with IL-6 and bind gp130 on the surface of a cell and effectively transduce signal (*Id.*).

[0065] The homology of hIL-6R and mIL-6R at the protein level is only about 54%; the transmembrane domain has a homology of about 79%, whereas the cytoplasmic domain has a homology of about 54% (Sugito *et al.* (1990)).

[0066] The natural ligand for the IL-6R, IL-6, was first isolated from cultures of HTLV-1-transformed T cells (see, Hirano et al. (1985) Purification to homogeneity and characterization of human B cell differentiation factor (BCDF or BSFp-2), Proc. Natl. Acad. Sci. USA 82:5490-5494). A human cDNA for the IL-6 gene was cloned at least twice, once as BSF-2 (see, Hirano et al. (1086) Complementary DNA fro a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin, Nature 324:73-76) and once as IFNβ 2 (see, Zilberstein et al. (1986) Structure and expression of cDNA and genes for human interferon-beta-2, a distinct species inducible by growth-stimulatory cytokines, EMBO 5:2529-2537), although it has since been demonstrated that recombinant human IL-6 exhibits no detectable IFN activity.

[0067] Human IL-6 is a 184-amino acid protein that exhibits only about 42% homology with mouse IL-6, although the genomic organization of the human and mouse genes are basically the same, and the promoter regions of the human and mouse genes share a 400-bp stretch that is highly conserved (see, Tanabe et al. (1988) Genomic Structure of the Murine IL-6 Gene: High Degree Conservation of Potential Regulatory Sequences between Mouse and Human, J. Immunol. 141(11):3875-3881).

[0068] The human IL-6 gene is about 5 kb (Yasukawa et al. (1987) Structure and expression of human B cell stimulatory factor-2 (BSC-2/IL-6) gene, EMBO J. 6(10):2939-2945), whereas the mouse IL-6 gene is about 7 kb (Tanabe et al. (1988) Genomic Structure of the Murine IL-6 Gene: High Degree Conservation of Potential Regulatory Sequences between Mouse and Human, J. Immunol. 141(11):3875-3881). The mouse and human IL-6 genes reportedly share highly conserved 5'-flanking sequence important to regulation. A schematic diagram of the human and mouse IL-6 genomic loci is shown in FIG. 1 (not to scale). Exons I, II, III, IV, and V (in both human and mouse) are indicated by closed boxes to the right in the figure. Selected putative regulatory regions are indicated by open boxes to the left in the

figure. The putative regulatory regions for humans are, from left to right, a glucocorticoid element from -557 to -552; an IFN enhancer core sequence from -472 to -468; a glucocorticoid element from -466 to -461; an AT-rich region from -395 to - 334, a consensus AP-1 binding site from -383 to -277; an IFN enhancer core sequence from -253 to -248; a GGAAA-containing motif from -205 to -192; a c-fos SRE homology sequence from -169 to -82 containing an IFN enhancer core sequence, a cAMP-response element, a GGAAA motif, a CCAAT box, and a GC-rich region; and AP-1 binding site from -61 to -55; and a CCAAT box from -34 to -30. The putative regulatory regions for mouse are, from left to right, a GC rich region from - 553 to -536, a glucocorticoid element from -521 to -516 and from -500 to -495; a Z-DNA stretch from -447 to -396; an AP-1 binding site overlapping an IFN enhancer core sequence from -277 to -288, a GGAAA motif overlapping an IFN enhancer core sequence from -210 to -195; a c-fos SRE homology region from -171 to -82 containing a cAMP response element, a GGAAA motif overlapping an IFN enhancer core sequence, and a GC-rich region; and, an AP-1 binding site from -61 to -55. Mouse codons I-V have lengths 19, 185, 114, 150, and 165, respectively. Mouse intron lengths are: I-II, 162 bp; II-III, 1253 bp; III-IV, 2981 bp; IV-V, 1281 bp. Human codons I-V have lengths 19, 191, 114, 147, and 165. Human intron lengths are I-II , 154; II-III, 1047; III-IV, 706; IV-V, 1737. Genomic organization data are from Tanabe *et al.* (1988), and Yasukawa et al. (1987) Structure and expression of human B cell stimulatory factor-2 (BSF-2/IL-6) gene, EMBO J. 9(10):2939-2945.

[0069] It might be reasonable to presume that the mouse and human IL-6 genes appear to be similarly regulated based on the similarity of their 5'-flanking sequence. A variety cell types exhibit enhanced IL-6 expression in response to IL-1, TNF, PDGF, IFNβ, serum, poly(I)poly(C), and cycloheximide (see, Tanabe *et al.* (1988). IL-6 in humans mediates the acute phase response, hematopoiesis, B cell differentiation, T cell activation, growth and/or differentiation and/or activation of a variety of cell types (e.g., hepatocytes, fibroblasts, endothelial cells, neurons, pituitary cells, lymphomas, myelomas, breast carcinomas, NK cells, macrophages, osteoclasts, *etc.*) (reviewed in, *e.g.*, Heinrich *et al.* (1990), Kishimoto *et al.* (1989), and Keller *et al.* (1996); Sugita et al. (1990) Functional Murine Interleukin Receptor with Intracisternal A Particle Gene Product at its Cytoplasmic Domain, J. Exp. Med. 171:2001-2009).

[0070] In practice, however, mice transgenic for human IL-6 exhibit a panoply of substantial and debilitating pathologies, reflecting a significant pleiotropy of the IL-6 gene. Transgenic mice comprising a 6.6-kb fragment containing the human IL-6 gene and a μ enhancer (Eμ) produce high concentrations of hIL-6 and extremely high IgG1 levels (120- to 400-fold over wild-type mice), reflecting an IL-6 deregulation that is accompanied by plasmacytosis, mesangio-proliferative glomerulonephritis, and high bone marrow megakaryocyte levels (Suematsu et al. (1989) IgG1 plasmacytosis in interleukin 6 transgenic mice, Proc. Natl Acad. Sci. USA 86:7547-7551). Aberrant regulation of IL-6 and/or IL-6R is associated with myelomas, plastocytomas, rheumatoid arthritis, Castleman's disease, mesangial proliferative glomerulonephritis, cardiac myxoma, plams cell neoplasias, psoriasis, and other disorders (see, Kishimoto, T. (1989) The Biology of Interleukin-6, Blood 74(1):1-10; Sugita *et al.* (1990); also, Hirano et al. (1990) Biological and clinical aspects of interleukin 6, Immunology Today 11(12):443-449)). IL-6 is also implicated in sustaining levels of intra-prostatic androgens during androgen deprivation treatment of prostate cancer patients by a paracrine and/or autocrine mechanism, potentially providing castration-resistant prostate tumor growth (Chun et al. (2009) Interleukin-6 Regulates Androgen Synthesis in Prostate Cancer Cells, Clin. Cancer Res. 15:4815-4822).

[0071] The human protein is encoded as a 212 amino acid protein, in mature form a 184 amino acid protein following cleavage of a 28 amino acid signal sequence. It contains two N-glycosylation and two O-glycosylation sites, and human IL-6 is phosphorylated in some cells. The mouse protein is encoded as a 211 amino acid protein, in mature form a 187 amino acid protein following cleavage of a 23 amino acid signal sequence. O-glycosylation sites are present, but not N-glycosylation sites. (See reviews on IL-6, e.g., Heinrich et al. (1990) Interleukin-6 and the acute phase response, Biochem. J. 265:621-636.)

[0072] IL-6 function is pleiotropic. The IL-6 receptor is found on activated B cells but reportedly not on resting B cells. In contrast, IL-6R is found on resting T cells and can reportedly promote T cell differentiation, activation, and proliferation, including the differentiation of T cells into cytotoxic T lymphocytes in the presence of IL-2.

Humanized IL-6/IL-6R Ectodomain Mice and IL-6-Mediated Acute Phase Response

[0073] In humans, IL-6 induces the acute phase response. Early studies with human hepatocytes established that IL-6 induces acute phase proteins such as, e.g., C-reactive protein (CRP) and serum amyloid A (SAA) in a dose-dependent and time-dependent manner (reviewed in Heinrich et al. (1990) Interleukin-6 and the acute phase response, Biochem. J. 265:621-636). Non-human animals, e.g., mice or rats, comprising humanized IL-6 and IL-6R genes are therefore useful systems for measuring the acute phase response mediated by human IL-6. Such animals are also useful for determining whether a substance induces an IL-6-mediated acute phase response, by exposing a humanized IL-6/IL-6R animal as described herein to the substance, and measuring a level of one or more acute phase response proteins (or RNAs). In one embodiment, the humanized animal is exposed to the substance in the presence of an antagonist of a human IL-6R, and a level of one or more acute phase response proteins (or RNAs) is measured, wherein a reduction in a level of an acute phase response protein (or RNA) in the presence of the human IL-6R antagonist indicates a human

IL-6R-mediated acute phase response.

[0074] Human IL-6 can bind both human IL-6R and mouse IL-6R; mouse IL-6 binds mouse IL-6R but not human IL-6R (no binding of mIL-6 to hIL-6R detectable, whereas hIL-6 can compete with mIL-6 for binding mIL-6R; Coulie et al. (1989) High-and low-affinity receptors for murine interleukin 6. Distinct distribution on B and T cells, Eur. J. Immunol. 19:2107-211); see also, *e.g.*, Peters et al. (1996) The Function of the Soluble Interleukin 6 (IL-6) Receptor In Vivo: Sensitization of Human Soluble IL-6 Receptor Transgenic Mice Towards IL-6 and Prolongation of the Plasma Half-life of IL-6, J. Exp. Med. 183:1399-1406). Thus, human cells that bear hIL-6R in a mouse (*e.g.*, in a xenogenic transplant) cannot rely on endogenous mIL-6 to carry out IL-6-mediated functions, including but not limited to the role of IL-6 blood cell or lymphocyte development (*e.g.*, hematopoiesis, B cell activation, T cell activation, *etc.*).

[0075] In a mixed *in vivo* system comprising a wild-type mouse IL-6 gene and a human IL-6R gene (but no mouse IL-6R gene), an acute phase response inducer is not expected to induce detectable levels of acute phase proteins that would indicate an acute phase response. However, a humanized mouse as described herein, comprising a humanized IL-6 gene and an IL-6R gene comprising a humanized ectodomain sequence will respond to an acute phase response inducer and exhibit acute phase response proteins in serum. Mice wild-type for IL-6/IL-6R tested for acute phase proteins in the presence or absence of the acute phase inducer turpentine showed a turpentine-dependent increase in acute phase proteins. Mice with humanized IL-6 gene, but not IL-6R, showed no acute phase response in the presence of turpentine. But mice bearing both a human IL-6 gene and an IL-6R gene with a humanized ectodomain exhibited a strong acute phase response (FIG. 2). The IL-6-mediated acute phase response was IL-6 dependent in both wild-type mice (FIG. 3, top) and in humanized IL-6/IL-6R ectodomain mice (FIG. 3, bottom), as evidenced by the ability of the appropriate anti-IL-6R antibody to abrogate the acute phase response at a sufficiently high antibody dose. Thus, a double humanization of IL-6 and IL-6R recapitulates the wild-type IL-6-mediated acute phase response with respect to serum acute phase proteins.

Genetically Modified Mice

[0076] Genetically modified mice are provided that express a human IL-6 and/or a humanized IL-6 receptor from endogenous mouse loci, wherein the endogenous mouse IL-6 gene and/or the endogenous mouse IL-6 receptor gene have been replaced with a human IL-6 gene and/or a human sequence comprising a sequence that encodes an ectodomain of a human IL-6 receptor. The genetically modified mice express the human IL-6 and/or humanized IL-6 receptor from humanized endogenous loci that are under control of mouse promoters and/or mouse regulatory elements. The replacement(s) at the endogenous mouse loci provide non-human animals that express human IL-6 and a humanized IL-6 receptor in a manner that does not result in the panoply of substantial pathologies observed in IL-6 transgenic mice known in the art.

[0077] Transgenic mice that express human IL-6 are known in the art. However, they generally suffer from significant pathologies that severely limit their usefulness. Humanized mice as described herein express a human IL-6 and/or humanized IL-6 receptor under the control of endogenous mouse regulatory elements at endogenous mouse IL-6 and IL-6Rα loci. These mice, in contrast, exhibit expression patterns with respect to these genes that are different from transgenic mice known in the art.

[0078] Replacement of non-human genes in a non-human animal with homologous or orthologous human genes or human sequences, at the endogenous non-human locus and under control of endogenous promoters and/or regulatory elements, can result in a non-human animal with qualities and characteristics that may be substantially different from a typical knockout-plus-transgene animal. In the typical knockout-plus-transgene animal, an endogenous locus is removed or damaged and a fully human transgene is inserted into the animal's genome and presumably integrates at random into the genome. Typically, the location of the integrated transgene is unknown; expression of the human protein is measured by transcription of the human gene and/or protein assay and/or functional assay. Inclusion in the human transgene of upstream and/or downstream human sequences are apparently presumed to be sufficient to provide suitable support for expression and/or regulation of the transgene no matter where in the animal's genome the transgene winds up. But in many cases the transgene with human regulatory elements expresses in a manner that is unphysiological or otherwise unsatisfactory, and can be actually detrimental to the animal. In contrast, the inventors demonstrate that a replacement with human sequence at an endogenous locus under control of endogenous regulatory elements provides a physiologically appropriate expression pattern and level that results in a useful humanized animal whose physiology with respect to the replaced gene are meaningful and appropriate and context of the humanized animal's physiology.

[0079] Fertilized mouse eggs injected with a construct having the MHC class I promoter H2 and a β-globin intron driving expression of a 695-bp mouse IL-6 gene reportedly produce mice that constitutively express mouse IL-6 at relatively high levels (as compared with wild-type mice) (see, Woodrofe et al. (1992) Long-Term Consequences of Interleukin-6 Overexpression in Transgenic Mice, DNA and Cell Biology 11 (8):587-592). But these mice are prone to develop lymphomas associated with the intestines, lymph nodes, and kidney, as well as splenic amyloid deposits. They also exhibit abnormal B cell maturation (see, Woodrofe *et al., Id.*), so that studies of B cell function are compromised.

In contrast, mice as described herein that comprise a replacement of the mouse IL-6 gene with a human IL-6 gene at the mouse IL-6 locus are not prone to develop these lymphomas, and the mice exhibit apparently normal B cell populations.

