Human hematopoietic stem cell maintenance and myeloid cell development in next-generation humanized mouse models

Trisha R. Sippel,1,2 Stefan Radtke,1,3 Tayla M. Olsen,1,2 Hans-Peter Kiem,1,3-5 and Anthony Rongvaux1,2,6,7

1Clinical Research Division, 2Program in Immunology, and 3Stem Cell and Gene Therapy Program, Fred Hutchinson Cancer Research Center, Seattle, WA; 4Department of Medicine and 5Department of Pathology, University of Washington School of Medicine, Seattle, WA; 6Immunotherapy Integrated Research Center, Fred Hutchinson Cancer Research Center, Seattle, WA; and 7Department of Immunology, University of Washington, Seattle, WA

Key Points

- Next-generation humanized mice differentially support human HSPC maintenance and myelopoiesis.
- MISTRG mice support long-term human HSPC maintenance demonstrated by quaternary transplantation and development of human tissue macrophages.

Introduction

Mice repopulated with a human hematopoietic system provide valuable tools for in vivo studies of human hematopoiesis and immunity.1-3 Such humanized mice are generated by transplantation of human CD34+ (hCD34+) hematopoietic stem and progenitor cells (HSPCs) into preconditioned, immunodeficient mice.4,5 Prkdcscid mutation or Rag deficiency make recipient mice devoid of T and B lymphocytes. They also lack natural killer cells because of inactivation of the Il2rg gene, essential for interleukin-15 (IL-15)–dependent natural killer cell development.5 Mouse-to-human phagocytic tolerance is achieved by expression of the NOD-specific variant of the mouse Sirpa gene or human SIRPA gene, which encode signal regulatory protein α (SIRPα) “don’t eat me” receptors that bind human CD47.7,8 Conventional humanized mouse models (NSG: NOD Scid Il2rg−/−9,10; SRG, hSIRPA Rag2−/−Il2rg−/−8,11) present incomplete replacement of the hematopoietic system and inefficient human myelopoiesis.1 Thus, improved models have recently been developed.

We have compared human HSPC maintenance and myelopoiesis in 3 of the newer models: NSG-SGM3 (also designated NSGS), NSGW41, and MISTRG. NSGS transgenic mice support human myelopoiesis by supplying supraphysiological concentrations of 3 cytokines: stem cell factor (SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-3.12-14 NSGW41 mice favor human engraftment by reducing mouse HSPCs through the W41/W41 inactivating mutation of the Kit gene, thus opening the bone marrow (BM) niche to human HSPC engraftment and differentiation. This strategy achieves a form of genetic preconditioning and alleviates the requirement for pretransplantation irradiation.15 MISTRG mice combine genetic preconditioning and cytokine-mediated support through knockin gene replacement, removing mouse cytokine-encoding genes and replacing them with their human counterparts. As a result, MISTRG mice express physiological concentrations of 4 human cytokines: macrophage colony-stimulating factor (M-CSF), IL-3, GM-CSF, and thrombopoietin.16-21

Methods

NSGS13 and NSGW4115 mice were obtained from The Jackson Laboratory. MISTRG mice were previously reported.20,21 CD34+ cells were transplanted intrahepatically in newborn mice4,5,20; the mice were bled at several time points, and euthanized and analyzed 22 weeks after transplantation, as approved by the Institutional Animal Care and Use Committee (50941). Details are provided in the supplemental Methods.

