Renaissance for mouse models of human hematopoiesis and immunobiology

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More than 20 years after the first successful engraftment of human leukocytes and hematopoietic organs in mice, scientists met for the 2nd International Workshop on Humanized Mice to discuss progress and to highlight expectations in this dynamic field.

Progress in biomedical research toward the development of efficient new therapeutics requires the study of human physiology and pathology *in vivo*. However, rigorous human disease experimentation is often necessarily hampered by ethical restrictions and by the lack of appropriate predictive model systems. Mice represent the most frequently used experimental mammalian model system, but they are separated from humans by millions of years of evolution. Although nonhuman primate models are evolutionarily closer, they too show clear genetic differences from humans and further suffer from logistic and ethical constraints. Moreover, the species-specific susceptibility and transmission of certain coevolved infectious agents condition the development of subsequent disease pathology in animal models. This limitation became particularly evident with the rise of the human immunodeficiency virus (HIV) pandemic and was a driving force for the generation of small-animal models with genetic and/or cellular components of the human hemato-lymphoid system. The development of these so-called 'humanized mice' was directly related to and benefited from more basic fundamental work by developmental biologists who had identified mouse strains with defects in immune function.

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The fact that immunodeficient mice have become standard tools in hematology and immunology research is thanks in part to initiatives started in the early 1970s to reunite researchers in the field. One example of those is the International Workshops on Immune-Deficient Animals (held nine times between 1972 and 1997), spearheaded by Tatsuji Nomura from the Central Institute for Experimental Animals (Kawasaki, Japan). These meetings were a forum for researchers to compare models, and they catalyzed advances in the field. In a more recent permutation of this idea, Tatsuji Nomura and Mamoru Ito held the International Workshop on Humanized Mice in Tokyo in 2006 to compare results obtained with the latest immunodeficient mouse strains. On 3–6 April 2009, about 200 scientists from 21 nations met in Amsterdam for the second installment of this workshop, the 2nd International Workshop on Humanized Mice (IWHM II), which focused on present 'physiology' of human hemato-lymphoid cell differentiation, maintenance and function in humanized mice and ongoing efforts to improve the engineering of human tissue grafts. This IWHM II meeting report summarizes recent breakthroughs in this dynamic field and discusses the potential effect of these models on basic as well as applied research on human disease.

Present state of the art

IWHM II was opened by a keynote lecture from one of the founders in the field, Joseph M. McCune (San Francisco), who reviewed the evolution of mouse models for HIV research and presented a personal scrapbook of the early days of humanized mouse research using the humanized severe combined immunodeficiency (SCID-hu) model (**Fig. 1**), and also recounted the first exciting scientific leaps, as well as the strong public reactions surrounding this work in the late 1980s. Although it is clear that the SCID-hu model was a catalyst for HIV research in subsequent years, the model was imperfect and required pristine animal facilities as well as access to human fetal tissues. Fortunately, improved immunodeficient hosts for human cell engraftment were being developed by Leonard D. Shultz at the Jackson Laboratories, by Mamoru Ito at the Central Institute for Experimental Animals in Japan and by others. Most importantly, these new models were often made rapidly available to qualified researchers, thereby allowing scientific strides to be made in a timely fashion.

The choice of the present immunodeficient mouse models used for humanized-mouse research is the result of incremental advances in the understanding of the immune response to xenografts[1–3]. Three major steps were involved and were summarized in presentations by Mike McCune, Leonard Schultz and Mamoru Ito.

The description of the *Prkdc*^{scid} mutation in CB17 mice that causes B cell and T cell deficiency due to inappropriate DNA repair during B cell and T cell antigen receptor rearrangement was followed by the breakthrough findings that human peripheral blood mononuclear cells, human fetal hematopoietic tissues, and human hematopoietic progenitors engraft in these mice, generating the human peripheral blood leukocyte–SCID[4], SCID-hu[5] and human SCID-repopulating cell–SCID[6] models. However, engraftment was low,