[0080] Mice (C57BL/6) transgenic for hIL-6 due to a random insertion of a 6.6-kb (BamHI-Pvu II fragment) length of human DNA containing the hIL-6 gene coupled with an IgM enhancer have been reported (see, Suematsu et al. (1989) IgG1 plasmocytosis in interleukin 6 transgenic mice, Proc. Natl. Acad. Sci. USA 86:7547-7551). The mice express hIL-6 at between 800 pg/mL and 20,000 pg/mL in serum, where wild-type mice typically express only about 100 pg/mL IL-6. The mice exhibit an increase in serum Ig (120 to 400-fold over wild-type mice) and a decrease in albumin as they age. The mice suffer from a massive plasmacytosis, exhibit splenomegaly and lymph node enlargement, as well as exhibiting plasma cells and increased megakaryocytes in bone marrow. Upon inspection, what appear to be enlarged lymph nodes are instead massed of compacted abnormal plasma cells. Both spleen and thymus exhibit massive proliferation of plasma cells, which also infiltrate portions of the lung, liver, and kidney. Kidney in these mice also exhibits IL-6-stimulated mesangial cell proliferation typical of mesangio-proliferative glomerulonephritis. Similarly, mice (BALB/c) transgenic for a trimmed hIL-6 cDNA driven by a mouse H-2L^{d} promoter randomly inserted into the genome display severe plasmacytosis (see, Suematsu et al. (1992) Generation of plasmacytomas with the chromosomal translocation t(12;15) in interleukin 6 transgenic mice, Proc. Natl. Acad. Sci. USA 89:232-235). Although C57BL/6 mice that overexpress hIL-6 do not develop transplantable plasmacytomas (they do exhibit plasmacytosis), transgenic BL/6 mice back-crossed into BALB/c mice reportedly do.

[0081] Random transgenesis of a hIL-6 cDNA driven by a glial fibrillary acidic protein (GFAP) gene promoter reportedly results in hIL-6 overexpression in the mouse central nervous system, which also leads to significant pathologies (see, Campbell et al. (1993) Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6, Proc. Natl. Acad. Sci. USA 90:10061-10065). These mice exhibit extensive neuropathology and reactive astrocytosis resulting from IL-6 expression in the CNS due to loss of control as the result of random integration of an IL-6 transgene at an apparently CNS-permissive transcriptional locus. Although expression of hIL-6 cDNA linked to a β-globin 3'-UTR and driven by a neuron-specific enolase promoter microinjected into fertilized mouse eggs (F1 C57BL/6 x BALB/c) produced mice with a normal lifespan and without apparent neurological defects that expressed hIL-6 in neurons but not elsewhere (see, Fattor et al. (1994) IL-6 Expression in Neurons of Transgenic Mice Causes Reactive Astrocytosis and Increase in Ramified Microglial Cells But No Neuronal Damage, Eur. J. Neuroscience 7:2441-2449), the mice exhibited high levels (20- to 30-fold higher than wild-type) of activated and enlarged astrocytes with increased processes throughout the brain, as well as a 10- to 15-fold increase in ramified microglial cells in white matter. Thus, brain expression of IL-6 reportedly leads to conditions that range from reactive astrocytosis to frank and profound neuropathology.

[0082] Microinjection into fertilized eggs of an F1 cross of C57BL/6x"DBAII" mice of a 639-bp hIL-6 cDNA linked to a β-globin 3'-UTR and a mouse MT-1 promoter reportedly produced a transgenic mouse in which the hIL-6 gene was randomly integrated produced a weakened and diseased mouse that dies young of kidney failure (see Fattori et al. (1994) Blood, Development of Progressive Kidney Damage and Myeloma Kidney in Interleukin-6 Transgenic Mice, Blood 63(9):2570-2579). Transgenic mice expired at 12-20 weeks and exhibited elevated levels of α1 and β-globulins in plasma, hypergammaglobulinemia, elevated megakaryocytes in spleen (3-fold higher than wild-type) and bone marrow, plasmacytosis of lymphoid organs (spleen, thymus, and lymph nodes) characterized by abnormal and compactly arranged plasmocytoid cells, and glomerulonephritis leading to glomerulosclerosis similar to multiple myeloma.

[0083] Microinjection into fertilized eggs of a C57BL/6J mouse of a H-2L^{d}-driven hIL-6 cDNA caused IL-6-dependent muscle wasting in mice, characterized in part by a significantly lower gastrocnemius muscle weight in transgenic mice as compared to weight-matched controls, a difference that was ameliorated by treatment with an IL-6 antagonist (see, Tsujinaka et al. (1996) Interleukin 6 Receptor Antibody Inhibits Muscle Atrophy and Modulates Proteolytic Systems in Interleukin 6 Transgenic Mice, J. Clin. Invest. 97(1):244-249). At 12 weeks these mice displayed serum hIL-6 levels of more than 600,000 pg/mL. The transgenic mice also had livers that weighed about 1,242 mg, as compared to control livers that were about 862 mg. Transgenic mice treated with IL-6 antagonist had livers that weighed about 888 mg. Muscle cathepsins B and B+L were significantly higher (20-fold and 6.2-fold) in transgenic mice than in controls, a phenomenon that was eliminated in transgenic mice treated with an IL-6 antagonist. cathepsin B and L mRNAs were estimated to be about 277% and 257%, respectively, as compared with wild-type mice; the difference was significantly reduced with IL-6 antagonist treatment.

[0084] Mice comprising a hIL-6 minigene driven by a mouse MHC class I H-2Ld promoter and a hIL-6R minigene driven by a chicken β-actin promoter, and a gp130 gene, exhibited pathologies typical of hIL-6 transgenic mice (*e.g.*, hepergammaglobulinemia, splenomegaly, mesangial proliferative glomerulonephritis, lung lymphoid infiltration) as well as ventricular hypertrophy (Hirota et al. (1995) Continuous activation of gp130, a signal-transducing receptor component for interleukin 6-related cytokines, causes myocardial hypertrophy in mice, Proc. Natl Acad. Sci. USA 92:4862-4866). The ventricular hypertrophy is believed to be mediated by a continuous activation of gp130 (*Id.*). The role of IL-6 is reportedly to help strengthen the cytokine receptor complex and induce dimerization of gp130, which is the signal transducing component responsible for transducing the IL-6 signal (Paonessa et al. (1995) Two distinct and independent sites on IL-6 trigger gp130 dimer formation and signalling, EMBO J. 14(9):1942-1951). The activated complex is believed

to be a hexamer composed of two IL-6, each IL-6 bound to one IL-6Rα and two gp130 (each IL-6 contains two independent gp130-binding sites) exhibiting a 2:2:2 stoichiometry, wherein the dimerization of gp130 causes activation of JAK-Tyk tyrosine kinases, phosphorylation of gp130 and STAT family transcription factors and other intracellular substrates (*Id.*; Stahl, N. (1994) Association and Activation of Jak-Tyk Kinases by CNTF-LIF-OSM-IL-6 β Receptor Components, Science 263:92-95), consistent with a general model of cytokine receptor complex formation (see, Stahl, N. and Yancopoulos, G. (1993) The Alphas, Betas, and Kinases of Cytokine Receptor Complexes, Cell 74:587-590; Davis et al. (1993) LIFRβ and gp130 as Heterodimerizing Signal Transducers of the Tripartite CNTF Receptor, Science 260:1805-1808; Murakami et al. (1993) IL-6-Induced Homodimerization of gp130 and Associated Activation of a Tyrosine Kinase, Science 2601808-1810).

[0085] Mice transgenic for human sIL-6R driven by a rat PEP carboxykinase promoter and human IL-6 driven by a mouse metallothionein-1 promoter are reportedly markedly smaller that mice transgenic for human IL-6 alone or human sIL-6R alone (Peters et al. (1997) Extramedullary Expansion of Hematopoietic Progenitor Cells in Interleukin(IL-)-6-sIL-6R Double Transgenic Mice, J. Exp. Med. 185(4):755-766), reflected in reduced body fat and reduced weight (20-25 g vs. 40 g). Double transgenic mice reportedly also exhibit spleen (5-fold) and liver (2-fold) enlargement as compared with reportedly normal organ weights for single transgenic mice, apparently due to extramedullary proliferation of hematopoeitic cells of spleena and liver but not bone marrow, as well as elevated megakaryocytes in spleen and plasmacellular infiltrates in all parenchymal organs (*Id.*). Double transgenics also exhibit livers with an increase of about 200- to about 300-fold in granulocytes, macrophages, progenitor cells, and B cells as compared with single transgenics; in contrast, IL-6 single transgenic mice exhibited lesser increases in macrophages (15-fold) and B cells (45-fold) (*Id.*). The extraordinary findings are presumably due to stimulation of growth and differentiation of hematopoietic progenitor cells by activating gp130 signal transduction (*Id.*).

[0086] Further, double transgenic (mouse metallothionine promoter-driven hIL-6/rat PEP carboxykinase promoter-driven hIL-6R) mice exhibit a hepatocellular hyperplasia that is reportedly identical to human nodular regenerative hyperplasia with sustained hepatocyte proliferation that strongly suggests that IL-6 is responsible for both hepatocyte proliferation and pathogenic hepatocellular transformation (Maione et al. (1998) Coexrpession of IL-6 and soluble IL-6R causes nodular regenerative hyperplasia and adenomas of the liver, EMBO J. 17(19):5588-5597). Because hepatocellular hyperplasia is reportedly not observed in single transgenic hIL-6 mice and hIL-6 can bind mIL-6R, the finding may appear paradoxical until it is considered that the double transgenic may result in higher levels of hIL-6 complexed to soluble IL-6R (here, soluble hIL-6R), which complex is a more potent inhibitor that IL-6 alone (*Id.*).

[0087] In contrast to mice that are transgenic for human IL-6, humanized IL-6 mice that comprise a replacement at an endogenous mouse IL-6 locus, which retain mouse regulatory elements but comprise a humanization of IL-6-encoding sequence, do not exhibit the severe pathologies of prior art mice. Genetically modified mice that were heterozygous or homozygous for hIL-6 were grossly normal.

[0088] Mice with a humanized IL-6 gene (MAID 760) as described in the Examples were immunophenotyped and found to have normal B cell numbers in FACS analyses (lymphocyte-gated) of spleen B cells using a pan B cell marker (CD445R(B220)) (FIG.4). For spleen, wild-type mice exhibited 63% B cells; hIL-6 heterozygote mice exhibited 63% B cells; and mice homozygous for hIL-6 at the endogenous mouse locus exhibited 63% B cells. B cell numbers for homozygous hIL-6 mice immunized with TNP-KLH were also normal (65% for wild-type, and 61% for hIL-6 homozygotes).

[0089] Splenic T cells were also about the same as wild-type (FIG.5). Percentages of splenic T cells for Thelper/Tcytoxic were, for wild-type 20%/40% (ratio of 1.4:1); for hIL-6 heterozygotes 23%/14% (ratio of 1.6:1); for hIL-6 homozygotes 21%/15% (ratio of 1.4:1) (markers were CD8a-APC; CD4-FITC). Homozygous hIL-6 mice immunized with TNP-KLH exhibited similar splenic T cell numbers to wild-type mice, i.e., Thelper/Tcytotoxic were 22%/20% (ratio of 1.1:1) as compared with 21%/19% for wild-type (also a ratio of 1.1:1).

[0090] Humanized IL-6 mice also exhibited about normal levels of splenic NK cells on FACS analysis (CD11b and DX5) (FIG. 7). hIL-6 heterozygotes exhibited 2.2% NK cells, and hIL-6 homozygotes exhibited 1.8% NK cells, whereas wild-type mice exhibited 2.4% NK cells. Following immunization with TNP-KLH, homozygotes exhibited 1.6% splenic NK cells, whereas wild-type mice exhibited 2.1% splenic NK cells.

[0091] Humanized IL-6 mice also exhibited normal levels of splenic Ly6G/C(Gr1) cells (FIG. 6). hIL-6 heterozygotes exhibited 7.0% $GR1^+$ cells (1.3% $Gr1^{hi}$); homozygotes exhibited 6.8% $Gr1^+$ + cells (0.9% $Gr1^{hi}$), whereas wild-type mice exhibited 8.0% $Gr1^+$ cells (1.8%$Gr1^{hi}$). Immunized IL-6 homozygotes (immunized with TNP-KLH) exhibited 11% Gr1+ cells (4.0% $Gr1^{hi}$), whereas wild-type mice exhibited 10% $Gr1^+$ cells (3.0% $Gr1^{hi}$).

[0092] Humanized IL-6 mice also exhibited normal blood B and T cell numbers in FACS analysis (FIG. 8 and FIG. 9). FACs with a pan B cell marker (CD445R(B220)) revealed that homozygous hIL-6 mice exhibited 52% B cell as compared with wild-type 53%; heterozygotes exhibited 38% (an average of two different stainings of 29% and 47%). Homozygous hIL-6 mice immunized with TNP-KLH gave similar B cell numbers (43%, as compared with 45% for wild-type mice).

[0093] Humanized IL-6 mice exhibited normal blood T cell numbers in FACS analysis as measured by CD8a and CD4 staining. Heterozygous hIL-6 mice exhibited Thelper/Tcytotoxic numbers of 39%/26% (ratio of 1.5:1); homozygous hIL-6 mice exhibited Th/Tc numbers of 24%/20% (ratio of 1.2:1), whereas wild-type mice exhibited Th/Tc numbers of 26%/20%

(ratio of 1.3:1). Homozygous hIL-6 mice immunized with TNP-KLH had Th/Tc numbers of 29%/21% (ratio of 1.4:1), whereas wild-type immunized mice had Th/Tc numbers of 28%/23% (1.2:1).

[0094] Humanized IL-6 mice also exhibited myeloid cell numbers in blood that were similar to wild-type mice as measured by FACS analysis of naïve and immunized mouse blood stained with Ly6G/C(Gr1) and CD11 b, as well as CD11 b and DX5 (FIG. 10, FIG. 11, and FIG 12). Heterozygous hIL-6 mice exhibited %Gr+ cells of 10.8%, homozygotes 6.9%, whereas wild-type mice exhibited 9.7%. Immunized hIL-6 homozygotes exhibited M1(Ly6G/C(Gr) of 10^1-10^4)/ M2(Ly6G/C(Gr) staining of about 10^2-10^3) numbers of 43%/34%, whereas wild-type mice had numbers of 45%/38%. FACS plots of CD11 b (vertical axis) vs. Ly6G/C (horizontal axis) for immunized homozygous hIL-6 mice showed cell percentage in quadrants (upper left/upper right/lower right) of 16%/8%/3%, which were identical to immunized wild-type quadrant numbers.