Results and discussion

We transplanted fetal hCD34+ cells intrahepatically into newborn recipient mice by following a standard protocol.4,5 We preconditioned NSGS and MISTRG mice with an 80-cGy dose of gamma irradiation. Because NSGW41 mice do not require preconditioning,15 we performed transplantation for NSGW41 mice and a second group of MISTRG mice without using irradiation. Engraftment was successful (at >10% hCD45+ cells in the blood 15 weeks after transplantation) in almost all (82%-100%) animals in all 4 experimental groups (supplemental Figure 1). NSGS mice supported high-level chimerism as early as 10 weeks after transplantation, with >75% hCD45+ cells in the blood; human cell
Figure 1. NSGS, NSGW41, and MISTRG mice support high-level multilineage human hematopoietic development but show differential maintenance of functional HSCs. Newborn mice were preconditioned (80 cGy) or not (0 cGy), and fetal CD34+ cells were transplanted by intrahepatic injection. (A) Blood human CD45+ immune cell chimerism measured over time in successfully engrafted mice. The dotted line indicates 10% engraftment; mice below this threshold at 15 weeks were excluded from the analysis. Error bars indicate mean ± standard deviation (SD) (n = 7-23; ****P < .0001 for NSGS vs all groups of mice at week 10; **P = .0248 for NSGS vs MISTRG mice [80 cGy]; and P = .0046 for NSGS vs MISTRG mice [0 cGy]). (B) Frequency and (C) absolute numbers of hCD45+ cells in the BM of recipient mice. Each symbol represents an individual mouse, and the bars indicate (B) mean ± SD or (C) geometric mean ± geometric SD (NSGS mice, n = 4; irradiated MISTRG [80 cGy] mice, n = 12; NSGW41 mice, n = 9; nonirradiated MISTRG [0 cGy] mice, n = 9; one-way ANOVA with Tukey’s multiple comparison test). (D) Composition of human white blood cells 10 weeks after transplantation in the same mice as in panel A (n = 7-23; error bars indicate mean ± standard error of the mean [SEM]). (E) Red blood cell (RBC) counts in the blood of nonhumanized (nonirradiated and noninjected) or successfully humanized mice (from panel A) 10 weeks after transplantation (n = 3-7 for nonhumanized mice; n = 7-23 for humanized mice; unpaired Student t test comparing NSGS and NSGW41 nonhumanized with humanized mice; one-way ANOVA with Tukey’s multiple comparison test).
chimerism steadily increased to ~80% at the 22-week end point in the other groups (Figure 1A). Similarly, the BM of most recipients contained >80% hCD45^+ cells (Figure 1B), with higher absolute hCD45^+ cell counts in the BM of NSGW41 mice (Figure 1C). All strains exhibited multilineage immune cell differentiation (Figure 1D), including T and B lymphocytes, and varying frequencies of CD33^+ myeloid cells (described below).

Mouse RBCs are susceptible to in vivo destruction by human phagocytes, resulting in anemia. The presence of a human immune system had only mild or negligible effects on RBC counts in NSGS and NSGW41 mice but resulted in anemia in MISTRG mice (Figure 1E). However, there was no significant difference in the long-term (~22 weeks) survival of the 4 groups of humanized mice (Figure 1F). This observation is in contrast to the original
Figure 2. NSGS, NSGW41, and MISTRG mice differentially support the development of human myeloid cell lineages. (A) Representative flow cytometry analysis and (B) frequency of human myeloid CD33\(^+\) cells populations in the BM of recipient mice 22 weeks after engraftment, distinguishing granulocytic (CD33\(^+\)SSC\(^{-}\)) cells and monocytic (CD33\(^+\)SSC\(^{+}\)) cells based on live 7-AAD\(^-\)mTer119\(^+\)hCD45\(^-\)CD45\(^+\) cells. (A) Numbers and (B) bars indicate frequencies among hCD45\(^+\) cells expressed as mean ± SD (NSGS mice, n = 4; irradiated MISTRG mice, n = 12; NSGW41 mice, n = 9; nonirradiated MISTRG mice: n = 9). Representative flow cytometry analysis and (D) frequency of human myeloid CD33\(^+\) cells in the blood of recipient mice from panel A compared with human healthy donor blood (n = 6).