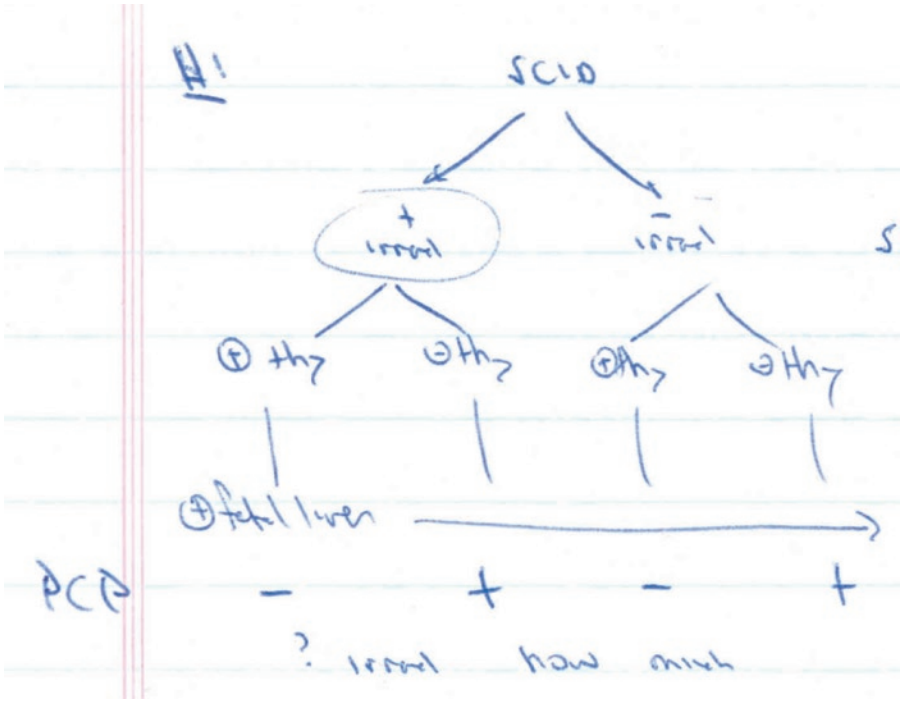

Figure 1 The kernel of an idea: early experiments using SCID-hu mice (from the laboratory notebook of Mike McCune, April 1987).

limited over time and mostly confined to the transplanted tissues.

Second, placement of the SCID mutation on the nonobese diabetic (NOD) background led, somewhat unexpectedly, to further immunodeficiency that improved the engraftment of human cells. This was due to diminished function of natural killer cells, complement and macrophages, and in the 1990s, the NOD-SCID model was the standard for evaluating 'long-term' (that is, several months) engraftment of human hematopoietic stem and progenitor cells. However, human T cells did not develop in humanized NOD-SCID mice, and B cells did not mature into immunoglobulin-producing cells (probably because of lack of T cell help). Nevertheless, this deficiency could be overcome by the transplantation of fetal tissues (liver and thymus) with hematopoietic stem cells from the same human donor (generating NOD–SCID–fetal liver cell (FLC) mice[7] or NOD–SCID–bone marrow–liver–thymus (BLT) mice[8]), which allows human T cells as well as antibody-secreting B cells to be made. Still, BLT- or FLC-humanized mice have some limitations (difficulties in obtaining fetal tissues; low throughput) and are based on NOD-SCID recipient mice that develop lethal thymomas with age.

A third critical improvement involved the development in 1995 of mice with null alleles for the common cytokine receptor γ-chain (*Il2rg*$^{-/-}$ (called '$\gamma_c^{-/-}$' here)); γ_c is required for signaling by interleukin 2 (IL-2), IL-4, IL-7, IL-9, IL-15 and IL-21. The placement of γ_c mutations on the NOD-SCID background or combination with mutations of recombination-activating gene 2 (*Rag2*) on the BALB/c background generates new alymphoid mouse recipients (lacking T cells, B cells and natural killer cells) that develop robust human lymphopoiesis with mature human T cells and B cells in mouse secondary lymphoid organs after transfer of CD34$^+$ human hematopoietic stem cells (HSCs)[9–12].

Thus, after a long series of sequential improvements, a relatively simple and reproducible mouse model was available that could generate a 'human immune system' (commonly referred to as 'HIS mice'). The two main HIS models (NOD-SCID-γ_c (NOG) and BALB/c *Rag2*$^{-/-}\gamma_c^{-/-}$) represent the most widely used models available at present for studying human hematology and immunology in mice *in vivo*. Along with the aforementioned BLT mice, these HIS models (and their derivatives) form the foundation for the studies of human immune function in mice discussed at IWHM II.