[0095] Homozygous TNP-KLH-immunized humanized IL-6 mice exhibited CD11 b vs. DX5(NK) staining FACS plots that were similar to immunized wild-type mice. Quadrant analysis blood FACS plots (CD11b vertical axis, DX5(NK) horizontal axis) revealed upper left/upper right/lower right numbers of 9.5%/17%/10% for hIL-6 homozygotes and 6.5%/17.3%/14% for wild-type mice.

[0096] Humanized IL-6 mice exhibited an isotype response that was essentially the same as observed in wild-type mice. Early and final IgG1, IgG2a, IgG2b, IgG3, IgA, IgE, and IgM levels were about the same as observed in wild-type mice. In one experiment, final IgM was slightly higher in humanized mice; final IgG3 was also elevated in humanized mice.

[0097] B cell development in naive hIL-6 mice was essentially indistinguishable from development in wild-type mice based on FACS analysis of bone marrow IgM/CD24/B220 staining (FIG. 13). Immunophenotyping of immune mice revealed that marker populations for various cell types in the B cell development progression were essentially normal in hIL-6 mice. Progression of cells from hematopoietic stem cells, common lymphoid progenitors, ProB cells, PreB cells, and immature and mature B cells is normal in hIL-6 mice (FIG. 14 and FIG. 15)

EXAMPLES

Example 1: Replacement of Endogenous Mouse IL-6 Gene with hIL-6 Gene

[0098] The 4.8-kb human IL-6 gene containing exons 1 through 5 of the human IL-6 gene replaced 6.8 kb of the murine IL-6 gene locus.

[0099] A targeting construct for replacing the mouse with the human IL-6 gene in a single targeting step was constructed using VELOCIGENE® genetic engineering technology (see, Valenzuela et al. (2003) High-throughput engineering of the mouse genome coupled with high-resolution expression analysis, Nature Biotech, 21(6):652-659). Mouse and human IL-6 DNA were obtained from bacterial artificial chromosome (BAC) RPCI-23 clone 368C3, and from BAC CTD clone 2369M23, respectively. Briefly, a NotI linearized targeting construct generated by gap repair cloning containing mouse IL-6 upstream and downstream homology arms flanking a 4.8 kb human IL-6 sequence extending from ATG in exon 1 to exon 5 with 16 nucleotides of 3' downstream sequence (genomic coordinates: NCBIh37.1: ch7:22,766,882 to 22,771,637) and a floxed neo selection cassette, was electroporated into F1 H4 mouse embryonic stem (ES) cells (C57BL/6 x 129 F1 hybrid). Correctly targeted ES cells (MAID 790) were further electroporated with a transient Cre-expressing vector to remove the drug selection cassette. Targeted ES cell clones without drug cassette (MAID 1428) were introduced into an 8-cell stage mouse embryo by the VELOCIMOUSE® method (see, US Pat. No. 7,294,754, 7,576,259, 7,659,442, and Poueymirou et al. (2007) F0 generation mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analyses Nature Biotech. 25(1):91-99). VELOCIMICE® (F0 mice fully derived from the donor ES cell) bearing the humanized IL-6 gene were identified by genotyping for loss of mouse allele and gain of human allele using a modification of allele assay (see, Valenzuela et al. (2003)).

[0100] Correctly targeted ES cell clones were identified by a loss-of-native-allele (LONA) assay (Valenzuela *et al.* 2003) in which the number of copies of the native, unmodified 116 gene were determined by two TaqMan™ quantitative polymerase chain reactions (qPCRs) specific for sequences in the mouse 116 gene that were targeted for deletion. The qPCR assays comprised the following primer-probe sets (written 5' to 3'): upstream forward primer, TTGCCGGTTT TCCCTTTTCT C (SEQ ID NO:1); upstream reverse primer, AGGGAAGGCC GTGGTTGTC (SEQ ID NO:2); upstream probe, FAM-CCAGCATCAG TCCCAAGAAG GCAACT-BHQ (SEQ ID NO:3); downstream forward primer, TCAGAGT-GTG GGCGAACAAA G (SEQ ID NO:4); downstream reverse primer, GTGGCAAAAG CAGCCTTAGC (SEQ ID NO:5); downstream probe, FAM-TCATTCCAGG CCCTTCTTAT TGCATCTG-BHQ (SEQ ID NO:6); in which FAM refers to the 5-carboxyfluorescein fluorescent probe and BHQ refers to the fluorescence quencher of the black hole quencher type (Biosearch Technologies). DNA purified from ES cell clones that that have taken up the targeting vector and incorporated in their genomes was combined with TaqMan™ Gene Expression Master Mix (Life Technologies) according to the manufacturer's suggestions in a 384-well PCR plate (MicroAmp™ Optical 384-Well Reaction Plate, Life Technologies) and cycled in an Applied Biosystems Prism 7900HT, which collects fluorescence data during the course of the PCRs and determines a threshold cycle (Ct), the fractional PCR cycle at which the accumulated fluorescence reaches a pre-

set threshold. The upstream and downstream //6-specific qPCRs and two qPCRs for non-targeted reference genes were run for each DNA sample. The differences in the Ct values (ΔCt) between each //6-specific qPCR and each reference gene qPCR were calculated, and then the difference between each ΔCt and the median ΔCt for all samples assayed was calculated to obtain ΔΔCT values for each sample. The copy number of the *Il6* gene in each sample was calculated from the following formula: copy number = $2 \bullet 2^{-\Delta\Delta Ct}$. A correctly targeted clone, having lost one of its native copies, will has an 116 gene copy number equal to one. Confirmation that the human *IL6* gene sequence replaced the deleted mouse 116 gene sequence in the humanized allele was confirmed by a TaqMan™ qPCR assay that comprises the following primer-probe sets (written 5' to 3'): the human forward primer, CCCCACTCCACTGGAATTTG (SEQ ID NO:7); the human reverse primer, GTTCAACCACAGCCAGGAAAG (SEQ ID NO:8); and the human probe, FAM-AGCTACAACTCATTGGCATCCTGGCAA-BHQ (SEQ ID NO:9).

[0101] The same LONA assay was used to assay DNA purified from tail biopsies for mice derived from the targeted ES cells to determine their 116 genotypes and confirm that the humanized 116 allele had transmitted through the germline. Two pups heterozygous for the replacement are bred to generate a mouse that is homozygous for the replacement of the endogenous mouse IL-6 gene by the human IL-6 gene. Pups that are homozygous for the replacement are used for phenotyping.

[0102] The upstream junction of the murine locus and the sequence containing the hIL-6 gene is designed to be within 5'-AATTAGAGAG TTGACTCCTA ATAAATATGA GACTGGGGAT GTCTGTAGCT CATTCTGCTC TGGAGCCCAC CAAGAACGAT AGTCAATTCC AGAAACCGCT ATGAACTCCT TCTCCACAAG TAAGTGCAGG AAATCCTTAG CCCTGGAACT GCCAGCGGCG GTCGAGCCCT GTGTGAGGGA GGGGTGTGTG GCCCAGG (SEQ ID NO:10), wherein the final mouse nucleotide prior to the first nucleotide of the human gene is the "T" in CCGCT, and the first nucleotide of the human sequence is the first "A" in ATGAA. The downstream junction of the sequence containing the hIL-6 gene and the murine locus is designed to be within 5'-TTTTAAAGAA ATATTTATAT TGTATTTATA TAATGTATAA ATGGTTTTTA TACCAATAAA TGGCATTTTA AAAAATTCAG CAACTTTGAG TGTGTCACGC TCCCGGGCTC GATAACTATA ACGGTCCTAA GGTAGCGACT CGAGATAACT T-3' (SEQ ID NO:11), wherein the final nucleotide of the human sequence is with the final "G" in TCACG and the first nucleotide of the mouse sequence is the first "C" in CTCCC; the downstream junction region also contained a loxP site at the 3' end (the beginning of which is shown) for removal of a floxed ubiquitin promoter-driven neo cassette. The junction of the neo cassette with the mouse IL-6 locus is designed to be within 5'-TATACGAAGT TATCCTAGGT TGGAGCTCCT AAGTTACATC CAAACATCCT CCCCCAAATC AATAATTAAG CACTTTTTAT GACATGTAAA GTTAAATAAG AAGTGAAAGC TGCAGATGGT GAGTGAGA (SEQ ID NO:12), where the final "C" of AGCTC is the final nucleotide of the neo cassette; the first nucleotide of the mouse genome following the cassette is the initial "C" of CTAAG.

Example 2: Immunophenotyping of Naive and Immunized hIL-6 Mice: B Cells

[0103] Mice homozygous for the hIL-6 gene replacement were analyzed for B cells (DC445R(B220). Lymphocyte-gated fractions from spleen cell preparations of naive and immunized (TNP-KLH) hIL-6 mice were stained and immunophenotyped using flow cytometry. FACS analysis showed that the percentage of B cells of the spleen cell preparation as measured by CD45R(B220)-FITC staining were about the same (63% of cells) for preparations from naive wild-type, hIL-6 heterozygotes, and hIL-6 homozygotes. For immunized mice, B cells accounted for about 65% of total cells of the spleen cell preparation in wild-type mice, and about 61% of total cells in hIL-6 homozygotes. Spleens of hIL-6 mice (both naive and immunized) contain a population of B cells that is about the same size as the splenic B cell population in wild-type mice.

[0104] Bone marrow of wild-type, hIL-6 heterozygotes, and hIL-6 homozygotes was stained with B cell markers (CD45R(B220)-APC, CD24(HSA)-PE, or CD43 conjugated to a dye and/or IgM (IgM-FITC). B cell development in bone marrow of normal mice will be reflected in surface markers as cells progress from stem cells to early pro-B cells to late pro-B cells, to large pre-B cells to small pre-B cells to immature B cells and finally, to mature B cells. Common lymphocyte progenitor pro-B cells will express CD45R, and in later stages will express IgM as immature and later as mature B cells. Thus, CD45R-stained and anti-IgM-stained B cells should reveal a pattern characteristic of B cell development. Bone marrow of hIL-6 heterozygotes and homozygotes displayed a pattern of CD45R(B220)-APC and anti-IgM-FITC staining that was essentially indistinguishable from wild-type bone marrow, showing populations of B cells that stained positive for CD45R(B220) and IgM, or CD45R(B220) alone. B cell sub-populations in bone marrow of hIL-6 mice revealed by FACS staining were similar to those in wild-type mice (Table 1; see also, FIG. 13).

	Table 1. B Cells in Bone Marrow of Naive Mice		
	Wild-type Mouse (%)	hIL-6 Mouse	
		Heterozygote (%)	Homozygote (%)
CLP-ProB	40	29	32
PreB-ImmatureB	12.3	19.3	15.3
Mature B	6.4	6.5	6.7

[0105] Staining for CD24 (see FIG. 14) revealed the (normal) pattern shown in Table 2, indicating normal development in bone marrow.

	Table 2. B Cells in Bone Marrow of Naive Mice		
	Wild-type Mouse (%)	hIL-6 Mouse	
		Heterozygote (%)	Homozygote (%)
Developing HSC-CLP	46.6	46	43
Mature CLP/early ProB	10.2	9.0	10.1
Late ProB, PreB, Immature B	7.2	11.6	10.7
Mature B	14.1	14.9	17

[0106] Staining for CD43 (see FIG. 15) revealed the (normal) pattern shown in Table 3, indicating normal development in bone marrow.

	Table 3. B Cells in Bone Marrow of Naive Mice		
	Wild-type Mouse (%)	hIL-6 Mouse	
		Heterozygote (%)	Homozygote (%)
PreBII-Immature B cells	28.4	21.4	21.2
Mature B cells	8.1	11.5	8.0
ProB-PreBI	3.4	4.3	4.7

[0107] Thus, immunophenotyping of naïve hIL-6 mice revealed that B cell development in such mice is essentially normal.

Example 3: Replacement of Endogenous Mouse IL-6Rα Ectodomain Gene Sequence with hIL-6Rα Ectodomain Gene Sequence

[0108] The 45 kb human IL-6Rα gene containing exons 1 through 8 of the human IL-6Rα gene replaced 35.4 kb of the murine IL-6Rα gene locus. Mouse exons 9 and 10 were retained; only exons 1-8 were humanized. In total, 35,384 nt of mouse sequence was replaced by 45,047 nt of human sequence.

[0109] A targeting construct for replacing the mouse with the human IL-6Rα gene in a single targeting step was constructed using VELOCIGENE® genetic engineering technology (see, Valenzuela et al. (2003) High-throughput engineering of the mouse genome coupled with high-resolution expression analysis, Nature Biotech, 21(6):652-659). Mouse and human IL-6Rα DNA were obtained from bacterial artificial chromosome (BAC) RPCI-23 clone 125J8, and from BAC CTD clone 2192J23, respectively. Briefly, a NotI linearized targeting construct generated by gap repair cloning containing mouse IL-6Rα upstream and downstream homology arms flanking a 45 kb human IL-6Rα sequence extending from ATG in exon 1 to exon 8 with 69 nucleotides of 3' downstream sequence and a floxed neo selection cassette, was electroporated into F1 H4 mouse embryonic stem (ES) cells (C57BL/6 x 129 F1 hybrid). Correctly targeted ES cells (MAID 794) were further electroporated with a transient Cre-expressing vector to remove the drug selection cassette. Targeted ES cell clones without drug cassette (MAID 1442) were introduced into an 8-cell stage mouse embryo by the VELOCIMOUSE® method (see, US Pat. No. 7,294,754, 7,576,259, 7,659,442, and Poueymirou et al. (2007) F0 gen-

eration mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analyses Nature Biotech. 25(1):91-99). VELOCIMICE® (F0 mice fully derived from the donor ES cell) bearing the humanized IL-6Rα gene were identified by genotyping for loss of mouse allele and gain of human allele using a modification of allele assay (see, Valenzuela et al. (2003)).