(E) Representative flow cytometry analysis and (F) frequency of blood human neutrophils (CD66\(^b\)CD16\(^+\)) within the CD33\(^+\)SSC\(^{+}\) population shown in the blue gate in panel C. The insets show representative images of sorted human CD33\(^+\)SSC\(^{+}\) granulocytic cells stained by Diff-Quik (scale bar, 20 μm). Representative images of sorted human CD33\(^+\)SSC\(^{+}\) monocytes of the indicated CD14/CD16 phenotype stained by Diff-Quik (scale bar, 20 μm). (I) Representative images of sorted human CD33\(^+\)SSC\(^{+}\) monocytes of the indicated CD14/CD16 phenotype stained by Diff-Quik (scale bar, 20 μm).

Frequency of cytokine-producing monocytes among human blood or MISTRG mouse blood cells measured by intracellular cell staining for tumor necrosis factor α (TNFα) and IL-6 after ex vivo stimulation with lipopolysaccharide (LPS; 100 ng/mL) or R848 (10 μg/mL) for 24 hours. (K) Tissue macrophages in the lungs and livers of recipient mice compared with normal human tissues, as identified by immunohistochemistry for human CD68. Histograms (B,D,F,H,J) represent mean ± SD.
description of MISTRG mice, in which anemia was lethal when engraftment reached ~50%.20 Here, we are using a lower dose of radiation (80 cGy instead of 150 cGy), and we are transplanting fewer fetal CD34+ cells (20 000-50 000 instead of 100 000 cells). We also observed MISTRG mice by embryo transfer, which may have eliminated a pathobiologic anemia-related lethality.

In humans, the BM CD34+ cell fraction contains human hematopoietic stem cells (HSCs) capable of life-long self-renewal and multilineage differentiation.22 As previously reported,12 the transgenic overexpression of human cytokines in NSGS mice induces HSC exhaustion, and human CD34+ cells are rare in the BM of these humanized mice (Figure 1G-H). In contrast, the human LinCD34+CD38low population, known to be enriched in HSCs,23 is present in comparable numbers in the BM of NSGW41 and MISTRG mice (Figure 1G-H; supplemental Figure 2). The stem and progenitor properties of CD34+ cells can be functionally assessed in vitro in colony-forming unit (CFU) assays and in vivo by serial transplantation. Because of the quasi-absence of CD34+ cells in NSGS mice, we performed these assays with total BM hCD45+ cells isolated from irradiated humanized NSGS and MISTRG mice. Human BM cells from NSGS mice had almost undetectable capacity to form in vitro CFUs or repopulate secondary recipients (Figure 1I-J). In contrast, BM hCD45+ cells from MISTRG mice efficiently formed CFUs, including CD45+CD66+CD16− monocytes/macrophages, megakaryocytes (CFU-GEMM) (Figure 1I), and total BM hCD45+ cells (Figure 1J) or purified hCD45+ cells (Figure 1K) efficiently engrafted secondary recipients. Because high frequencies of BM hCD34+ cells were present in the BM of the latter group, we serially transplanted those mice and were able to detect multilineage hCD45+ cells up to quaternary recipients (Figure 1K), demonstrating long-term maintenance of human HSCs in preconditioned MISTRG mice. When comparing NSGW41 with MISTRG mice (0 cGy), we observed that BM CD34+ cells from both recipient strains had similar functional properties in a CFU assay (Figure 1L). In vivo, CD34+ cells from NSGW41 mice showed a trend toward more efficient secondary transplantation compared with CD34+ cells from nonirradiated MISTRG primary recipients (Figure 1M).