Improving human immune responses

Despite the breakthrough they afforded in allowing development of human lymphocytes (B cells and T cells) and dendritic cell (DC) subsets, at present HIS models are limited in their capacity to generate robust cellular and humoral immunity after immunization or infection. James Di Santo (Paris) summarized the different factors that limit immunity in HIS mice, including mechanisms that result in ongoing rejection of human cells, and species-specific incompatibilities that compromise their overall homeostasis in the mouse environment.

IL-7 and IL-15 have a major role in lymphocyte development and are critical for the maintenance of mature lymphocytes that reside in secondary lymphoid tissues. Although mouse IL-7 and IL-15 are both considered to be cross-reactive with their cognate human receptors, several groups have shown that lymphopoiesis in HIS mice is limited by the availability of these human cytokines. The development of innate human lymphocytes, and especially natural killer cells, can be improved by treatment with exogenous agonists of human IL-15 and IL-15 receptor α-chain[13]. As for human IL-7, three groups (those of Anja van Lent (Amsterdam), Stephanie Eisenbarth (New Haven, Connecticut, USA) and David Baltimore (Pasadena, California, USA)) have found that human T cells in mice are susceptible to IL-7 at several stages of development, which suggests that the efficacy of cytokine delivery may be conditioned by the cell type producing the soluble factor as well as the activation state of the responding human T cell.

Lymphoid homeostasis is critically dependent on interactions with major histocompatibility complex (MHC) molecules both during their selection and in the periphery. In HIS mice, both mouse (H-2) and human (HLA) MHC molecules are available, which has led to the question of whether human lymphocytes are selected and restricted to mouse or human MHC. This is an important question, as a future application of HIS mice might include screening of candidates for human vaccines. As for the coevolution of human MHC and human antigen receptors, interactions between developing human lymphocytes and human MHC would be expected to result in more physiological signaling. Takeshi Takahashi (Sendai, Japan) reported that elimination of host MHC class II expression has little effect on human CD4$^+$ T cell in HIS mice, which suggests a mechanism whereby engrafted HLA-DR$^+$ cells promote intrathymic CD4$^+$ T cell selection. In terms of CD8$^+$ T cells, a transgene for HLA-A0201 was shown to promote the selection and homeostasis of human CD8$^+$ T cells in HIS mice (Nick Huntington, Paris). Several groups are now deriving HIS mouse models that integrate human HLA class I and class II as transgenes (groups led by James Di Santo (Paris), Hergen Spits (Amsterdam,) and

Canals of Amsterdam, the site of IWHM II. Photo: Alcidie Mommers.

Lenny Schultz (Bar Harbor, Maine, USA)) or by the replacement of endogenous loci (groups led by Richard Flavell (New Haven, Connecticut, USA), Markus Manz (Zurich, Switzerland) and Sean Stevens (Tarrytown, New York, USA)).

Improving human hematopoiesis in mice

The present immunodeficient mouse models allow robust human lymphopoiesis from engrafted HSCs but generate few human myeloid cells and essentially no human erythrocytes or platelets. This bias is explained in part by the fact that the recipient mice have compromised lymphoid compartments but normal myeloid, erthyroid and megakaryocytoid cell development. Moreover, mouse macrophages can eliminate human xenografts , but phagocytosis is supressed by interactions between CD47 and SIRPα. Interestingly, the NOD allele encoding SIRPα has a high affinity for human CD47, which suggests that improved engraftment in NOD-SCID mice may be explained by less macrophage phagocytosis[14]. Expression of human SIRPα as a transgene in recipient mice or expression of mouse CD47 in human HSCs before engraftment may substantially improve the durability of the engraftment of human hematopoietic cells in HIS mouse models.

The immunodeficient mouse recipients now in use are limited by their inability to maintain long-term self-renewal and function of human HSCs, leading to an ultimate failure of human hematopoiesis. A substantial fraction of endogenous mouse HSCs is quiescent; however, this characteristic is not seen in transferred human HSCs in humanized mice (Hitoshi Takizawa, Bellinzona, Switzerland), which suggests that human-specific factors may be missing. Several groups reported approaches aimed at improving the engraftment and maintenance of human HSCs. Mutations affecting HSC maintenance (W/W^v alleles of the c-Kit receptor for stem cell factor) enhance the engraftment of human HSCs in NOG mice even in the absence of preconditioning irradiation (Mamoru Ito, Kawasaki, Japan), although the long-term survival of the mouse recipient is compromised. The incorporation of human transgenes for thrombopoietin and IL-3–granulocyte-monocyte colony-stimulating factor (Anthony Rongvaux and Tim Villinger, New Haven, Connecticut, USA) or treatment of BALB/c *Rag2*$^{-/-}\gamma_c^{-/-}$ HIS mice with stem cell factor (Elwin Rombouts, Rotterdam, The Netherlands) enhances human myeloid differentiation with positive effects on HSC maintenance. These results suggest that humanized mice with a more balanced development and long-term maintenance of diverse human hematopoietic lineages may be achieved in the near future.