[0110] Correctly targeted ES cell clones were identified by a loss-of-native-allele (LONA) assay (Valenzuela et al. 2003) in which the number of copies of the native, unmodified Il6 gene were determined by two TaqMan™ quantitative polymerase chain reactions (qPCRs) specific for sequences in the mouse Il6 gene that were targeted for deletion. The qPCR assays comprised the following primer-probe sets (written 5' to 3'): upstream forward primer, GCCCTAGCAT GCAGAATGC (SEQ ID NO:13); upstream reverse primer, AAGAGGTCCC ACATCCTTTG C (SEQ ID NO:14); upstream probe, CCCACATCCA TCCCAATCCT GTGAG (SEQ ID NO:15); downstream forward primer, GAGCTTGCCC CCAGAAAGG (SEQ ID NO:16); downstream reverse primer, CGGCCACATC TCTGGAAGAC (SEQ ID NO:17); downstream probe, CATGCACTGC CCCAAGTCTG GTTTCAGT (SEQ ID NO:18). DNA purified from ES cell clones that that have taken up the targeting vector and incorporated in their genomes was combined with TaqMan™ Gene Expression Master Mix (Life Technologies) according to the manufacturer's suggestions in a 384-well PCR plate (MicroAmp™ Optical 384-Well Reaction Plate, Life Technologies) and cycled in an Applied Biosystems Prism 7900HT, which collects fluorescence data during the course of the PCRs and determines a threshold cycle (Ct), the fractional PCR cycle at which the accumulated fluorescence reaches a pre-set threshold. The upstream and downstream IL-6Rα-specific qPCRs and two qPCRs for non-targeted reference genes were run for each DNA sample. The differences in the Ct values (ΔCt) between each IL-6Rα-specific qPCR and each reference gene qPCR were calculated, and then the difference between each ΔCt and the median ΔCt for all samples assayed was calculated to obtain ΔΔCt values for each sample. The copy number of the Il6 gene in each sample was calculated from the following formula: copy number = 2 • 2-ΔΔt. A correctly targeted clone, having lost one of its native copies, will have an IL-6Rα gene copy number equal to one. Confirmation that the human IL-6Rα gene sequence replaced the deleted mouse IL-6Rα gene sequence in the humanized allele was confirmed by a TaqMan™ qPCR assay that comprises the following primer-probe sets (written 5' to 3'): the human forward primer, GGAGAGGGCA GAGGCACTTA C (SEQ ID NO:19); the human reverse primer, GGCCAGAGCC CAAGAAAAG (SEQ ID NO:20); and the human probe, CCCGTTGACT GTAATCTGCC CCTGG (SEQ ID NO:21).

[0111] The same LONA assay was used to assay DNA purified from tail biopsies for mice derived from the targeted ES cells to determine their IL-6Rα genotypes and confirm that the humanized IL-6Rα allele had transmitted through the germline. Pups heterozygous for the replacement are bred to generate a mouse that is homozygous for the replacement of the endogenous mouse IL-6Rα gene by the human IL-6Rα (ectodomain) gene. Pups that are homozygous for the replacement are used for phenotyping.

[0112] The upstream junction of the murine locus and the sequence containing the hIL-6Rα gene is designed to be within 5'-CGAGGGCGAC TGCTCTCGCT GCCCCAGTCT GCCGGCCGCC CGGCCCCGGC TGCGGAGCCG CTCTGCCGCC CGCCGTCCCG CGTAGAAGGA AGCATGCTGG CCGTCGGCTG CGCGCTGCTG GCTGCCCTGC TGGCCGCGCC GGGAGCGGCG CTGGCCCCAA GGCGCTGCCC TGCGCAGGGT AAGGGCTTCG G (SEQ ID NO:22), wherein the final mouse nucleotide prior to the first nucleotide of the human gene is the "C" in GAAGC, and the first nucleotide of the human sequence is the first "A" in ATGCT. The downstream junction of the sequence containing the hIL-6 gene and the murine locus is designed to be within 5'-CAAGATTATT GGAGTCTGAA ATGGAATACC TGTTGAGGGA AATCTTTATT TTGGGAGCCC TTGATTTCAA TGCTTTTGAT TCCCTATCCC TGCAAGACCC GGGCTCGATA ACTATAACGG TCCTAAGGTA GCGACTCGAG ATAACTTC-3' (SEO ID NO:23), wherein the final nucleotide of the human sequence is with the final "A" in CAAGA and the first nucleotide of the mouse sequence is the first "C" in CCCGG; the downstream junction region also contained a loxP site at the 3' end for removal of a floxed ubiquitin promoter-driven neo cassette. The first nucleotide of the loxp site is the first "A" in ATAAC. The junction of the neo cassette with the mouse IL-6Rα locus is designed to be within 5'-TATACGAAGT TATCCTAGGT TGGAGCTCTA CTCCATATGC TCACTTGCCG TTGTTTGCTA CGATACGGTG AGGCCCGTGC GAAGAGTGGC ACAGATCAGG AGGCTTATGT GGTCAGTCCA CAGTATGGC (SEQ ID NO:24), where the final "C" of AGCTC is the final nucleotide of the neo cassette; the first nucleotide of the mouse genome following the cassette is the initial "T" of TACTC.

SEQUENCE LISTING

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Claims

1. A genetically modified murine animal comprising a replacement at an endogenous murine IL-6 locus of a murine gene encoding IL-6 with a human gene encoding human IL-6, wherein the human gene encoding human IL-6 is under control of endogenous murine regulatory elements at the endogenous murine IL-6 locus.

2. The genetically modified murine animal of claim 1, wherein the human gene encoding human IL-6 comprises exons 1 through 5 of the human IL-6 gene found in the CTD-2369M23 bacterial artificial chromosome.

3. The genetically modified murine animal of claim 1, wherein the murine animal expresses a humanized IL-6Rα wherein the endogenous murine IL-6Rα gene has been replaced with a human sequence comprising a sequence that encodes an ectodomain of a human IL-6Rα.

4. The genetically modified murine animal of claim 1, wherein the murine animal does not exhibit a feature selected from plasmocytosis, glomerulosclerosis, glomerulonephritis, kidney failure, hypergammaglobulinemia, elevated megakaryocytes in spleen, elevated megakaryocytes in bone marrow, splenomegaly, lymph node enlargement, compacted abnormal plasma cells, and a combination thereof.

5. The genetically modified murine animal of claim 3, wherein the humanized IL-6Rα comprises murine transmembrane and intracellular domains.

6. A genetically modified murine animal, comprising a humanization of an endogenous murine IL-6Rα gene, wherein the humanization comprises a replacement of murine IL-6Rα ectodomain-encoding sequence with human IL-6Rα ectodomain-encoding sequence at the endogenous murine IL-6Rα locus, and wherein the humanized IL-6Rα gene is under control of endogenous murine regulatory elements.

7. The genetically modified murine animal of claim 6, further comprising a humanized IL-6 gene comprising a replacement at an endogenous murine IL-6 locus of a murine gene encoding IL-6 with a human gene encoding human IL-6.

8. A method for making a humanized murine animal, comprising replacing a murine gene sequence encoding murine IL-6 with a human gene encoding human IL-6 so that the human IL-6 gene is under control of endogenous murine regulatory elements.

9. A method for making a humanized murine animal, comprising replacing all murine exons encoding ectodomain sequences of murine IL-6Rα with a human genomic fragment encoding human IL-6Rα ectodomain to form a humanized IL-6Rα gene, wherein the humanized IL-6Rα gene is under control of endogenous murine regulatory elements.

10. A genetically modified murine animal comprising a humanized IL-6Rα gene comprising a replacement of a murine ectodomain-encoding sequence with a human ectodomain sequence, wherein the humanized IL-6Rα gene comprises murine transmembrane and intracellular domain sequences, wherein the murine animal further comprises a gene encoding a human IL-6, and wherein the genes encoding human IL-6 and humanized IL-6Rα are under control of endogenous murine regulatory elements.

Patentansprüche

1. Ein genetisch modifiziertes murines Tier, umfassend einen Austausch an einem endogenen IL-6-Locus eines mu-

rinen Gens, das für IL-6 kodiert, durch ein humanes Gen, das für humanes IL-6 kodiert, wobei das humane Gen, das humanes IL-6 kodiert, unter der Kontrolle von endogenen murinen regulatorischen Elementen am endogenen murinen IL-6-Locus steht.

2. Genetisch modifiziertes murines Tier aus Anspruch 1, wobei das humane Gen, das humanes IL-6 kodiert, die Exone 1 bis 5 des humanen IL-6-Gens enthält, das im bakteriellen artifiziellen Chromosom CTD-2369M23 vorgefunden wird.

3. Genetisch modifiziertes murines Tier aus Anspruch 1, wobei das murine Tier ein humanisiertes IL-6Rα exprimiert, worin das endogene murine IL-6Rα-Gen durch eine humane Sequenz ausgetauscht wurde, die eine Sequenz enthält, die für eine Ectodomäne eines humanen IL-6Rα kodiert.

4. Genetisch modifiziertes murines Tier aus Anspruch 1, wobei das murine Tier kein Merkmal aufweist, das ausgewählt ist aus Plasmozytose, Glomerulosklerose, Glomerulonephritis, Nierenversagen, Hypergammaglobulinämie, erhöhte Megakaryozyten in Milz, erhöhte Megakaryozyten im Knochenmark, Splenomegalie, Lymphknotenvergrößerung, komprimierte abnormale Plasmazellen und einer Kombination davon.

5. Genetisch modifiziertes murines Tier aus Anspruch 3, wobei das humanisierte IL-6Rα murine transmembrane und intrazelluläre Domänen umfasst.

6. Ein genetisch modifiziertes murines Tier, umfassend eine Humanisierung eines endogenen murinen IL-6Rα-Gens, wobei die Humanisierung einen Austausch einer murinen IL-6Rα Ectodomäne-kodierenden Sequenz durch eine humane IL-6Rα Ectodomäne-kodierende Sequenz am endogenen murinen IL-6Rα-Locus umfasst, und wobei das humanisierte IL-6Rα-Gen unter der Kontrolle von endogenen murinen regulatorischen Elementen steht.

7. Genetisch modifiziertes murines Tier aus Anspruch 6, weiterhin umfassend ein humanisiertes IL-6-Gen, das einen Austausch am endogenen murinen IL-6-Locus von einem murinen Gen, das für IL-6 kodiert, durch ein humanes Gen, das für humanes IL-6 kodiert, enthält.

8. Ein Verfahren zur Herstellung eines humanisierten murinen Tieres, umfassend das Austauschen einer murinen Gen-Sequenz, die für murines IL-6 kodiert, durch ein humanes Gen, das für humanes IL-6 kodiert, so dass das humane IL-6-Gen unter der Kontrolle von endogenen murinen regulatorischen Elementen steht.

9. Ein Verfahren zur Herstellung eines humanisierten murinen Tieres, umfassend das Austauschen aller murinen Exone, die für die Ectodomäne-Sequenzen von murinem IL-6Rα kodieren, durch ein humanes genomisches Fragment, das für die Ectodomäne von humanem IL-6Rα kodiert, um ein humanisiertes IL-6Rα-Gen zu bilden, wobei das humanisierte IL-6Rα-Gen unter der Kontrolle von endogenen murinen regulatorischen Elementen steht.

10. Ein genetisch modifiziertes murines Tier, umfassend ein humanisiertes IL-6Rα-Gen, das einen Austausch einer murinen Ectodomäne-kodierenden Sequenz durch eine humane Ectodomäne-kodierende Sequenz umfasst, wobei das humanisierte IL-6Rα-Gen murine transmembrane und intrazelluläre Domäne-Sequenzen umfasst, wobei das murine Tier weiterhin ein Gen umfasst, das für ein humanes IL-6 kodiert, und wobei die Gene, die für humanes IL-6 und für humanisiertes IL-6Rα kodieren, unter der Kontrolle von endogenen murinen regulatorischen Elementen stehen.

Revendications

1. Animal murin génétiquement modifié comprenant un remplacement au niveau d'un locus d'IL-6 murin endogène d'un gène murin codant pour l'IL-6 par un gène humain codant pour l'IL-6 humaine, dans lequel le gène humain codant pour l'IL-6 humaine est sous le contrôle d'éléments régulateurs murins endogènes au niveau du locus d'IL-6 murin endogène.

2. Animal murin génétiquement modifié selon la revendication 1, dans lequel le gène humain codant pour l'IL-6 humaine comprend les exons 1 à 5 du gène de l'IL-6 humain trouvés dans le chromosome artificiel bactérien CTD-2369M23.

3. Animal murin génétiquement modifié selon la revendication 1, l'animal murin exprimant un IL-6Rα humanisé dans lequel le gène de l'IL-6Rα murin endogène a été remplacé par une séquence humaine comprenant une séquence qui code pour un ectodomaine d'un IL-6Rα humain.

4. Animal murin génétiquement modifié selon la revendication 1, l'animal murin ne présentant pas une caractéristique choisie parmi une plasmocytose, une glomérulosclérose, une glomérulonéphrite, une insuffisance rénale, une hypergammaglobulinémie, des mégacaryocytes élevés dans la rate, des mégacaryocytes élevés dans la moelle osseuse, une splénomégalie, une hypertrophie des ganglions lymphatiques, des cellules plasmatiques anormales tassées, et une combinaison de celles-ci.

5. Animal murin génétiquement modifié selon la revendication 3, dans lequel l'IL-6Rα humanisé comprend des domaines transmembranaire et intracellulaire murins.

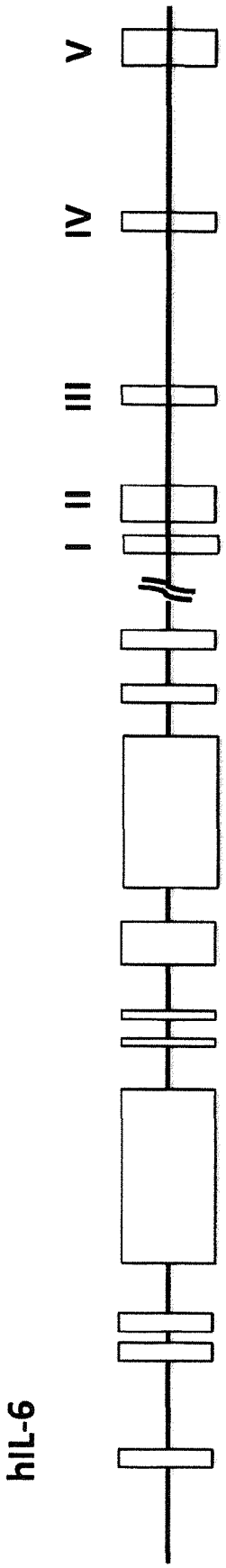
6. Animal murin génétiquement modifié, comprenant une humanisation d'un gène d'IL-6Rα murin endogène, dans lequel l'humanisation comprend un remplacement d'une séquence codant pour l'ectodomaine de l'IL-6Rα murin par une séquence codant pour l'ectodomaine de l'IL-6Rα humain au niveau du locus d'IL-6Rα murin endogène, et dans lequel le gène de l'IL-6Rα humanisé est sous le contrôle d'éléments régulateurs murins endogènes.

