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Advances in human myeloid-engrafting NSG-SGM3 and MISTRG mice

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Humanized mouse models with transgenic expression of human myelopoiesis-supporting growth factors have enhanced human myeloid cell engraftment and improved the study of human innate immune responses. Here, we discuss the remaining challenges associated with studying innate immunity in humanized NSG-SGM3 and MISTRG mice, as well as potential advances to overcome them.

Studying innate immunity in humanized myeloid-engrafting immunodeficient mice remains challenging

Species-specific differences between humans and mice hamper translational research, particularly in innate immunology. Studies of innate immunity in humanized mice (i.e., immunodeficient mice engrafted with a human immune system; Box 1) were initially limited due to challenges in engrafting human myeloid cells [1]. The transgenic expression of human myelopoiesis-supporting growth factors in multiple immunodeficient backgrounds promoted significantly improved human myeloid engraftment [1]. While this has allowed more detailed study of human innate immune responses to cancer, sterile inflammation, and infection, considerable challenges remain. Here, we discuss the effect of these challenges on infection-induced innate immunity in humanized NSG-SGM3 and MISTRG mice (Box 1), to date the two most commonly used models, highlighting current and potential future refinements to overcome them. While these two models form the main focus of this article, similar strategies have been applied with comparable success in other immunodeficient backgrounds, such as NOG strains (for more information, see [1]).

Incomplete development of the human immune system

Despite improved engraftment in transgene-expressing humanized mouse models, the human immune system remains incomplete, with some cells failing to engraft efficiently and others displaying functionally immature phenotypes [1]. For example, although human granulocytes, including mast cells, eosinophils, and basophils, do engraft in humanized mice, human neutrophils engraft poorly. Given their abundance in human peripheral blood and key roles in the innate immune response to infection, this significantly impacts the human immune response in these models. Recent enhancements to the MISTRG line, humanizing *GCSF* while simultaneously ablating the murine G-CSF receptor to generate MISTRGGR mice, have enabled improved engraftment of mature, functional human neutrophils [2]. Nevertheless, neutrophils still constitute a considerably smaller proportion of peripheral blood immune cells in humanized MISTRGGR mice than in humans. Furthermore, although the engrafted neutrophils mount functional immune responses, they remain incapable of fully clearing a *Pseudomonas aeruginosa* infection, highlighting the need for further improvement [2].

Cells of the erythroid and megakaryocyte lineages also fail to engraft efficiently in most humanized mouse models. While not considered classical immune cells, these cells have key roles in the pathogenesis of multiple infections; for example, platelets are particularly important in the severe stages of Dengue virus (DENV) infection. Platelet engraftment can be improved by ablation of the KIT receptor, as in NSGW41 mice. This has the additional benefit of depleting the murine hematopoietic stem cell pool, thus enabling human immune reconstitution in the absence of preconditioning [3]. Furthermore, additional supplementation with 17β-estradiol promotes the development of secondary lymphoid tissue upon humanization in this NSGW41 background, further enhancing human immune function [4]. Therefore, the recent introduction of this KIT receptor mutation onto the NSG-SGM3 background, creating NSG-SGM3-W41 mice, may provide an advanced model to study human immunity [5]. Similarly, NSG-QUAD mice, which additionally express human M-CSF on the NSG-SGM3 background, display improved engraftment of human monocytes [6]. Unlike their NSG-SGM3 predecessors, NSG-QUAD mice engraft human monocytes resembling all three of the known human monocyte subsets. These mice are also capable of inducing potent human proinflammatory responses to various stimuli, indicating that they may be suitable models of monocyte/macrophage-mediated immunity [6].

Although the above strategies improve overall engraftment of the human immune system, full immune functionality remains incomplete due to insufficient crosstalk between innate and adaptive immune cells. In these models, human T cells are educated in the murine thymus, restricting them to murine H2-presented antigens and limiting their communication with human antigen-presenting cells. This prevents humanized mice from mounting a complete human immune response and, therefore, may account for the lack of clinical phenotypes observed in these models upon infection with pathogens such as Ebola virus (EBOV) [7]. Multiple strategies

Box 1. Generation of humanized myeloid-engrafting mouse models

Humanized mice can be generated by engrafting human hematopoietic stem cells into an immunodeficient murine host. Multiple mouse strains can be used, all characterized by genetic deficiencies that impair murine B, T, and/or natural killer (NK) cell development. Functional B and T cell development is abrogated by defective DNA repair caused by the PkrdcSCID mutation or recombination activating gene (RAG) 1 or 2 deficiency, whereas NK cell development is prevented by disrupted IL-15 signaling due to mutations in IL-2 receptor gamma chain (IL-2Rγ). In addition, these mouse strains usually carry a polymorphism in Sirpα [as in non-obese diabetic (NOD) backgrounds] or a human SIRPA transgene to enable recognition of human cells as 'self' [1].

