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Forum

Humanized Mice for Modeling Human Infectious Disease: Challenges, Progress, and Outlook

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Over 800 million people worldwide are infected with hepatitis viruses, human immunodeficiency virus (HIV), and malaria, resulting in more than 5 million deaths annually. Here we discuss the potential and challenges of humanized mouse models for developing effective and affordable therapies and vaccines, which are desperately needed to combat these diseases.

Infectious diseases continue to heavily burden our global society. Chronic viral infections, including those caused by hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV), currently afflict more than 500 million people worldwide, cumulatively resulting in more than 3.5 million deaths per year. Bacterial and parasitic diseases have a similarly high impact; *Mycobacterium tuberculosis* frequently establishes persistent infections, with an estimated 2 billion carriers around the globe and an annual mortality of close to 1.7 million individuals. Endemic and epidemic malaria results in severe disease in an estimated half-a-billion people each year, and causes over 1.5 million deaths annually. Although progress has been made in the prevention and treatment of these infections, more effective, tolerable, and affordable therapies are urgently needed.

Many of these important pathogens display unique human tropism, and the development of novel intervention strategies has been hampered by the lack of robust, cost-effective, and predictive animal models that accurately reproduce the hallmarks of human infections. While rodents and nonhuman primates have been employed in biomedical research and drug/vaccine development, they often do not yield reliable preclinical results that translate into effective human treatments. Two important factors contribute to this failure: (1) on the microbial side, surrogate pathogens often differ significantly from highly restricted human counterparts; and (2) on the host side, the immune correlates of protection in nonhuman mammalian species often diverge from human responses.

For decades large primates, especially chimpanzees, have been used to study immunobiology and candidate therapies against human pathogens such as HIV, HBV, and HCV. Although the genomes of humans and chimpanzees are >98% identical, small differences can significantly influence disease pattern and outcome (Muchmore, 2001). For example, chimpanzees and humans do not share any human leukocyte antigen (HLA) class I alleles, the common human allele (HLA-A2) is completely absent in chimpanzees, and clear differences in the MHC class II region are also observed. As a consequence nonhuman primates often do not completely reproduce the pathophysiology of human disease. In addition, use of these large primates is expensive and banned in many countries due to ethical concerns. As a result, experiments using limited numbers of outbred animals are plagued by interindividual variability and consequently have limited reproducibility.

These deficiencies exacerbate the considerable roadblocks to vaccine development. Current regulations for human vaccine testing—among others the stringent requirement for good manufacturing practice (GMP) grade material for phase I studies—have led to an explosive increase in clinical development costs. To bridge the translational gap, novel strategies are needed for rapid and cost-efficient screening, selection, and prioritization of the most promising candidates. None of the existing animal models adequately addresses the needs of vaccine developers, nor do they provide the detailed understanding of the human immune response that is required for generation of vaccine strategies against pathogens that have closely co-evolved with humans. Investigation of human biology in vivo is hampered by severe ethical and practical limitations—including use of placebos and experimental drugs prone to resistance selection, the risk of discrimination or stigmatization as a consequence of having participated in the research, limited sample procurement, and heterogeneity of the study population. These limitations and lack of suitable animal surrogates create a seemingly insurmountable barrier to conducting essential preclinical investigations.

"Humanized" mice have recently emerged as powerful tools in the investigation of human disease (reviewed in Legrand et al., 2006; Manz, 2007; Shultz et al., 2007). These are amenable small animal models transplanted with human cells or tissues (and/or equipped with human transgenes) that may be ideally suited for direct investigation of human infectious agents. Successful engraftment depends on avoiding rejection and maximizing tissue function, ensured by correct localization and appropriate tissue support by host factors. Despite the challenges, humanized mouse technology has made rapid progress over the last few years, and it is now possible to achieve high levels of human chimerism in various host organs/tissues, particularly the immune system, liver, and muscle. Such humanized mice provide a new opportunity to perform preclinical studies of intractable human pathogens.

With a focus on HBV and HCV, HIV, tuberculosis, and malaria, we here discuss the current status and future prospects of mice carrying the target tissues of human pathogens, as well as bearing human immune components to react against them (Figure 1).

Toward Robust and Predictive Small Animal Models of Human Disease: Human Hematopoietic and Immune System Mice

Human immune system (HIS) mice principally recapitulate the development of human lymphoid compartment. HIS mice are generated by grafting immunodeficient animals with suspensions of hematopoietic progenitor cells and/or human peripheral blood cells, and potentially with supplemental human tissues supporting the generation of human immune cells (Gimeno et al., 2004; Ito et al., 2002; Melkus et al., 2006; Traggiai et al., 2004). HIS mice are already showing potential as the only available small animal challenge model for HIV infection, a valuable platform for testing the efficacy of antiviral compounds (Figure 1). Importantly, these mice can be infected with HIV not only intravenously but also by relevant intravaginal and intrarectal routes, making this model particularly attractive for preclinical validation of antimicrobial agents that are active at mucosal surfaces (Denton et al., 2008; Grant et al., 2008). Recent disappointing clinical trial outcomes with anti-HIV microbicides could have possibly been predicted and avoided if sufficient preclinical data of this type had been available (Grant et al., 2008).

