New models of human immunity

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Two humanized mouse models open up a plethora of research applications.

Progress in immunology is inextricably linked to technologies for modeling the human immune system in animals. Mice that fully recapitulated human immunity would be useful in a broad range of research, from investigation of immune function in health and disease to testing the safety and efficacy of new therapies, including vaccines. Two papers in this issue represent important steps toward this goal. Rongvaux *et al.*[1] and Lee *et al.*[2] describe improved humanized mouse models that enable new lines of research that have been difficult to pursue until now.

Although considerable effort has been devoted to mimicking human immunity in mice, existing models have a number of drawbacks[3]. For example, transplantation of hematopoietic stem and progenitor cells requires irradiation of recipient mice, which may cause unwanted damage to tissues important for optimal cell engraftment. In addition, the mice show suboptimal development of myeloid lineages of human immune cells and mount weak antibody responses to immunization. Two types of humanized mouse models are in wide use today[3,4]. In one, bone marrow, liver, thymus mice are generated by injection of human hematopoietic stem and progenitor cells from bone marrow a few weeks after transplantation of human fetal liver and thymus. In the other, human hematopoietic stem and progenitor cells are injected into newborn Balb/c *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ (BRG) or non-obese diabetic severe combined immunodeficient *Il2rg*$^{-/-}$ (NSG) mice. In these models, however, the cytokines that influence development of hematopoietic cells are produced mostly by mouse stromal cells, whereas the developing hematopoietic cells are of human origin. Because not all mouse cytokines efficiently cross-react with the human versions of their receptors, some hematopoietic cell lineages—especially innate immune cells, including myeloid cells and natural killer cells—are not well supported by the mouse microenvironment.

To overcome this limitation, Rongvaux *et al.*[1] undertook the gargantuan effort of systematically swapping endogenous cytokine genes in *Rag2*$^{-/-}$*Il2rg*$^{-/--}$ mice on a mixed Balb/c129 background for their human counterparts using VelociGene Technology, in collaboration with Regeneron Pharmaceuticals. They focused on the mouse cytokines that do not cross-react with human receptors (granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin(IL)-3, macrophage-colony stimulating factor (M-CSF)) or do so only weakly (thrombopoietin (TPO))[5]. Whereas GM-CSF, IL-3 and M-CSF predominantly support myeloid cell development, TPO supports the growth and survival of hematopoietic stem cells. Some of the mice also received a bacterial artificial chromosome transgene encoding human SIRPα, which is expressed on phagocytic cells and, upon binding to CD47, initiates a signal suppressing phagocytosis of the CD47-expressing cell. The reason for this modification is that SIRPα proteins encoded in the genome of most mouse strains (except non-obese diabetic mice) do not interact with human CD47, making human cells that populate immunodeficient mice sensitive to phagocytosis by mouse macrophages[6].

Rongvaux *et al.*[1] generated their mice by interbreeding existing single-cytokine knock-in mice. They refer to the resulting M-CSF$^{h/h}$IL-3/GM-CSF$^{h/h}$TPO$^{h/h}$ quadruple knock-in *Rag*$^{-/-}$*Il2rg*$^{-/-}$ mice as MITRG. MITRG mice that also bear the human SIRPA transgene are called MISTRG. After transplantation of human fetal liver CD34^{+} cells—even in the absence of irradiation of recipient animals—MITRG and MISTRG mice outperformed NSG mice, BRG mice and mice bearing single cytokine knock-ins, showing robust development of functional human myeloid cells, including monocytes, basophils, eosinophils, dendritic cells and natural killer cells in the peripheral blood as well as in lymphoid and nonlymphoid tissues. Whether innate lymphoid cell subsets, which influence innate immunity, tissue remodeling and homeostasis of myeloid cells, developed was not reported. As innate lymphoid cells can be found in NSG mice repopulated with

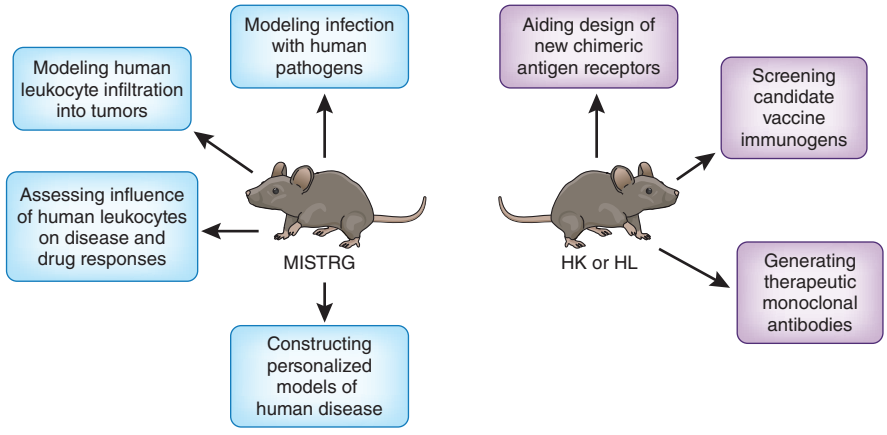

Figure 1 Potential applications of new models of human immunity. MISTRG mice described by Rongvaux *et al.*[1] and HK and HL mice presented by Lee *et al.*[2] will likely enable a variety of basic and applied research applications.

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fetal liver hematopoietic stem cells[7], it would be interesting to know whether their reconstitution is improved in MISTRG mice.

One drawback of the high levels of engraftment in MISTRG mice, however, is low numbers of mouse blood cells, which can cause anemia. Although anemia may limit the usefulness of these mice for long-term experiments, it can be decreased by not irradiating the recipient mice and by lowering the dose of injected human hematopoietic stem and progenitor cells.