While the combination of these features in strains such as NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG), NOD.Cg-Rag1tm1Mom Il2rgtm1Wjl/SzJ (NRG), and BALB/c $Rag2^{-/-}$IL-$2R\gamma c^{-/-}$ (BRG), enable xenotransplantation, efficient human myeloid engraftment requires expression of human myelopoiesis-promoting growth factors. This has been achieved using two strategies. In NSG-SGM3 mice, human stem cell factor (SCF), granulocyte–macrophage colony-stimulating factor (GM-CSF), and interleukin-3 (IL-3) are transgenically overexpressed on the NSG background. In MISTRG mice, the murine genes encoding macrophage colony-stimulating factor (M-CSF), IL-3, GM-CSF, thrombopoietin, and Sirpα have been humanized on the BRG background, enabling physiological expression [1].

have been developed to enhance innate–adaptive immune crosstalk in humanized mouse models, including engrafting human thymic tissue alongside hematopoietic stem cells and transgenically expressing human major histocompatibility complex (MHC) class I and II genes in place of endogenous murine genes [1]. These strategies may provoke a better human immune response to infection. Indeed, human HLA-A2-expressing NSG-A2 mice more faithfully recapitulate the clinical course of severe EBOV infection compared with NSG-SGM3 mice after human immune engraftment [8]. Based on this, expressing human MHC genes on NSG-SGM3 and MISTRG backgrounds may further improve the function of the engrafted immune system.

One key disadvantage of the improved human immune engraftment in transgene-expressing humanized mouse models is the onset of severe macrophage activation syndrome (MAS), characterized by hyperactivation of the engrafted human immune cells [9]. This particularly affects NSG-SGM3 mice and their derivatives, due to the supraphysiological levels of the human transgene-encoded cytokines. Expression of human transgenes from endogenous loci, as in MISTRG mice, can mitigate this to a large extent [1]. However, for some applications, the high level of human cytokines promotes expansion of relevant immune cell subsets. For example, the supraphysiological levels of SCF in NSG-SGM3 mice enables highly efficient engraftment of mast cells, allowing investigation of human allergic responses [10].

Incomplete host–immune system crosstalk

A second drawback of humanized mouse models is the incomplete crosstalk between the human immune system and murine host cells. This is particularly important when studying the immune response to infection, because tissue–immune cell communication is required for both efficient detection of the pathogen and perpetuation of the immune response. This communication is often absent in humanized mice, due, in part, to incomplete cross-reactivity between human and murine cytokines and their receptors, and in part to an inability of certain viruses to replicate in murine tissue cells [1]. As a result, engrafted human immune cells may be incapable of inducing targeted effects on murine tissues. Conversely, murine tissue cells may be unable to recruit human immune cells following infection. This may account for discrepancies in the pathogenesis of infectious diseases in humans compared with humanized mice, as seen upon EBOV infection in huNSG-SGM3 mice, which does not provoke clinical disease, despite inducing a potent human proinflammatory response [7].

One way to address this is by engrafting human tissues alongside the human immune system. This has been done with both hepatocytes and fetal lung fragments, enabling the investigation of both human tissue- and immune-mediated responses to infection. The dual benefit of this approach is that it allows infection with human-restricted nonhematopoietic targeting pathogens, such as hepatitis viruses in the liver and severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) in the lung [1]. Using these techniques in human myeloid-engrafting mice, as recently done with hepatocyte engraftment in the MISTRG background and lung organoid engraftment in NRGF mice (NRG mice deficient in Flt3, described in more detail below) [11,12], will allow more in-depth study of the human innate immune response to nonhematopoietic cell-targeting infections. Alternatively, expressing human receptors in immunodeficient mice can overcome species barriers, enabling the infection of humanized mice with tissue-targeting, species-restricted viruses. This approach facilitated SARS-CoV-2 infection in humanized MISTRG6 mice (MISTRG mice additionally expressing IL-6) following delivery of human ACE2 to the lungs [13].

Residual murine immunity

The third challenge to studying innate immune responses to infections in humanized mouse models is the confounding effect of residual murine immune responses. Immunodeficient mouse strains used for human xenotransplantation are primarily deficient in adaptive immunity and natural killer (NK) cells [1]. However, these strains retain some innate immune activity. In addition, whereas adaptive immunity is mediated exclusively by hematopoietic cells, nonhematopoietic cells can also mediate innate immune responses. Activation of these residual murine innate

immune responses during infection may interfere with the human immune response, confounding experimental readouts.