Next, we characterized human CD33+ myeloid cell populations. The BM contained granulocytic (CD33+SSC+) and monocytic (CD33+Ssc−) cells in all 4 models (Figure 2A-B). Peripheral granulocytes were defective in all strains (Figure 2C-F; supplemental Figure 3A-B). In NSGS mice, a poorly differentiated population lacking most markers (CD33+CD66+CD16+) and with predominantly band morphology was expanded. In NSGW41 mice, the frequency of blood granulocytes was extremely low, but a subset of these cells presented a mature immunophenotype (CD33+CD66−CD16+) and had segmented nuclei. Human monocytes (CD33+Ssc+) (Figure 2G-H; supplemental Figure 3C-D) expressed abnormally low or undetectable levels of CD14 and no CD16 in NSGS mice. In MISTRG mice, the 3 conventional subsets of monocytes, defined by CD14 and CD16 expression,24 were present but in proportions different than in human physiology. The morphology of each monocyte subset, isolated from the blood of MISTRG mice, resembled that of the corresponding cells in human healthy donor blood (Figure 2I). Furthermore, when stimulated in vitro with the toll-like receptor agonists lipopolysaccharide or R848, the cytokine response of MISTRG mouse and healthy donor blood monocytes was comparable (Figure 2J). CD14+ monocytes were present at lower frequencies in NSGW41 mice than in MISTRG mice, and they matured to CD16+ cells only at late time points (≥22 weeks; supplemental Figure 3D). Likely because of the transgenic overexpression of SCF, which is the ligand for CD117, mast cells (CD117+FcεR1+CD203clow cells) are expanded in NSGW41 mice (supplemental Figure 3E) but are barely detectable in blood of other mice and healthy humans. Finally, CD68+ tissue macrophages were absent in NSGW41 mice, present in sparse patches in NSGS mice, and were present at densities similar to those in human tissues in MISTRG mice (Figure 2K).

In conclusion, NSGS mice can be used for mast cell studies,25 but they are defective in HSC maintenance and myeloid cell maturation; NSGW41 mice are suitable models for human HSC studies; and MISTRG mice support engraftment and maintenance of HSPCs of fetal, newborn, and adult origins (supplemental Figure 4) and proliferation of human HSCs with immune properties in a CFU assay (Figure 3A-B). In NSGS mice, a poorly differentiated population lacking most markers (CD33+CD66+CD16+) and with predominantly band morphology was expanded. In NSGW41 mice, the frequency of blood granulocytes was extremely low, but a subset of these cells presented a mature immunophenotype (CD33+CD66+CD16+) and had segmented nuclei. Human monocytes (CD33+Ssc+) (Figure 2G-H; supplemental Figure 3C-D) expressed abnormally low or undetectable levels of CD14 and no CD16 in NSGS mice. In MISTRG mice, the 3 conventional subsets of monocytes, defined by CD14 and CD16 expression,24 were present but in proportions different than in human physiology. The morphology of each monocyte subset, isolated from the blood of MISTRG mice, resembled that of the corresponding cells in human healthy donor blood (Figure 2I). Furthermore, when stimulated in vitro with the toll-like receptor agonists lipopolysaccharide or R848, the cytokine response of MISTRG mouse and healthy donor blood monocytes was comparable (Figure 2J). CD14+ monocytes were present at lower frequencies in NSGW41 mice than in MISTRG mice, and they matured to CD16+ cells only at late time points (≥22 weeks; supplemental Figure 3D). Likely because of the transgenic overexpression of SCF, which is the ligand for CD117, mast cells (CD117+FcεR1+CD203clow cells) are expanded in NSGW41 mice (supplemental Figure 3E) but are barely detectable in blood of other mice and healthy humans. Finally, CD68+ tissue macrophages were absent in NSGW41 mice, present in sparse patches in NSGS mice, and were present at densities similar to those in human tissues in MISTRG mice (Figure 2K).

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Authorship

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ORCID profiles: T.R.S., 0000-0003-3439-3239; S.R., 0000-0002-2842-4820; H.-P.K., 0000-0001-5949-4947; A.R., 0000-0001-7142-6533.

Correspondence: Anthony Rongvaux, Fred Hutchinson Cancer Research Center, Clinical Research Division–Program in Immunology, 1100 Fairview Ave N, Mail Stop D3-100, Seattle, WA 98109; e-mail: rongvaux@fredhutch.org.
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