The more comprehensive immune system of MISTRG mice will likely enable a variety of basic and translational research applications (**Fig. 1**). For example, because the mice permit engraftment of $CD34^+$ cells isolated from human cord blood or adult peripheral blood, one might be able to generate 'personalized' mice using $CD34^+$ cells from patients with diseases of interest. Combined with co-transplantion of pieces of tissues affected by the disease (for example, autoimmune target tissues), it might be possible to observe and perturb immune-mediated disease pathology in real time, determine whether immune cells play any role in the pathology or even screen candidate drugs to identify those most likely to be effective in a particular patient. Rongvaux *et al.*[1] in fact performed an experiment of this sort, focused on cancer rather than autoimmune disease. They showed that human myeloid cells infiltrate human tumor xenografts in MISTRG mice and that this infiltration correlates with tumor growth driven by vascular endothelial growth factor. That said, the tumor cells used were allogeneic, which may confound interpretations of the results.

Other potential applications of MISTRG mice relate to their robust development of human dendritic cells, which are key initiators of adaptive immune responses. For example, one could envision using the mice to model the immune response to clinically important pathogens, including HIV, both for basic research and for designing and testing vaccine candidates. However, as Rongvaux *et al.*[1] note, the humoral response in MISTRG mice is far from optimal in that antigen-specific antibodies were of the IgM isotype, indicating a lack of isotype switching, similar to what is seen with existing humanized mouse models. Because monoclonal antibodies obtained from immunized BRG mice are mostly in the germline configuration[8], it is likely that the IgM antibodies in MISTRG mice contain only a few somatic hypermutations, but this was not directly tested. For this reason, the mice are not suitable for analysis of the human antibody response to vaccination, nor are they likely to be useful for the generation of therapeutic human monoclonal antibodies. This is where the findings of Lee *et al.*[2] come in.

In the 1990s, several groups, using different techniques, generated mice able to produce human immunoglobulins[9]. The loci encoding mouse immunoglobulin variable regions were inactivated to prevent generation of mouse or chimeric mouse-human immunoglobulin molecules. Indeed, these mouse strains enabled development of several human monoclonal antibodies that were approved for use in the clinic or are in clinical trials. However, the mice have drawbacks that preclude their use in several important applications, including the testing of vaccine candidates. Some of these relate to the insertion of DNA encoding human immunoglobulin constant region segments; because human constant regions interact poorly with key mouse signaling molecules, the mice show suboptimal human B-cell development and function. In addition, inserting transgenes into the genome can result in genetic instability, and deletion of mouse variable regions can cause collateral damage due to deletion of genes important for mouse survival and fertility. Finally, because the mice lack the complete repertoire of human heavy and light chain variable segments, they cannot model the full spectrum of immune diversity seen in human immune responses.

More recently, several biotech companies have generated 'second generation' mice that may address several of these shortcomings[10]. However, many of these strains have not been described in peer-reviewed publications. Lee *et al.*[2] describe just such a second-generation strain. To generate it, they inserted all of the human immunoglobulin variable gene segments into their corresponding location in the mouse genome using repetitive cycles of genome engineering—called sequential recombinase-mediated cassette exchange—in embryonic stem cells. They left the mouse constant region intact. To avoid deleting intervening DNA that might encode essential genes while simultaneously minimizing incorporation of mouse V segments into human antibodies, they inverted the heavy chain locus relative to the mouse constant regions.

The resulting mice were fertile and showed robust B-cell development. After breeding mice created in this way, Lee *et al.*[2] obtained mice expressing heavy-chain variable regions and either κ (HK mice) or λ (HL mice) light-chain variable regions. In response to immunization with a variety of antigens, the mice produced isotype-switched somatically hypermutated antibodies whose features (such as V gene segment usage and CDR3 length) and affinities were similar to those of antibodies generated in humans, indicating that the B-cell repertoire of the mice faithfully represents that of humans.

Accordingly, this may be an ideal model for testing human vaccine candidates. Various recent approaches aim to design immunogens able to elicit broadly neutralizing antibodies against clinically important pathogens, including respiratory syncytial virus (RSV), influenza virus and HIV. For example, based on information about the structure of RSV epitopes recognized by a broadly neutralizing antibody isolated from a healthy adult human, one study outlined a path to rational design of immunogens for vaccination against this pathogen[11]. Such candidate immunogens could be administered in combination with various adjuvants to the humanized mice described by Lee *et al.*[2]. Interrogation of the resulting antibody response would reveal which immunogens in which formulations elicit broadly neutralizing antibodies similar to those observed in infected humans.

The mice should also be useful for generating human antibodies against antigens of interest. If specific for tumor antigens, the antibodies might even be converted into chimeric antigen receptors for applications in adoptive T-cell immunotherapy. The features of the mice—including the diversity of human variable regions represented and the robustness of B-cell response—indicate that they may provide an excellent platform for production of fully human therapeutic antibodies. Of course, this would require replacing the mouse constant region with its human counterpart.

Notably, unlike proprietary second-generation mice at biotech companies, the mice described by Lee *et al.*[2], although also generated by a commercial entity, will be made accessible to the academic community through a unique public access program (http://www.kymabaccess.org/). The mice of Rongvaux *et al.*[1] have been deposited at Jackson Laboratories. The availability of the two new mouse strains should facilitate their integration both into basic research programs focused on understanding the cellular and molecular mechanisms underpinning the human immune system in health, autoimmune disease and infection, and into applied research programs aimed at developing new therapies, including vaccines.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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