7. Animal murin génétiquement modifié selon la revendication 6, comprenant en outre un gène d'IL-6 humanisé comprenant un remplacement au niveau d'un locus d'IL-6 murin endogène d'un gène murin codant pour l'IL-6 par un gène humain codant pour l'IL-6 humaine.

8. Procédé de production d'un animal murin humanisé, comprenant le remplacement d'une séquence de gène murin codant pour l'IL-6 murine par un gène humain codant pour l'IL-6 humaine de telle façon que le gène de l'IL-6 humain est sous le contrôle d'éléments régulateurs murins endogènes.

9. Procédé de production d'un animal murin humanisé, comprenant le remplacement de tous les exons murins codant pour les séquences de l'ectodomaine de l'IL-6Rα murin par un fragment génomique humain codant pour l'ectodomaine de l'IL-6Rα humain pour former un gène d'IL-6Rα humanisé, dans lequel le gène d'IL-6Rα humanisé est sous le contrôle d'éléments régulateurs murins endogènes.

10. Animal murin génétiquement modifié comprenant un gène d'IL-6Rα humanisé comprenant un remplacement d'une séquence codant pour l'ectodomaine murin par une séquence d'ectodomaine humaine, dans lequel le gène d'IL-6Rα humanisé comprend des séquences des domaines transmembranaire et intracellulaire murins, l'animal murin comprenant en outre un gène codant pour l'IL-6 humaine, et dans lequel les gènes codant pour l'IL-6 humaine et l'IL-6Rα humanisé sont sous le contrôle d'éléments régulateurs murins endogènes.



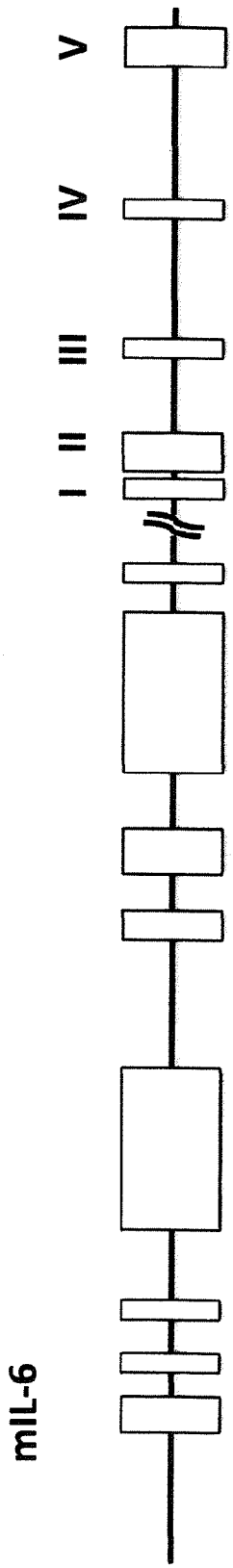


FIG. 1

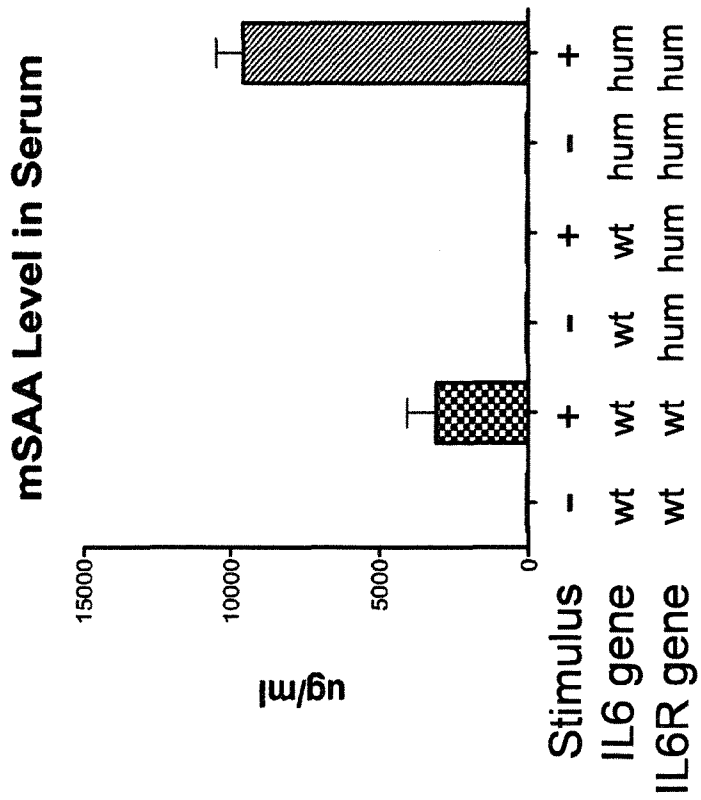

mSAA Level in Serum
ug/ml
15000
10000
5000
0
Stimulus - + - + - +
IL6 gene wt wt wt wt hum hum
IL6R gene wt wt hum hum hum hum

FIG. 2

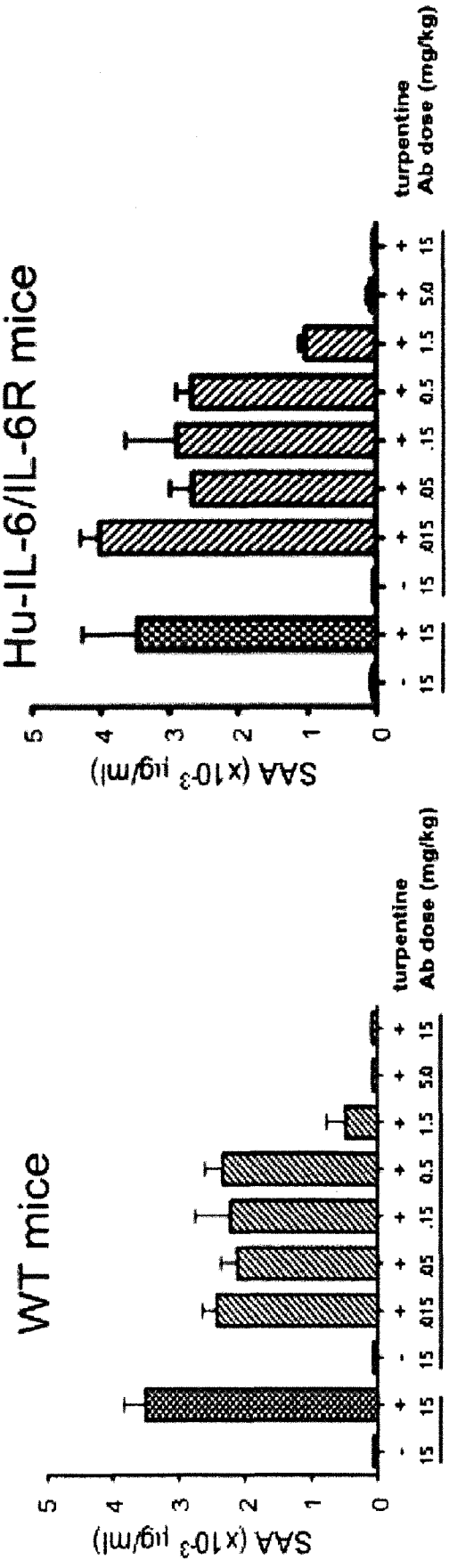

Hu-IL-6/IL-6R mice
SAA (x10-3 µg/ml)
0
1
2
3
4
5
turpentine
Ab dose (mg/kg)
15 15 - + - + + + + + + + +
- + + - + + + + + + + +
15 15 .015 .05 .15 .5 1.5 5.0 15
Anti-human IL6Rα
WT mice
SAA (x10-3 µg/ml)
0
1
2
3
4
5
turpentine
Ab dose (mg/kg)
15 15 - + - + + + + + + + +
- + + - + + + + + + + +
15 15 .015 .05 .15 .5 1.5 5.0 15
Anti-mouse IL6Rα

FIG. 3

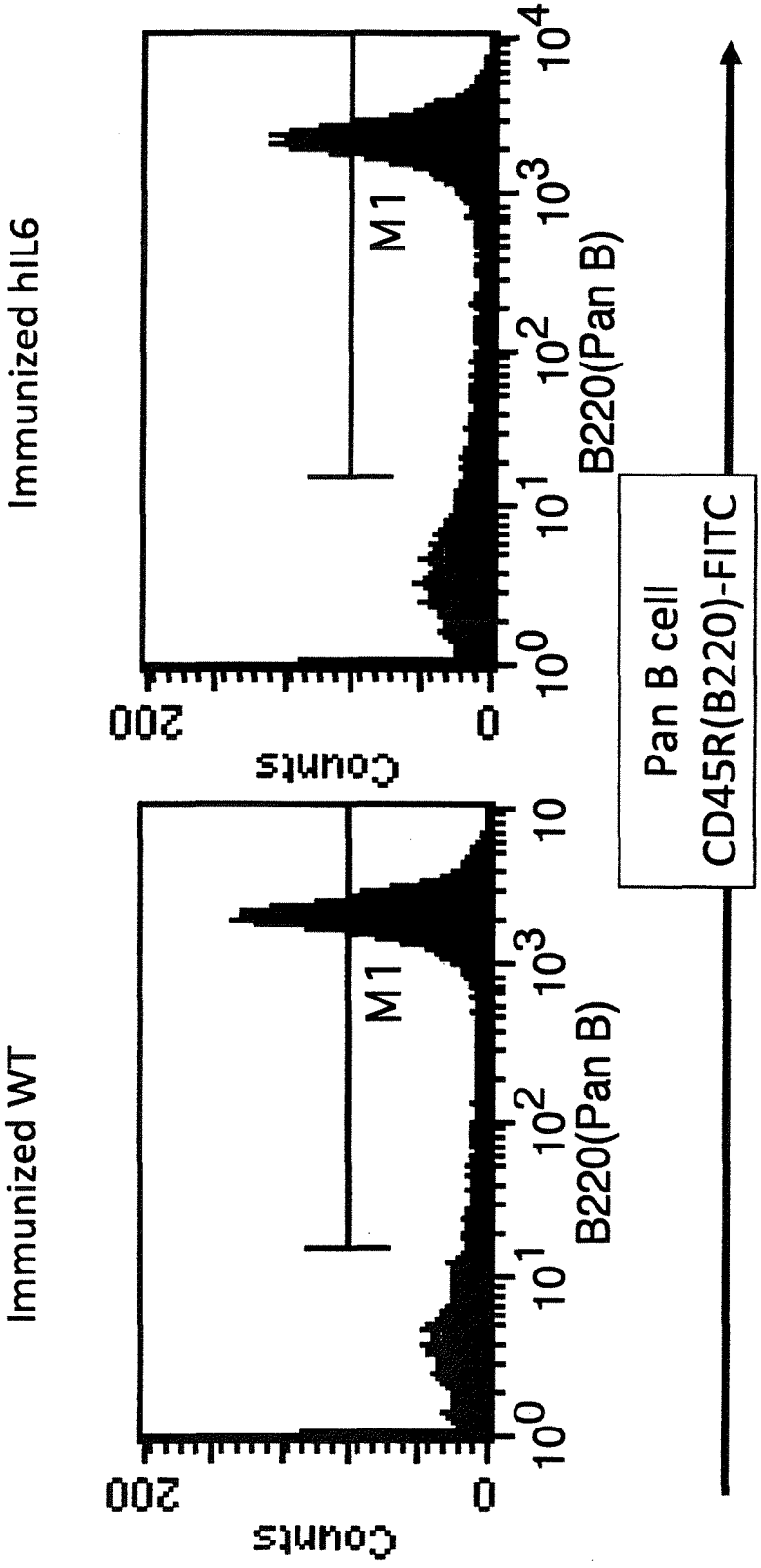

Immunized hIL6
Immunized WT
Counts
200
0
M1
B220(Pan B)
10^0
10^1
10^2
10^3
10^4
10
Pan B cell
CD45R(B220)-FITC