Despite their promise, immune responses to infection remain suboptimal in HIS mice. For instance, tetanus toxoid immunizations result in lower antibody levels in HIS mice than those achieved in human adults (Traggiai et al., 2004). Only very few HIV-infected HIS mice develop virus-specific antibodies, and thus far HIV-specific T cell responses have not been observed (Baenziger et al., 2006). Epstein Barr virus (EBV) infection of HIS mice results in activation of T and B cells, but can progress to B cell lymphoma, indicating that these mice cannot control the infection (Traggiai et al., 2004). Taken together these data suggest that immunity in humanized mice is not yet comparable to humans and further development is required before these mice can be employed in screening human vaccine candidates.

Currently, the total amount of human cells in HIS mice is below the desired level. Likewise, IgM and IgG serum titers are substantially lower in HIS mice as compared to humans. Hematopoietic stem cells are insufficiently maintained, and differentiation into particular lineages, such as erythromyeloid cells, is ineffective (Manz, 2007). The inadequate formation of higher order lymphoid structures may be central to this limited immune response. Generation of a robust predictive model for human immune responses will therefore require significant improvement of HIS mouse reconstitution and function. Strategies to improve reconstitution include enhancing the ablation of endogenous mouse subsets to create "space" for human cells, providing exogenous cytokines to overcome impaired biological crossreactivity between mouse and human, counteracting active graft destruction, and expressing human MHC molecules to ensure proper T cell education and homeostasis (Legrand et al., 2006; Manz, 2007). Although each of these modifications may only incrementally improve the functionality of HIS mice, the additive (and potentially synergistic) effects of these different improvements create optimism that a predictive model for the human immune response in mice can be achieved in the near future. Use of these advanced animal models for vaccine testing is expected to enhance our ability to predict performance in humans, and will thereby enable rapid and efficient selection of the best vaccine candidates for translation into the clinical development pipeline.

Creating Human Liver Chimeric Mice for the Study and Intervention of Human Hepatotropic Pathogens

The human liver serves as the reservoir for several important pathogens, including hepatitis B and C viruses and the malaria parasite *Plasmodium falciparum*, which together are responsible for more than half-a-billion infections and between 2.5 and 4.5 million deaths annually. Furthermore, the liver plays a critical role in drug metabolism and detoxification, with human hepatocytes showing unique enzymatic profiles that are not recapitulated in other mammalian species. Interest in these critical avenues of human health has spurred efforts to create animals with human livers. A high degree of human liver chimerism can be achieved by transplanting human hepatocytes into

