Chapter 2
Humanized Mice as Models for Human Disease

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2.1 Introduction

It has been over a quarter century since "humanized mouse" models were first introduced. As before, such models facilitate the preclinical analysis of therapeutic compounds and vaccines, and otherwise assist in decision-making processes as ideas move from the bench to the clinic. At best, these models even provide a more complete understanding of pathologic mechanisms in vivo, enabling the focused discovery of better interventions for use in treating human disease. Conversely, and as has been evident throughout the history of humanized mice, it is important that their limitations be acknowledged, especially by investigators close to the field, lest inappropriate experiments be designed and/or inaccurate predictions be made.

This book is devoted to state-of-the-art descriptions of the potential utility of these models, and this introductory chapter frames that potential in terms of ultimate goals and hurdles yet to cross. In doing this, two general messages emerge: first, great strides have been made in the development and optimization of these models in recent years and, secondly, more work needs to be done before their full potential and practical applications can be fully realized.

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2.2 The Rationale Design of Animal Models

By definition, a model is something that is designed to mimic something else. In the case of an animal model of a human disease, the immediate contradiction arises: since no two humans are alike, who should the animal "model"? To circumvent this issue, it is perhaps more pragmatic to instead pose the question: what problem is being addressed? The answer to this question dictates the design and development of the model, not to mention the need for its creation at all.

Likely as not, different mouse models will be used to address dissimilar questions and some questions will not be answerable through the use of any existing mouse model. By example, the human fetal and adult immune systems are distinct from one another in function if not phenotype, emanating in the fetus from a multilineage hematopoietic stem cell (HSC) in the liver and bone marrow that gives rise to tolerogenic (e.g., FoxP3+ Treg) T cells, and from another HSC population in the adult bone marrow that gives rise to immunoreactive (e.g., Th1 and Th2) T cells [1, 2]. It follows that the human fetal immune response would best be studied in a mouse model constructed with human fetal tissues whereas adult-type immune responses would be more appropriately studied in a model constructed with adult bone marrow-derived HSCs. As another example, it is clear that the lymphoid architecture of immunodeficient mice (including $Prkdc^{scid}/Prkdc^{scid}$, hereafter abbreviated as *scid* mice, and especially *scid* $IL2rg^{null}$ mice) is underdeveloped relative to that of wild-type mice or, for that matter, normal humans, making it unlikely that it can support physiologic trafficking and differentiation of human lymphoid cells following human HSC engraftment [3, 4]. Until and unless this problem is solved, it is difficult to imagine how "normal" human immune function can be optimally studied using such mice.

Once a question has been posed and a model designed, it would seem best to satisfy certain other criteria:

1. *The model must be shown to mimic that aspect of human physiology and/or pathophysiology that it is designed to "model."* This demonstration is the type of iterative work that forms the foundation of translational research, and there is no more important venue for it to occur than in the design and use of animal models. Thus, careful clinical observation should frame the design, development, and optimization of a given model. To the extent that similar (and, hopefully, identical) observations can be made in the model, it is more likely that new data obtained from it will be pertinent to the human case.
2. *The model must be constructed in a way that provides for reproducible results.* In general, this means that there should be openly available and standardized procedures that lead to definable mouse xenogeneic chimeras with measurable parameters of human hematolymphoid engraftment and immune function, ones that meet the usual level of reproducibility demanded of any experimental endeavor. External validation of novel findings is otherwise difficult. This criterion prompts careful consideration of two linked issues. First, since there is broad interindividual variation in almost every feature of human biology (including, for example,

the capacity of cells or hematolymphoid organs to engraft in a mouse and/or to sustain HIV replication thereafter), [5] it follows that reproducible results will be most likely obtained within a given cohort of mice if all members of the cohort are prepared with cells and tissues from the same human donor. Since data from humanized mouse models would ideally be applicable to more than a single individual, it follows also that it would be important to have the ability to repeat experiments in the context of multiple cohorts of humanized mice, bearing cells and tissues from multiple human donors.