FIG. 4

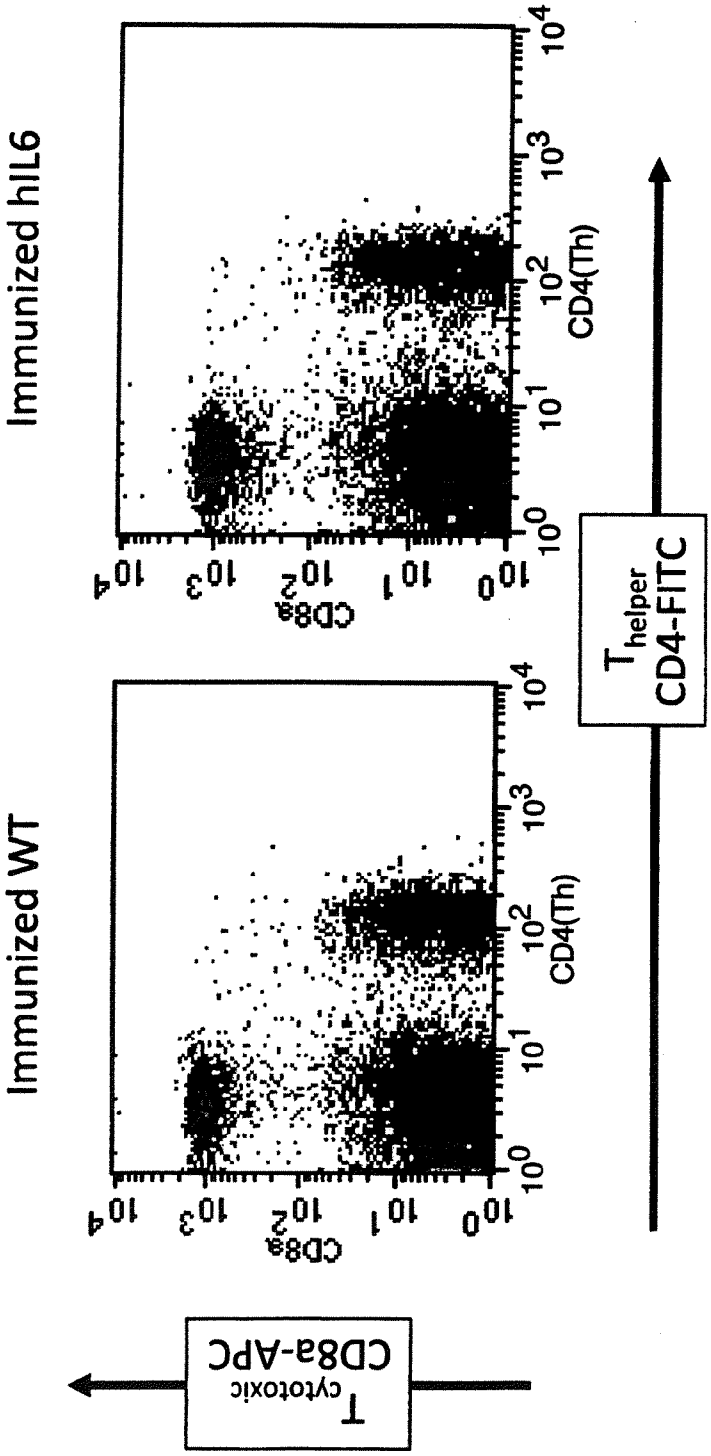

Immunized hIL6
Immunized WT
CD8a
CD4(Th)
10^0 10^1 10^2 10^3 10^4
T_{helper} CD4-FITC
$T_{cytotoxic}$ CD8a-APC

FIG. 5

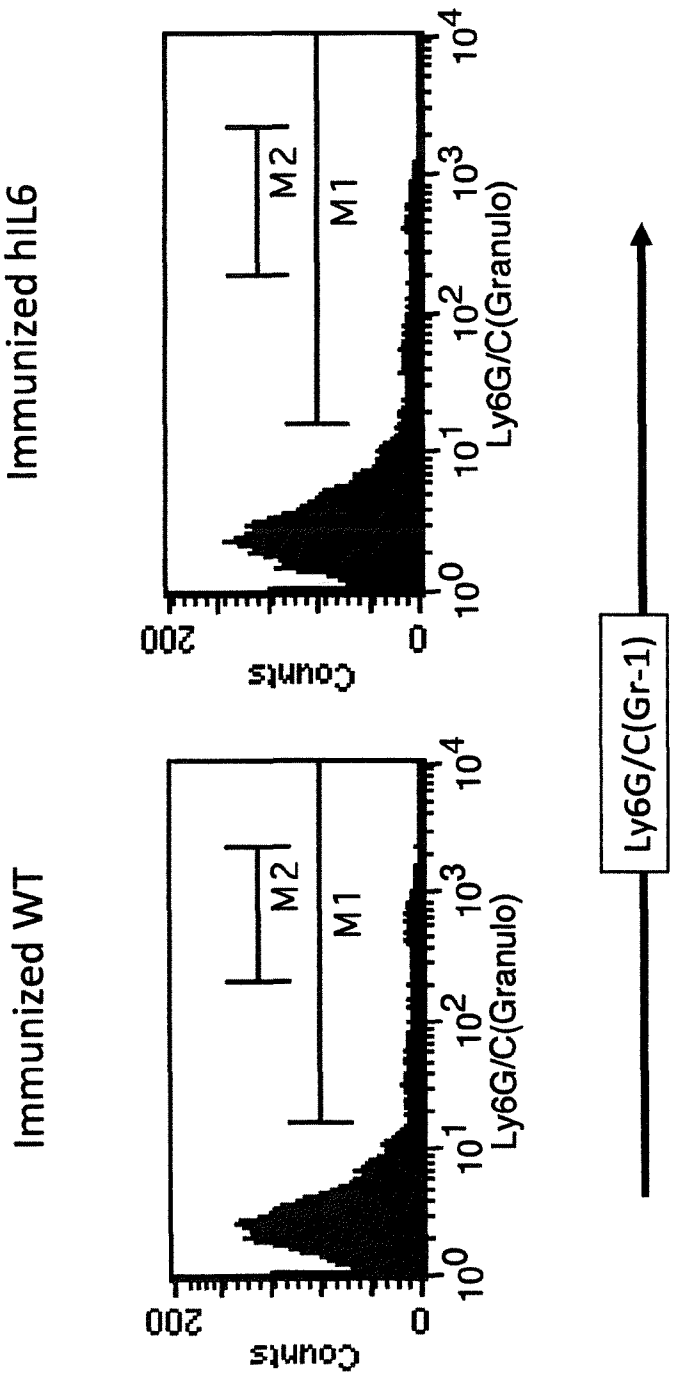

Immunized hIL6
Immunized WT
Counts
200
0
M1
M2
Ly6G/C(Granulo)
10^0
10^1
10^2
10^3
10^4
Ly6G/C(Gr-1)

FIG. 6

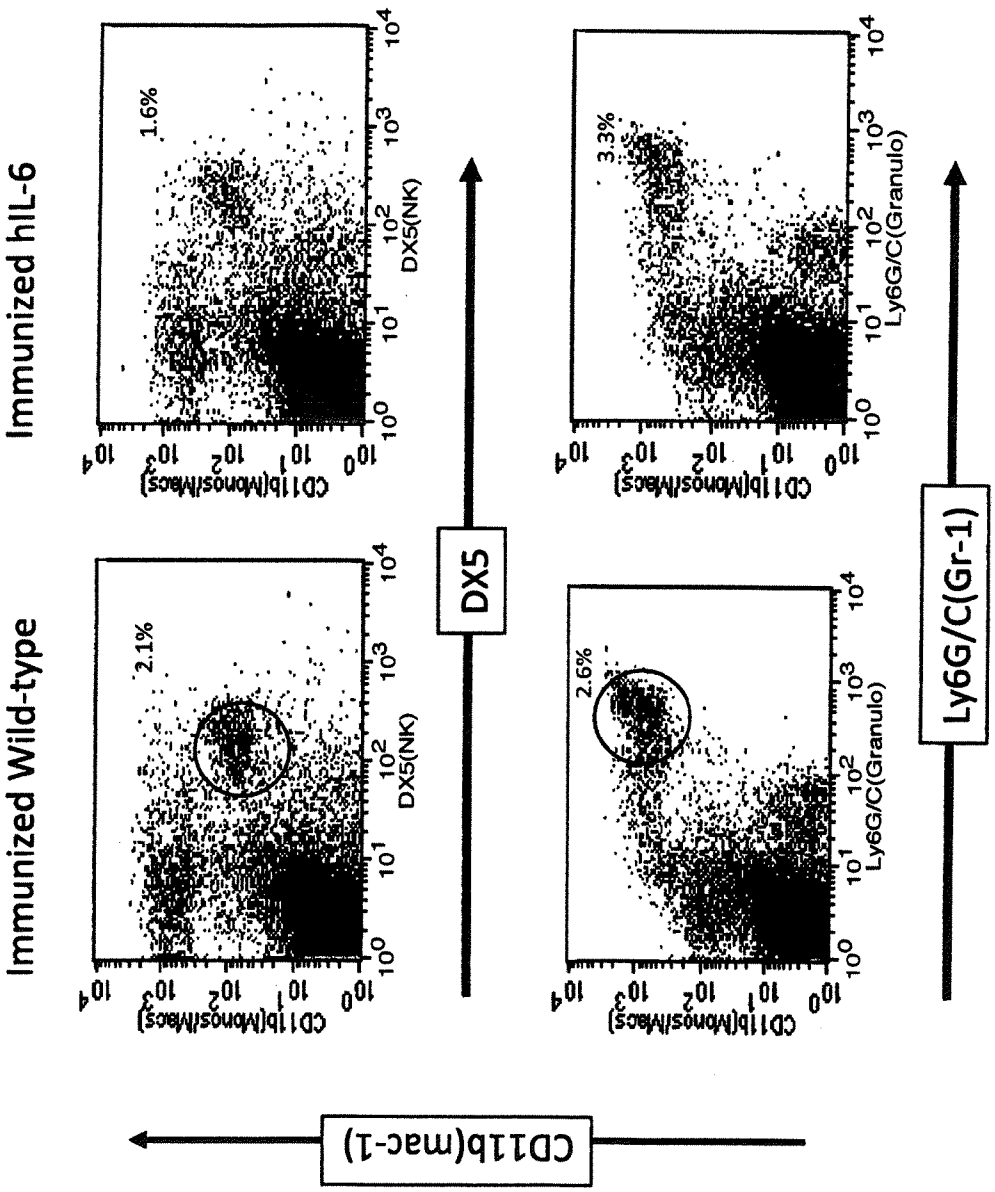

Immunized hIL-6
Immunized Wild-type
1.6%
2.1%
3.3%
2.6%
DX5(NK)
Ly6G/C(Granulo)
CD11b(Monos/Macs)
DX5
Ly6G/C(Gr-1)
CD11b(mac-1)

FIG. 7

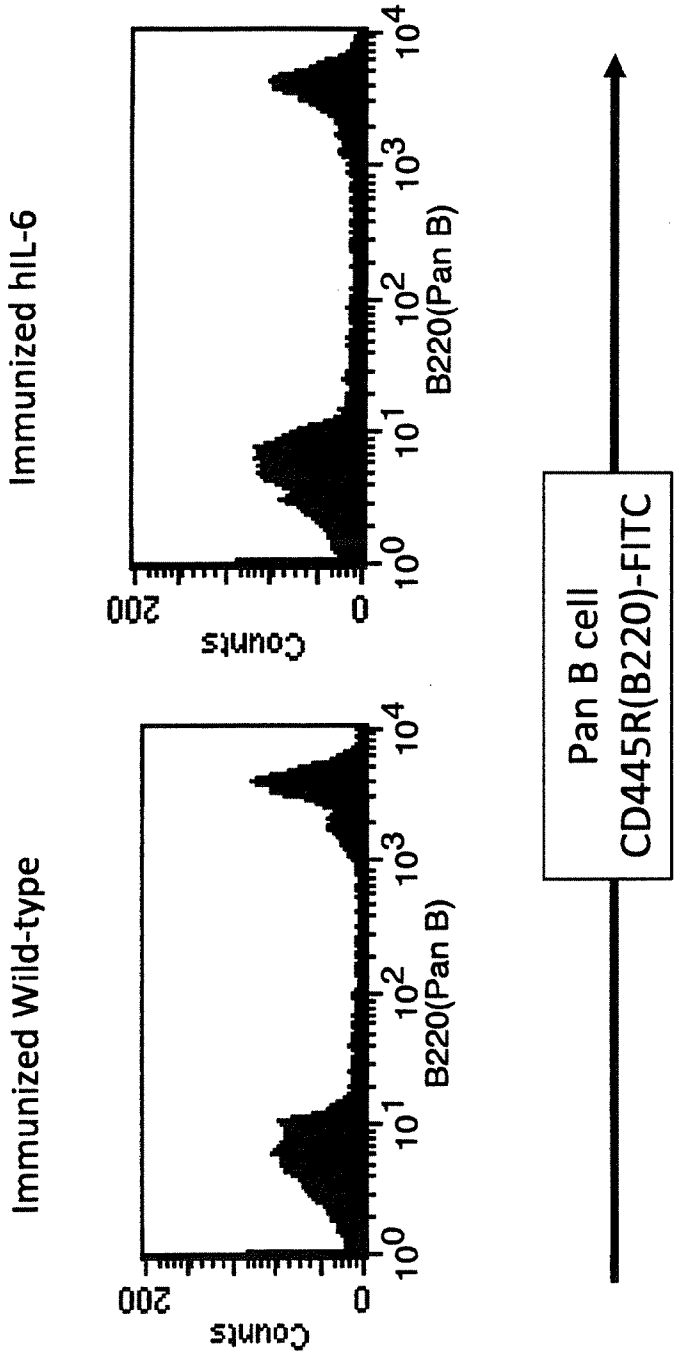

Immunized hIL-6
Counts
200
0
B220(Pan B)
10^0
10^1
10^2
10^3
10^4
Immunized Wild-type
Counts
200
0
B220(Pan B)
10^0
10^1
10^2
10^3
10^4
Pan B cell
CD445R(B220)-FITC

FIG. 8

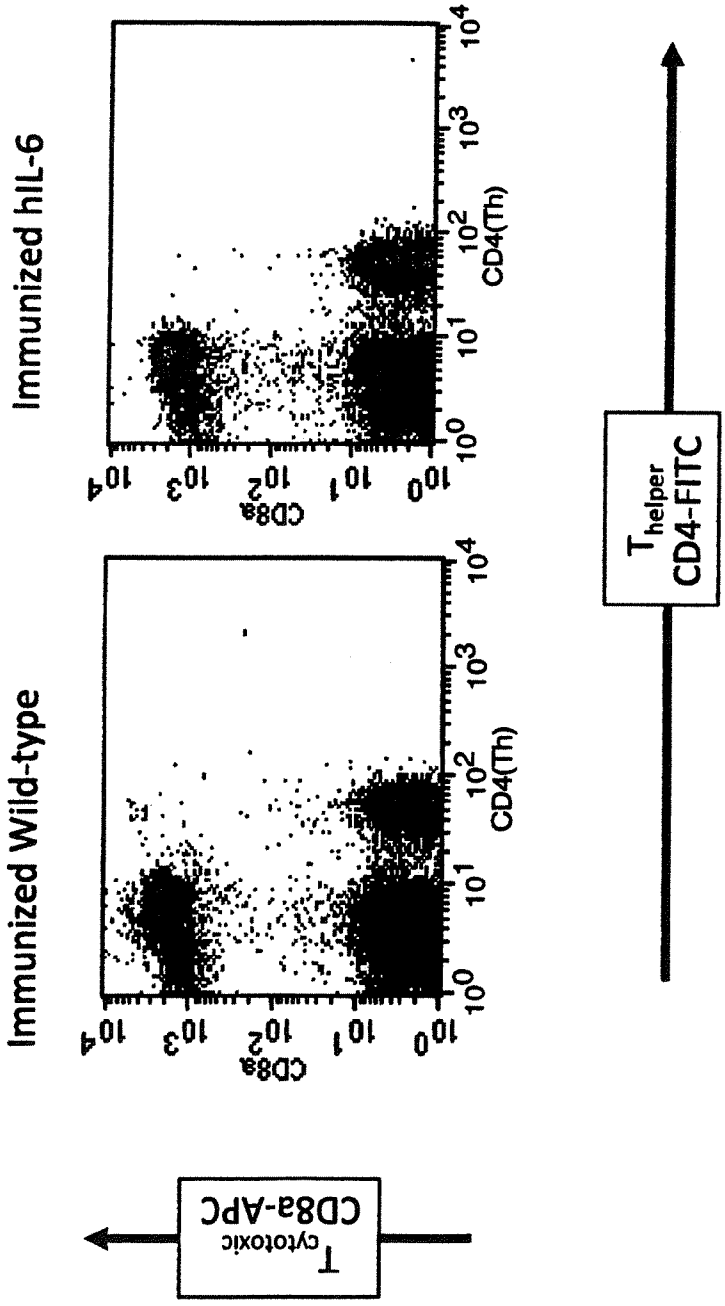

Immunized hIL-6
Immunized Wild-type
CD8a
CD4(Th)
10^0
10^1
10^2
10^3
10^4
$T_{cytotoxic}$ CD8a-APC
T_{helper} CD4-FITC