Human infectious disease in mice

Evolution shapes not only single species but also their coevolving commensal organisms and infectious pathogens. Pathogens might either be species-specific or cause species-specific pathologies, with some of the pathologies not even being directly caused by the pathogen but instead being caused by the immune reaction in the infected individual. Into these categories fall the following major global health problems: infection with HIV and hepatitis B and C viruses (with combined about 500 million people infected and 3.5 million deaths per year); epidemic malaria caused by *Plasmodium falciparum* (with about half a billion infected and 1.5 million deaths per year); and infection with *Mycobacterium tuberculosis* (with an estimated 2 billion carriers and 1.7 million deaths per year). The magnitude of human suffering caused by these pathogens is enormous and calls for an equally important dedication to developing predictive preclinical testing systems, as well as more adequate preventive and therapeutic measures. Mice carrying components of the human hemato-lymphoid system are obvious testing tools for the study of infectious pathogens with species-specific tropisms for human cellular targets.

As hepatitis B and C viruses do not infect mouse liver cells, humanized mouse models that incorporate human hepatocytes are useful for studying these pathologies and for screening therapeutics with activity against these viruses. The transplantation of human hepatocytes or pluripotent stem cell–derived liver cells into immunodeficient recipient mice that have controlled liver damage was demonstrated (Mamoru Ito (Kawasaki, Japan) and Hongkui Deng (Beijing)). These humanized hepatocyte mouse models complement existing systems[15] in having less intrinsic toxicity. Placing these new human hepatocyte models on the NOG or BALB/c *Rag2*$^{-/-}\gamma_c^{-/-}$ background should allow the development of chimeric systems that are susceptible to infection by human hepatotropic viruses and also potentially 'read out' the human immune response to infection.

HIS mice have been extremely useful for studying pathogens that directly target the human hemato-lymphoid system, particularly HIV and Epstein-Barr virus (EBV). Published work has demonstrated that long-term productive HIV infection can be achieved by either systemic or mucosal inoculation of HIS mice (generated with NOG, BALB/c *Rag2*$^{-/-}\gamma_c^{-/-}$ or BLT mice) with both chemokine receptor CXCR4– and CCR5–tropic HIV type 1 strains (Satoru Watanabe (Tokyo) and refs. 16–18). Detailed analysis of the immunopathology as well as the prevention and possible therapy of these infectious agents in HIS models were reported at IWHM II. Yoshio Koyanagi (Kyoto, Japan) showed that human effector memory T cells are 'preferentially' infected and depleted in HIV-infected HIS mice and Larisa Poluektova (Omaha, Nebraska, USA) reported that human macrophages in the brains of mice are readily infected and induced inflammatory responses, including neuronal loss, thus reflecting features of human HIV-associated central nervous system disease ('neuro-AIDS'). Efficient pre-

IWHM II co-organizers James Di Santo and Kees Weijer enjoy herring while visiting the canals of Amsterdam. Photo: Markus G. Manz.

exposure prophylaxis with HIV fusion inhibitors in SCID–hu mice was demonstrated by Cheryl Stoddart (San Francisco), whereas Paul Denton and Victor Garcia (Dallas) showed results in BLT mice for clinically available HIV antiviral compounds. The therapeutic use of highly active anti-retroviral therapy (Roberto Speck, Zurich, Switzerland) and a chimeric construct consisting of a gp120-directed aptamer with virus-neutralizing ability and small interfering RNA with proven efficacy against viral transcripts encoding the transactivators Tat and Rev (Ramesh Akkina (Fort Collins, Colorado, USA) and ref. 19) were all shown to be effective in limiting HIV infection in HIS mice.