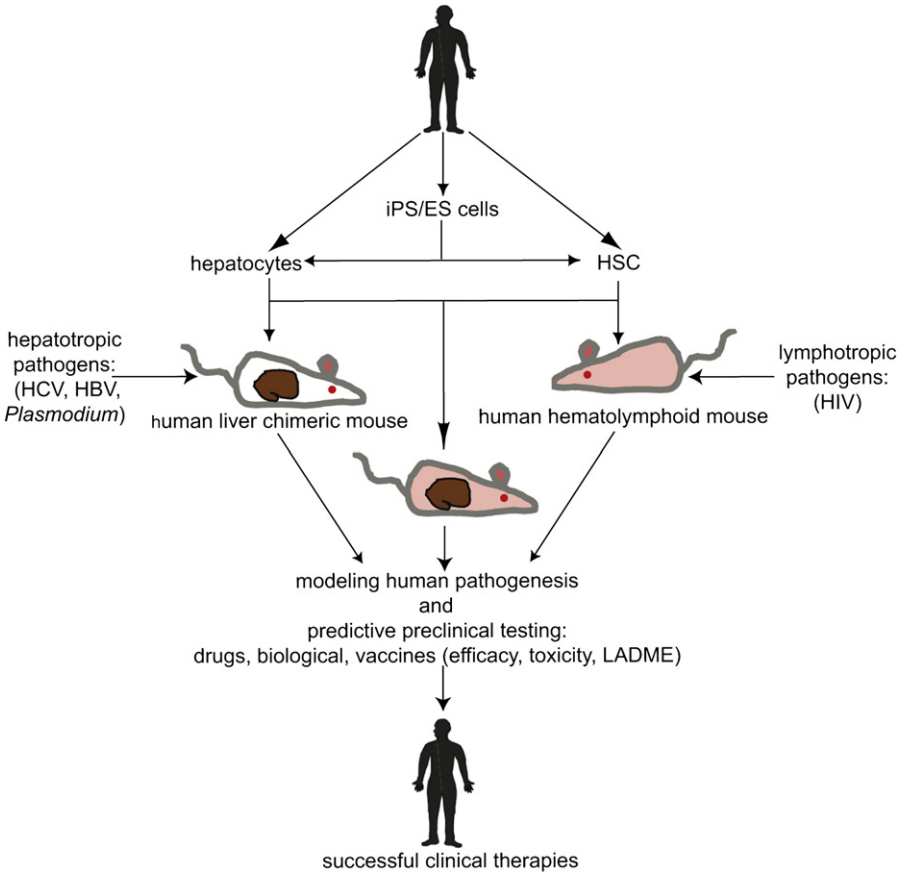

Figure 1. Strategies for the Creation and Optimization of Humanized Mice for the Study of Human Infectious Diseases
The generation of pathogen-specific humanized mouse models is a long-term goal in order to better understand human pathogenesis and to test therapeutic interventions in a preclinical setting. iPS, induced pluripotent stem cells; ES, embryonic stem cells; HSC, hematopoietic stem cells; LADME, liberation, absorption, distribution, metabolism, excretion.

immunodeficient mice with liver injuries to ablate endogenous murine hepatocytes. The most advanced model—and best characterized so far—is the urokinase-type plasminogen activator (uPA) mouse, in which a transgene driven by the albumin enhancer/promoter directs high-level hepatocyte-specific expression of the toxin uPA (Mercer et al., 2001; Meuleman et al., 2005). This creates an environment for the transplanted, transgene-free, human hepatocytes (HuHEP) to populate the repairing mouse liver and to promote survival of the animal. Indeed, HuHEP mice are susceptible to hepatotropic human pathogens and are therefore invaluable tools for basic research and preclinical applications (Mercer et al., 2001; Meuleman et al., 2005) (Figure 1). Due to the immunodeficient background of the recipient, however, neither pathogen- nor vaccine-induced immune responses can be studied. Combination of HuHEP mice with the above described HIS mouse model will be critical to surmount this shortcoming (Figure 1). Beyond their use in studying host-pathogen interactions, human liver chimeric mice have been employed for preclinical evaluation of efficacy, toxicology, and pharmacokinetic profiles of small molecule drug candidates and biologicals, including antibodies targeting host and pathogen components (Katoh and Yokoi, 2007). The observed drug metabolism profiles indicate that HuHEP mice will be useful for predicting human drug interactions, as well as endogenous enzyme induction and inhibition, all of which are serious concerns in drug development.

Despite proven utility in a variety of applications, and the fact that this technology was first described almost a decade ago, human liver chimeric mice have not found wide-spread distribution. Major obstacles to the availability and application of HuHEP mouse technology include very low throughput of chimeric mice generation and substantial variation in levels of human chimerism achieved (Mercer et al., 2001; Meuleman et al., 2005). Both issues can be attributed to the substantial technical challenges of humanizing the mouse liver, which requires advanced surgical techniques as well as intricate logistics to match availability of scarce primary human hepatocytes and suitable murine recipients. Increasing the time window for transplant by generating models in which liver injury is controllable, for example by using inducible versions of the albumin or major urinary protein (MUP) promoters that drive uPA transgene expression later in postnatal development, could increase flexibility in the generation of HuHEP mice. Another promising alternative is to create mice with targeted disruptions in fumarylacetoacetate hydrolase (FAH),

a gene responsible for hereditary tyrosinemia and liver failure in humans (Azuma et al., 2007). FAH knockout mice can be rescued by treatment of pregnant mothers with 2(2-nitro-4-trifluoromethylbenzoyl)-1,3 cyclohexane dione (NTBC) and liver injury can be induced at will by withdrawal of the drug. It remains to be seen, however, if any of these methods can reproducibly achieve the high hepatic reconstitution levels essential for human infectious disease modeling and downstream applications such as pharmaceutical screening and identification of lead compounds.