Alongside human tissue transplantation models, two additional strategies have been used to decrease the impact of residual murine innate immune responses. The first is depletion of residual murine myeloid cells, which can both improve human immune cell engraftment and enable infection models in humanized mice. For example, Flt3 deficiency in NRG mice (generating NRGF mice) reduces the number of murine dendritic cells and improves human myeloid engraftment when combined with human Flt3L supplementation. This strategy provoked an increased human immune response to the live attenuated yellow fever virus vaccine YFV-17D [14]. The same strategy can be applied using pre-existing myeloid-engrafting immunodeficient mouse strains by pharmacological targeting of residual murine cells, as was done to deplete murine neutrophils in MISTRGGR mice, resulting in a human-specific neutrophil response to *P. aeruginosa* infection [2]. The second strategy used to reduce residual murine immunity is impairing murine innate immune signaling. For example, TLR4 deficiency on the NSG background prevents the murine response to lipopolysaccharide (LPS) [15]. Applying these, or similar strategies to human cytokine-expressing hosts, such as NSG-SGM3 mice, may allow more precise study of human-specific innate immune responses.

Concluding remarks

The development of myeloid-engrafting humanized mouse hosts has significantly advanced the study of human-specific innate immunity. However, acknowledging the challenges that remain allows us to further refine these models to aid unraveling the complexities of innate immunity. To this end, the humanized mouse models presented here (Figure 1) highlight both current and potential strategies to allow a more in-depth characterization of human innate immune responses in the future.

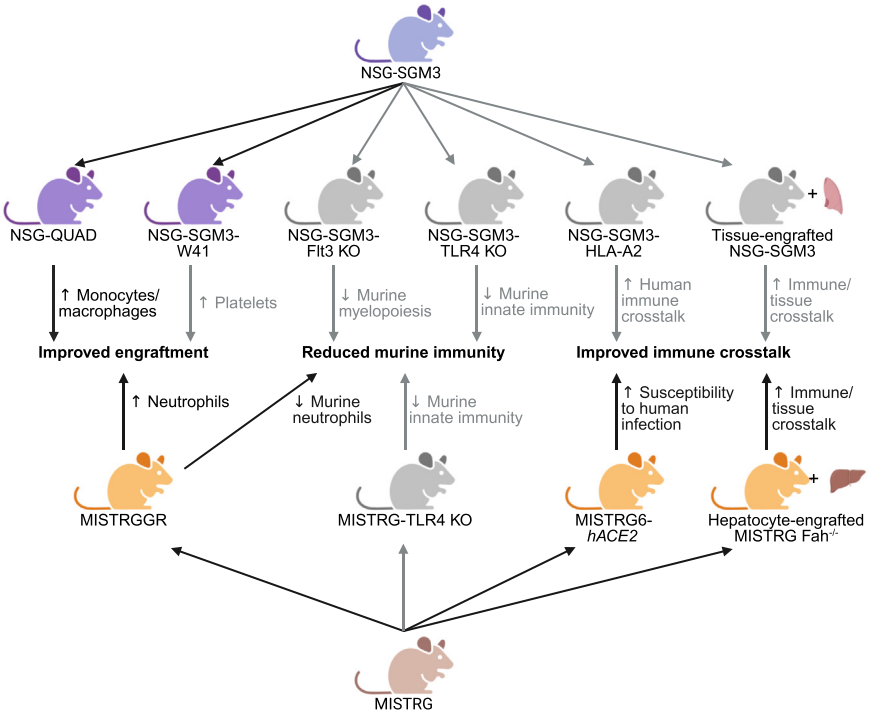

Figure 1. Current and potential advances in human myeloid-engrafting mice. Schematic overview of current and potential derivatives of both NSG-SGM3 and MISTRG mice, which could be used to refine humanized mouse models to overcome the three main challenges discussed in the main text. Gray mouse models indicate potential lines that have not yet been established. Comparable models have also been developed in other immunodeficient backgrounds, such as NOG strains, including NOG-EXL, NOG-W41, and NOG-hG-CSF KI mice, which are not included in this figure. Despite being beyond the scope of this article, these models provide similar advantages to those depicted here. Abbreviations: KO, knockout; MISTRG Fah$^{-/-}$, MISTRG mice deficient in fumarylacetoacetate hydrolase; MISTRG-TLR4 KO, MISTRG mice with Toll-like receptor 4 knockout; MISTRG6-*hACE2*, MISTRG mice with humanized IL-6 and expression of human ACE2; MISTRGGR, MISTRG mice with humanized G-CSF and murine G-CSFR knockout; NK cells, natural killer cells; NSG-QUAD, NSG-SGM3 mice with additional human CSF1 transgene; NSG-SGM3-Flt3 KO, NSG-SGM3 mice with additional Flt3 knockout; NSG-SGM3-HLA-A2, human HLA-A2 knockin on NSG-SGM3 background; NSG-SGM3-TLR4 KO, Toll-like receptor 4 knockout on NSG-SGM3 background; NSG-SGM3-W41, NSG-SGM3 mice with additional cKIT mutation. Figure created using BioRender (biorender.com).

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Declaration of interests

The authors do not have any competing interests to declare.

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