FIG. 9

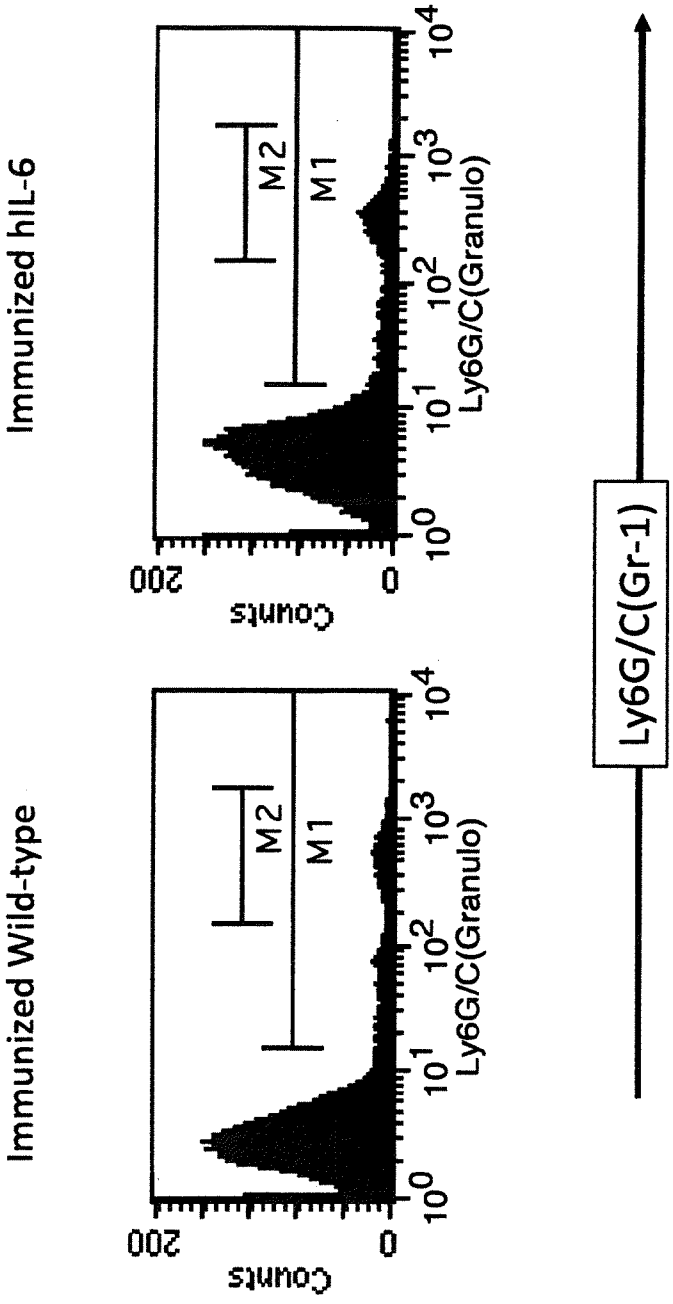

Immunized hIL-6
Immunized Wild-type
Counts
200
0
M1
M2
Ly6G/C(Granulo)
10^0
10^1
10^2
10^3
10^4
Ly6G/C(Gr-1)

FIG. 10

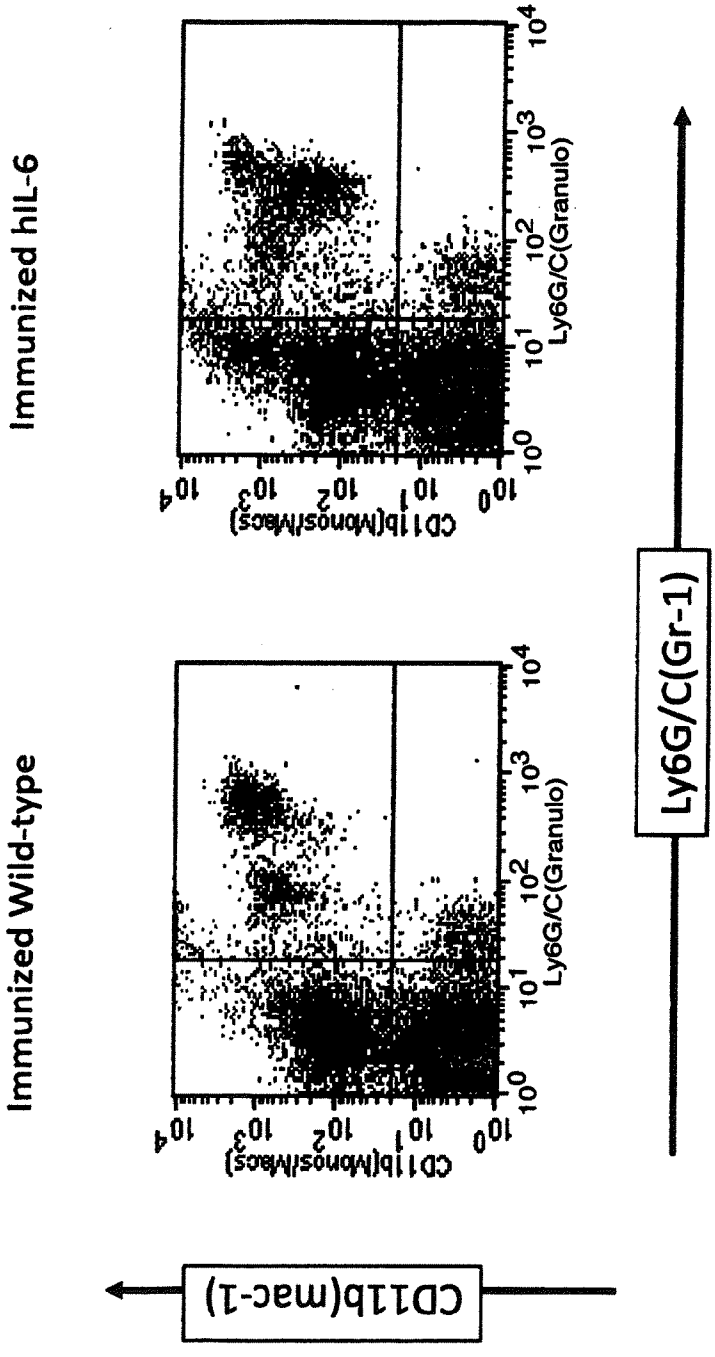

Immunized hIL-6
Immunized Wild-type
CD11b(Monos/Macs)
Ly6G/C(Granulo)
CD11b(mac-1)
Ly6G/C(Gr-1)

FIG. 11

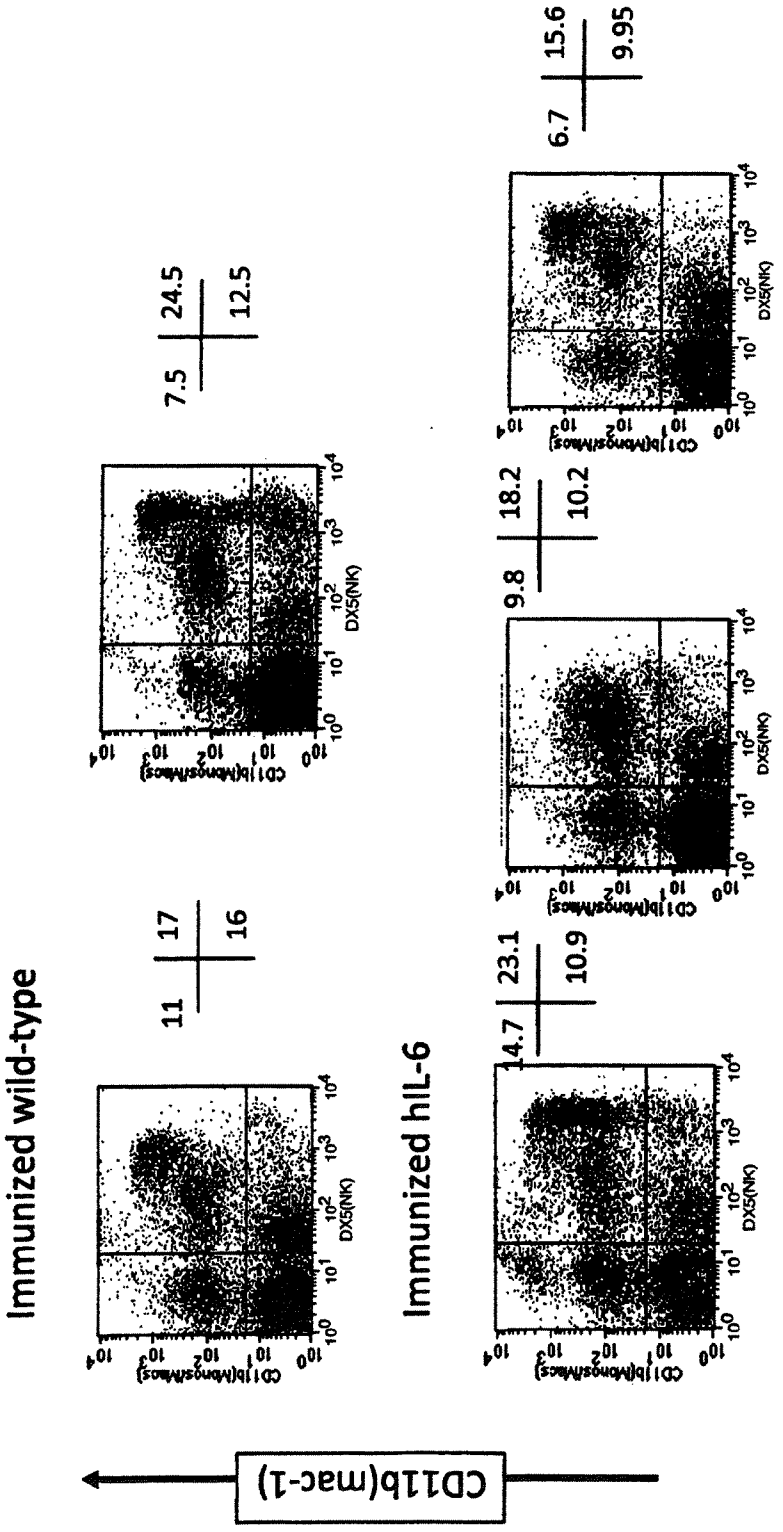

Immunized wild-type
Immunized hIL-6
CD11b(mac-1)
DX5
11
17
16
7.5
24.5
12.5
14.7
23.1
10.9
9.8
18.2
10.2
6.7
15.6
9.95
CD11b(Mac-1)
DX5(NK)

FIG. 12

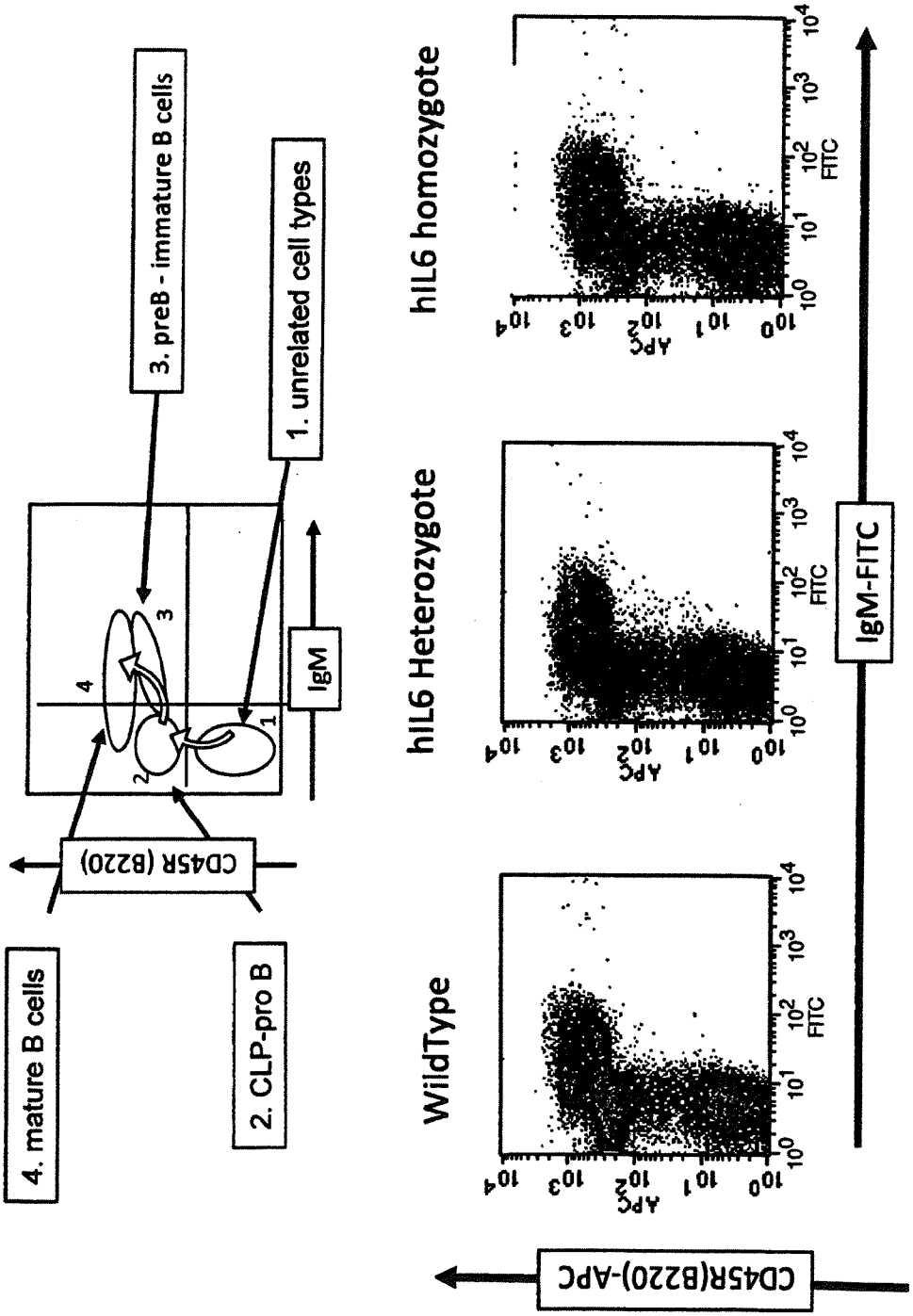

4. mature B cells
2. CLP-pro B
CD45R (B220)
IgM
1. unrelated cell types
3. preB - immature B cells
1
2
3
4
WildType
hIL6 Heterozygote
hIL6 homozygote
CD45R(B220)-APC
IgM-FITC
APC
FITC