3. *In most instances, the model should be capable of relatively high throughput analysis of interventions* such as drugs and vaccines, amenable to the analysis of different congeners in varying doses and by different routes, with appropriate positive and negative controls, and with a sufficient number of nearly identical animals in each subgroup to enable statistical analysis of the data. Thus, while isolated observations made in one or two humans have occasionally offered profound insights into normal and abnormal human biology (consider, for instance, the first vaccinations against smallpox by Jenner or the first use of insulin by Banting and Best), [6] it seems far less likely that statistically nonverifiable results from small numbers of humanized mice will have lasting clinical impact.

The bottom line: in the idealized case, it would be possible to make multiple cohorts of many mice, with each cohort using tissue from the same human donor, each with roughly similar quantitative and qualitative levels of engraftment, and all accurately mimicking a given aspect of human physiology or pathophysiology that is of interest to study and otherwise not approachable in vitro.

2.3 A Case in Point: The SCID-hu Thy/Liv Model

When first confronted with the above issues some 25 years ago, there was interest in designing an animal model that would enable the preclinical evaluation of antiviral compounds and vaccines against HIV. With this goal in mind, it was important that the animal be small (so that issues of cost and number could be minimized), that it harbor human hematolymphoid organs (e.g., thymus, lymph node, spleen, and bone marrow) and constituent primary cells that are normally infected by HIV in people, and that it be permissive for infection with primary isolates of HIV. Given these constraints, an attempt was made to engraft interactive human hematolymphoid organs into a mouse in such a way that they would not be rejected. Thus was born the SCID-hu Thy/Liv mouse and its various derivatives, e.g., those engrafted with bone, lymph node, bone–thymus–spleen (the "BTS" mouse), or all of the above (the "full house mouse") [7–11].

The decision to implant human fetal tissues and human HSC into the immunodeficient CB17 *scid/scid* (SCID) mouse was prompted by the following logic: (1) it seemed less likely that (human versus mouse) graft-versus-host disease (GVHD) would occur if human fetal HSC were allowed to differentiate into T and B cells

in the mouse environment and, hence, come to see the mouse as "self"; [12] (2) it seemed more likely that physiologic human immune cell maturation would occur within implanted human, as opposed to endogenous murine, parenchymal microenvironments; and, not least (3) successful engraftment of a functional human immune system could be easily ascertained: CB17 *scid/scid* mice, but not other stocks of immunodeficient mice available at the time, were known to otherwise succumb to *Pneumocystis carinii* pneumonia [13].

Initial experiments using the SCID-hu model showed robust engraftment, vascularization, and growth of the conjoint Thy/Liv organ that was formed by coimplantation of human fetal liver and human fetal thymus under the kidney capsule [7, 8]; no signs or symptoms of xenogeneic GVHD (due to the unanticipated movement of murine myeloid cells into the human Thy/Liv organ, promoting negative selection of developing human T cells that recognized the H-2^d background of the CB17-*scid/scid* mouse) [7, 14–15]; detectable replication and spread of primary isolates of HIV [16]; human antibody production and class switching in engrafted human fetal lymph nodes [7, 17]; and, remarkably, the absence of *Pneumocystis carinii* pneumonia in all engrafted mice [7]. Given these results, countless attractive applications presented themselves for further consideration: from preclinical analysis of antiviral compounds and vaccines against a wide variety of human pathogens to isolation of human hematopoietic progenitor cells to dissection of the rules of tolerance induction in humans to creation of an expanded battery of humanized mouse models for the analysis of other human organ systems (e.g., the central nervous system).

Over the next decade, with funding from the National Institutes of Health (NIH) as well as from biotech/pharma, and with the hard work and good thought of many, these notions were put to test. Not surprisingly, some worked but most did not:

1. The SCID-hu Thy/Liv model could be optimized and standardized, and created in cohorts of 50 or more mice per human tissue donor for the preclinical analysis of antiviral compounds against HIV [18, 19]. With the help of a relatively large and committed staff of highly trained and expert researchers, it has been possible to create as many as 40 such cohorts on an annual basis and to infect, dose, and analyze the results of antiviral drugs within them in a reproducible manner. Given the data now on hand, the predictive powers of this model have proven to be considerable: antiretroviral compounds found to be active in it have also been found to be active in humans; those not active in it are also inactive in humans. In addition, use of the model has provided unique information about the bioavailability and mechanism of action of antiviral compounds in vivo.
2. The Thy/Liv model and its derivatives (including the SCID-hu "Bone" and "BTS" models) enabled the discovery and definition of human HSC for clinical use [20–22], including application of gene-modified HSCs for the treatment of HIV disease [23, 24]. This application and recent modifications continue to be in play, enabling, by example, the recent discrimination between fetal and adult human HSCs [1].