Given the feasibility and safety concerns for clinical gene therapy studies, it is particularly interesting that the present generation of humanized mice seem well suited as a preclinical testing system. Mireille Centlivre (Amsterdam) demonstrated that the transplantation of human $CD34^+$ cells transduced with lentiviral vectors encoding short hairpin RNA specific for HIV type 1 allows the *in vivo* development of T cells that have the potential to inhibit the replication of HIV type 1 in a sequence-specific fashion. Finally, it was shown that expression of tripartite motif protein 5–cyclophilin fusion proteins engineered from human components in human T cells leads to less viremia and blocks the destruction of human $CD4^+$ cells in mice (Jeremy Luban, Geneva, Switzerland).

Although all of the *in vivo* experiments mentioned above clearly indicate the utility of HIS mice as valuable tools for HIV research and therapy, they do not require a functional adaptive immune response, which is required for *in vivo* screening of prophylactic and therapeutic HIV vaccines. Indeed, human adaptive immune responses in HIV-infected NOG and BALB/c *Rag2*$^{-/-}\gamma_c^{-/-}$ HIS mice are rather rare and often undetectable, most probably due to inadequate T cell selection and homeostasis, as discussed above. In contrast, Andrew Tager (Charlestown, Massachusetts, USA) reported that infection of BLT mice with HIV generates robust HIV-specific cellular and humoral immune responses[20].

Till Strowig (New York) showed that EBV-infected NOG mice transgenic for human HLA-A2 and transplanted with HLA-$A2^+$ human HSCs can mount protective EBV-specific T cell responses that limit B cell lymphoma development[21]. Moreover, Christian Münz (Zurich) reported that targeting EBV antigens to human DCs in HIS mice promotes greater adaptive immune responses. These findings are important, as they suggest that present efforts to express human HLA class I and II in HIS mice may be crucial for using these mice as high-throughput tools for assessing functional human adaptive immune responses without the need to transplant human fetal thymus.

At present, HIS mice seem to be unsuitable for studying infection and disease-related pathology caused by *P. falciparum* or *M. tuberculosis*. For malaria blood stages, this is due to insufficient formation and survival of human red blood cells, whereas more robust human myeloid cell and T cell responses seem to be necessary for the typical granulomatous responses in tuberculosis. Despite these limitations, conference participants were confident that studies of HIS mice using these human pathogens will be achieved with the recipients expressing human cytokines and HLA now under development.

Engineering human immunity

A second keynote presentation was given by David Baltimore, who provided a conceptual approach for engineering human immunity for both preventative and therapeutic treatment of infectious disease and cancer. By taking advantage of the power of lentiviral gene transfer, Baltimore's approach involves targeting key cellular elements of the immune response (stem cells, B cells, T cells and DCs) to create an environment in which the desired responses are more robust. Strategies include the cotransfer of antigen-specific B cell and T cell antigen receptors with survival factors (IL-7 and IL-15) via multicistronic lentiviral vectors or the incorporation of antibodies to cell type–specific antigens (CD34 for hematopoietic progenitors, DC-SIGN for DCs, and CD20 for B cells) in pseudotyped viral coat proteins to selectively target lentiviruses *in vivo*[22,23]. Coupled with the HIS mouse model, these exciting new approaches could affect the testing of protective or curative therapies for several infectious diseases, including HIV.

As HIS mice generate human B cell responses, they offer the possibility of creating a wealth of new therapeutic agents, most notably human monoclonal antibodies. Nicolas Legrand (Amsterdam), presented an approach for generating human monoclonal antibodies with HIS mice using a new technique (involving expression of the transcriptional repressor Bcl-6 and antiapoptosis molecule Bcl-x_L) that immortalizes human antigen–specific memory B cells. Although such antigen-specific human monoclonal antibodies are generally of the immunoglobulin M subclass (and suggest that additional signals are needed to generate switched immunoglobulin subclasses in HIS mice), this proof of concept is a critical step for the more widespread use of HIS mice as 'translational' tools.

Concluding remarks

The IWHM II showed that the latest immunodeficient mice are excellent hosts for human tissues and stem cells that can generate a functional human hemato-lymphoid system suitable for the study of human infectious pathogens and human immune responses and as preclinical vaccine testing tools. Shortcomings that limit the existing HIS mouse models have been defined, and several new approaches seem to improve the capacity of these mice to recapitulate normal human innate and adaptive immune responses. The momentum in the field is great, and all look forward to IWHM III that will be hosted by Victor Garcia (Chapel Hill, North Carolina, USA) in 2010.

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