"Multi-tissue" Humanized Mouse Models

Mice with humanized immune systems already represent the model of choice for various lymphotropic pathogens, including HIV, EBV, and Kaposi's sarcoma-associated herpes virus (KSHV). The addition of human hepatic tissue holds promise for the study of hepatotropic pathogens, such as HBV, HCV, dengue virus, yellow fever virus, and malaria (Shultz et al., 2007), all of which previously lacked accurate and amenable in vivo models (Figure 1). Other areas of investigation, such as autoimmune processes and human tumorigenesis, are also benefiting from humanized mouse technology (Shultz et al., 2007). Mouse models that combine several humanized compartments will increase the specific questions that can be addressed, and a collection of humanized mice "on demand" could be envisaged. For example, mice with a human immune system and supplementary human lung tissue could model tuberculosis or cytomegalovirus (CMV) pathogenesis. Furthermore, preclinical evaluation of vaccine potency could be performed in mice engrafted with tissues efficiently supporting various pathologies, and safety, efficacy, and pharmacokinetics studies of drugs and therapeutic biologicals could be addressed in a tissue- and cell subset-specific manner.

Combining immune and tissue subsets is only a starting point to further address the pathology, immune correlates, and mechanisms of persistence of highly specialized pathogens. HCV is one such uniquely human liver-tropic virus with limited treatment and prevention options. A humanized mouse model that can reliably "read-out" HCV-specific immune responses will find broad application in understanding the interaction between HCV and host, a process currently poorly understood at both the cellular and systemic levels. Understanding how HCV is detected by the immune system, why the majority of individuals fail to mount an effective response, the factors involved in chronic viral persistence versus resolution of infection, and whether immune responses can be stimulated to eliminate the virus will foster the development of preventive and possibly therapeutic vaccines. A suitable small animal model would be an invaluable complement to guide more challenging and expensive studies in humans and experimentally infected chimpanzees.

The construction of mice combining human immune and liver cells will require a highly specialized tissue procurement system. Technical concerns include the best sources of human tissue, timing of transplantation, identification of optimal murine hosts, and the extent of preengraftment conditioning regimens. To avoid the potential complication of tissue histo-incompatibilities, isolation of hematopoietic and hepatic progenitors should ideally be performed from the same donor material. While this can be achieved using human fetal liver tissue, availability of this material is limited due to legislative, ethical, and cultural issues. Alternative sources of progenitor cells exist, including hepatocytes isolated from adult liver, hematopoietic stem cells (HSC) harvested from umbilical cord blood, adult bone marrow, or adult mobilized peripheral blood, and material derived from differentiated embryonic stem cells and induced pluripotent stem (iPS) cell lines. Once protocols for directed differentiation of stem cells into hepatocytes and HSC yield reliable results, such cells would be an ideal source of transplantable material, since they can be generated in potentially unlimited quantities, further increasing the throughput, robustness, and reproducibility of these models (Figure 1).

Making Humanized Mouse Technology Broadly Accessible

In order to effectively integrate humanized mouse technology into the drug and vaccine development process, the technology must be widely accessible and highly reproducible and allow the production of large numbers of animals at a reasonable cost. Humanizing mice is demanding and not only requires advanced technical skills but also an intricate logistical setup. A practical and possibly cost-effective short-term solution could be large-scale generation of humanized mice by third party providers who are also equipped to execute custom-designed drug treatment and vaccination regimens against human pathogens, thereby reducing costly setup and maintenance of biocontainment facilities.

The scarcity of human primary material mandates better coordination of cord blood and fetal tissue procurement for nonclinical research and development. Hematopoietic stem cell and hepatocyte isolation and storage must be harmonized. A central distribution of quality-controlled, HLA-typed material, similar to clinical cord blood banks, would allow the most effective use of these limited resources. Additional innovative approaches will be required to create renewable "off-the-shelf" cell sources. Protocols for stepwise and directed differentiation of embryonic stem cells, and more recently iPS cells, into hepatocytes and HSCs, are currently being optimized. Future challenges lie in validating the in vivo engraftment potential and increasing the production throughput of these cells.

Concluding Remarks

The complex challenge of modeling the most devastating and intractable of human diseases can only be achieved through a concerted, multidisciplinary and multi-institutional effort. The scientific and technological competence required for such efforts covers a wide spectrum of expertise (technical, logistical, ethical), making international collaboration in this endeavor highly attractive. Under the constructive leadership of the Bill & Melinda Gates Foundation, efforts are shared between partners within each humanized mouse consortium, as well as between consortia. By gathering multiple fields of expertise from academic and industrial partners, the consortium structure ensures a true collaboration between highly motivated teams. Although this fresh approach at worldwide collaboration will doubtlessly promote novel models for infectious disease, it would be

shortsighted to rely exclusively on the support of philanthropic organizations. Given the significance of these medical problems and the potential impact of this technology, it is critical for policy makers and their federal funding agencies to invest in this area. Industries would also benefit from participating in existing multidisciplinary partnerships, since their engagement will ensure that the demands of pharmaceutical discovery pipelines are adequately addressed during the technology's development and provide them early access to it. These new tools, which are critical for the development of effective and affordable vaccines and therapeutics, will begin to tackle the urgent health problems confronting our global community.

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