FIG. 13

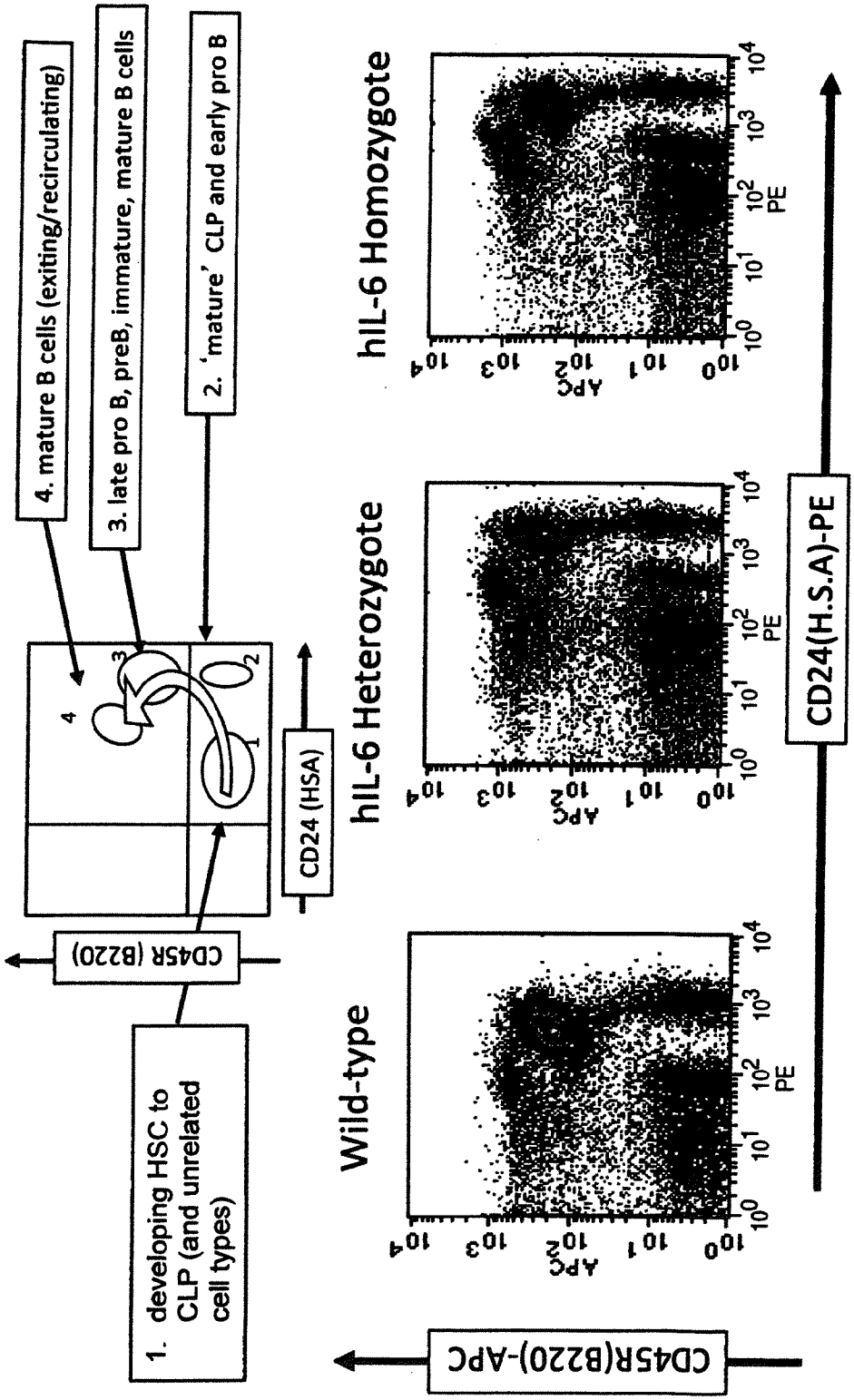

4. mature B cells (exiting/recirculating)
3. late pro B, preB, immature, mature B cells
2. 'mature' CLP and early pro B
1. developing HSC to CLP (and unrelated cell types)
CD45R (B220)
CD24 (HSA)
Wild-type
hIL-6 Heterozygote
hIL-6 Homozygote
CD45R(B220)-APC
CD24(H.S.A)-PE
APC
PE

FIG. 14

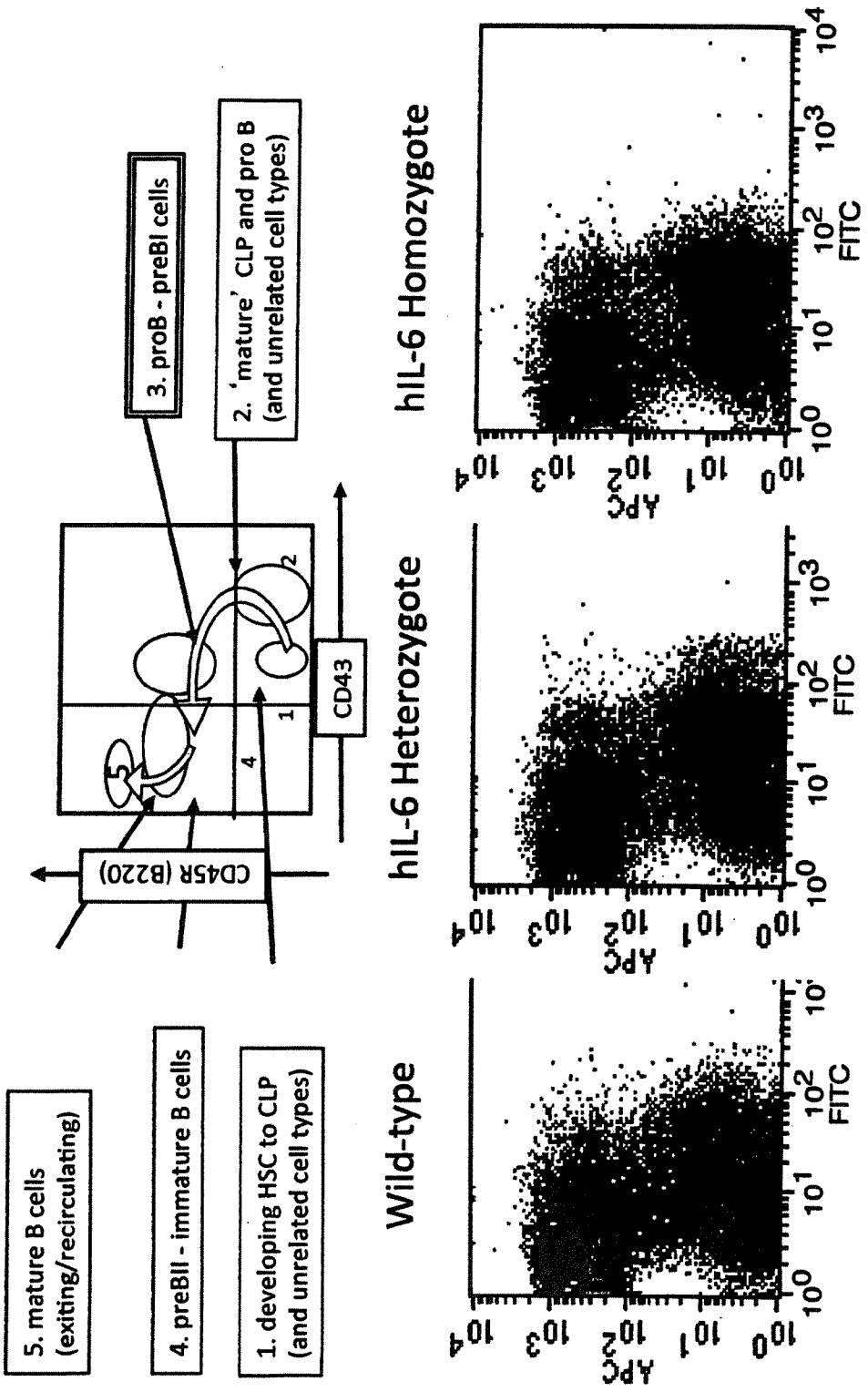

5. mature B cells (exiting/recirculating)
4. preBII - immature B cells
1. developing HSC to CLP (and unrelated cell types)
3. proB - preBI cells
2. 'mature' CLP and pro B (and unrelated cell types)
CD45R (B220)
CD43
1
2
3
4
Wild-type
hIL-6 Heterozygote
hIL-6 Homozygote
APC
FITC

FIG. 15

REFERENCES CITED IN THE DESCRIPTION

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EP2818478

HUMANIZÁLT IL-6 ÉS IL-6 RECEPTOR

SZABADALMI IGÉNYPONTOK

1. Genetikailag módosított rágcsáló, amely IL-6-ot kódoló rágcsálógén endogén rágcsáló-IL-6-lókuszán humán IL-6-ot kódoló humán génnel való helyettesítést tartalmaz, ahol a humán IL-6-ot kódoló humán gén az endogén rágcsáló-IL-6-lókuszon lévő endogén rágcsáló-szabályozóelemek szabályozása alatt áll.

2. Az 1. igénypont szerinti, genetikailag módosított rágcsáló, amelyben a humán IL-6-ot kódoló humán gén tartalmazza a CTD-2369M23 mesterséges bakteriális kromoszómán található humán IL-6-gén 1-5. exonjait.

3. Az 1. igénypont szerinti, genetikailag módosított rágcsáló, amely humanizált IL-6Rα-t expresszál, ahol az endogén rágcsáló-IL-6Rα-gén humán IL-6Rα ektodoménjét kódoló szekvenciát tartalmazó humán szekvenciával helyettesített.

4. Az 1. igénypont szerinti genetikailag módosított rágcsáló, amely nem mutat a következők közül választott jellemzőt: plazmocitózis, glomeruloszklerózis, glomerulonefritisz, veseelégtelenség, hipergammaglobulinémia, emelkedett megakariocita-szint a lépben, emelkedett megakariocita-szint a csontvelőben, lépmegnagyobbodás, nyirokcsomó megnagyobbodása, kompakt, abnormális plazmasejtek és kombinációik.

5. A 3. igénypont szerinti genetikailag módosított rágcsáló, ahol a humanizált IL-6Rα rágcsáló-transzmembrán- és intracelluláris domént tartalmaz.

6. Genetikailag módosított rágcsáló, amely endogén rágcsáló-IL-6Rα-gén humanizált változatát tartalmazza, ahol a humanizálás rágcsáló-IL-6Rα-ektodomént kódoló szekvencia humán IL-6Rα ektodomént kódoló szekvenciával való helyettesítését foglalja magában az endogén rágcsáló-IL-6Rα-lókuszon, és ahol a humanizált IL-6Rα-gén endogén rágcsáló-szabályozóelemek szabályozása alatt áll.

7. A 6. igénypont szerinti genetikailag módosított rágcsáló, amely IL-6-ot kódoló rágcsáló gén endogén rágcsálóIL-6 lókuszán humán IL-6-ot kódoló humán génnel való helyettesítést tartalmazó humanizált IL-6 gént tartalmaz.

8. Eljárás humanizált rágcsáló előállítására, amely magában foglalja a rágcsáló-IL-6-ot kódoló rágcsáló-génszekvencia helyettesítését humán IL-6-ot kódoló humán génnel oly módon, hogy a humán IL-6-gén endogén rágcsáló-szabályozóelemek szabályozása alatt áll.

9. Eljárás humanizált rágcsáló előállítására, amely magában foglalja valamennyi rágcsáló-IL-6Rα-ektodomén szekvenciáját kódoló rágcsálóexon helyettesítését humán IL-6Rα-ektodomént kódoló humán genomfragmenssel, ezáltal humanizált IL-6Rα-gén kialakítását, ahol a humanizált IL-6Rα-gén endogén rágcsáló-szabályozóelemek szabályozása alatt áll.

10. Genetikailag módosított rágcsáló, amely rágcsálóektodomént kódoló szekvencia humán ektodomén-szekvenciával való helyettesítését tartalmazó humanizált IL-6Rα-gént tartalmaz, ahol a humanizált IL-6Rα-gén rágcsáló-transzmembrán- és intracelluláris domén szekvenciát tartalmaz, ahol a rágcsáló humán IL-6-ot kódoló gént is tartalmaz, és ahol a humán IL-6-ot és a humanizált IL-6Rα-t kódoló gének endogén rágcsáló-szabályozóelemek szabályozása alatt állnak.