3. The Thy/Liv implant has been found to faithfully mimic the structure and physiology of the normal human thymus [7, 8, 25–29]. It accordingly represents a convenient model to study many aspects of human T cell differentiation and function that could not be studied in vitro and that are only studied with difficulty, if at all, in humans or in nonhuman primates.
4. The SCID-hu Bone model can be used to study the effects of irradiation [30] and exogenously provided cytokines [31] on human HSCs and for the analysis of species- and organ-specific metastasis of human malignancies [32, 33].

On the other hand, and after considerable effort, many other "potential applications" failed. Thus, peripheral human immune responses are observed but found to be neither useful nor relevant, in part because the level of human cell engraftment in the periphery is low but also because important cues provided by the murine lymphoid architecture (e.g., endothelial adhesion molecules, cytokines, chemokines, and the like) are incompatible with (i.e., do not bind to and/or appropriately activate) human cells; it is logistically difficult to carry out longitudinal studies in SCID-hu mice, e.g., sampling peripheral blood or implanted organs over time; and it is difficult to reproducibly engraft other important components of the human immune system, e.g., lymph node, spleen, liver, and gut associated lymphoid tissue. It was these deficiencies, indeed, that sparked interest in the development of secondary generations of humanized mouse models.

2.4 The Next Wave of Humanized Mice

Humanized mouse models developed to address the deficiencies of the SCID-hu models have been well summarized in recent reviews [3, 34–40] and extensively discussed in the remaining chapters of this book. An important distinguishing characteristic is their higher level of multilineage peripheral engraftment, manifest even in the peripheral blood. This attribute is obtained upon the use of mouse stocks that are even more immunodeficient than the CB17-*scid*/*scid* strain [e.g., NOD-*scid*/*scid Il2rg*^{null} (NSG), BALB/c-*Rag2*^{null}*Il2rg*^{null} (BRG), etc.], younger (e.g., engrafted with human cells shortly after birth), and/or irradiated [3]. As a consequence, it is now possible to sample human cells and to quantitate signs of HIV replication by drawing peripheral blood. This feature greatly simplifies the use of the models and enables experimental designs (e.g., longitudinal analyses) that cannot be carried out in the SCID-hu Thy/Liv mouse.

These advances not only open the door to a wide range of experimental possibilities, they also make the use of the models more widely accessible (provided that newly developed stocks of humanized mouse models are made readily available to all through a public repository). It is accordingly not surprising that they have been embraced with enthusiasm.

2.5 Barriers to Cross

Imagination being the powerful force that it is, investigators who are outside the field of humanized mouse technology will be motivated to pursue important applications that may or may not be feasible. It will be incumbent upon those who are working in the field to clearly discuss what can and cannot be done using currently available models. Working thereafter from a platform allowing continuous standardization, optimization, and iterative tests of relevance, the ultimate practical reality of the models will become evident over time. Likely as not, and as in the case of the SCID-hu models, some applications will be forthcoming and others will not.

At this juncture, the current battery of humanized mouse model presents three general problems, ones that should either be solved or accepted as insurmountable barriers to future use:

1. *The presence of clinical and subclinical GVHD:* In the same way that the "hu-PBL-SCID" mouse (in which adult human peripheral blood mononuclear cells were injected intraperitoneally into SCID mice) [41] developed high levels of GVHD [42, 43], many if not all of the current "humanized mouse" models are similarly affected. The frequency and manifestations of clinically apparent disease have been outlined in a number of recent reports [44, 45] and, until proven otherwise, it is reasonable to assume that subclinical GVHD may occur as well. If so, the associated levels of immune activation may make it easier to detect certain endpoints (e.g., viremia after infection with HIV), but it is not clear if a model harboring background levels of GVHD is relevant to the analysis of human diseases in which GVHD is not normally present. Furthermore, ongoing efforts to document and use primary human immune responses in these models should acknowledge that they are occurring in the context of GVHD. In future iterations of the current humanized mouse models, it will be important to understand why such GVHD occurs and how to prevent it from happening (e.g., by creating strains of SCID mice lacking murine MHC Class I and Class II molecules) [46]. Should that not be possible, the use of these models would most appropriately be focused on the analysis of xeno-GVHD.
2. *The absence of normal lymphoid structure and function:* In a normal immune response in mice or in humans, antigen presenting cells may move from distant sites through afferent lymphatics into draining lymph nodes, interacting thereafter with T and B cell subpopulations to prompt antigen-specific proliferation and differentiation of cells with cognate receptors; these then traffic out of the node through efferent lymphatics and into distal effector sites. Unfortunately, most of the infrastructure underlying such physiologic responses is not present in any of the humanized mouse models. In the *IL2rg*^{null} mouse models, for instance, the endogenous murine lymph nodes are poorly developed and unlikely to sustain normal levels of human immune cell trafficking and differentiation [3, 4]. Even in SCID-hu mice engrafted with human fetal lymph nodes, it is not clear whether the supporting vascular and lymphatic endothelium is human and/or mouse, and whether such endothelial structures support the physiologic trafficking of human

immune cells. Should humanized mice be developed for the analysis of human immune responses, e.g., to vaccines, this limitation should be addressed.

3. *The inability to create large cohorts of animals from single human donors:* To date, most if not all studies using the newer versions of humanized mice have been carried out with an unspecified number of human donors contributing to each cohort in a given experiment and, in some cases, as few as three mice in a given group. Alternatively, some studies have detailed the use of cohorts comprised of animals created from multiple human donors. Each approach is problematic. Use of a small number of test animals severely limits the number of conditions (e.g., of doses, controls, etc.) that might be tested in parallel in a single experiment and the number of animals that can be included in each subgroup. Such restrictions are even more compromising if there is a broad range of interindividual variation in engraftment, a degree of interassay variation in the measurement of endpoints, and/or attrition of animals during the course of the experiment. In future iterations of these humanized models, it will be important to develop and to optimize procedures and practices that allow for the practical and reproducible creation of larger cohorts. For instance, advances in the derivation of functional human hematopoietic stem cells and thymic epithelial cells from iPS cells may ultimately provide virtually unlimited numbers of cells for construction of humanized mouse models. In the meantime, it is important for investigators to state what criteria were used to decide which animals were selected for study (e.g., viral load, level of engraftment including *absolute* cell numbers, occurrence of GVHD, and criteria for removing animals from experiments).

The above issues highlight two general goals: first, it is important to show that a given humanized mouse model can be created in sufficient numbers to carry out experiments that are standardized, reproducible, and statistically verifiable; second, the events that are then documented in the model must be shown to have correlates in humans. It is only by achieving the first goal that the second can be addressed.

The steps and iterations required to optimize and to standardize these models will be numerous, time-consuming, and costly. Several approaches are possible. As occurred in the optimization and standardization of the SCID-hu Thy/Liv model, there can be an intensive, focused, stable, multimillion dollar investment made by the NIH and by private funders. Alternatively, collaborative data sharing amongst multiple academic investigators should provide an equally robust effort of similar strength. The current book provides a venue for such a collective effort, one that might lead to continued improvement of humanized mouse models and an ever greater capacity to apply them to the solution of unsolved problems of medical importance in humans.

At the end of the day, humanized mouse models will hopefully provide important input into the rapid advance of basic science into the clinic. They should support hypothesis-driven research that can inform meaningful and well-informed decisions along the course of this pathway. Even if such input is to show that a given intervention does not work, that is an incredibly useful (and time- and resource-saving) input to the multiyear, multimillion dollar pathway of drug development. To the extent that the humanized mouse models actually model humans, it may be possible to reach this dream.

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