National Institute of Allergy and Infectious Diseases

BIODEFENSE WORKSHOP SUMMARY
HUMANIZED MICE

June 13-14, 2005

Clarion Bethesda Park
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Abstract

NIAID convened a workshop on June 13-14, 2005 on humanized mouse models. Participants [*] presented research findings, discussed the current state of research and made recommendations on the potential use of humanized mouse models for human biomedical research. Topics included the development and use of humanized mice as models for:

- Immune System Reconstitution
- Autoimmunity and Atopy
- Microbial Infection
- Vaccine and Antimicrobial Drug Optimization
- Transplantation Tolerance

The participants expressed the need for support in the propagation and distribution of the existing animal models and solicitation mechanisms for the development and evaluation of new humanized mice and related research, with review panels willing to consider high risk, high impact work. Furthermore, they expressed a need for sponsorship of meetings

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and symposia to educate the scientific community at large about the potential benefits and applications of humanized mouse models.

Introduction

Investigation of the human hemato-lymphoid system, host-pathogen relationships, autoimmunity and transplantation has in the past been limited to *in vitro* assays and *in vivo* large and small animal models. A clear advantage of large animal models such as dogs, sheep, horses and non-human primates is their genetic closeness to humans and the large number of cells that can be obtained. However, ethical, practical and financial considerations greatly limit their use for research purposes. Although more genetically distant to humans, small animals such as mice do share substantial similarities with humans in terms of physiological parameters and genetic diversity; an added advantage is our ability to alter their genome almost at will. A wide variety of mouse models have indeed been instrumental in understanding human physiology, especially the human immune system. Nonetheless, the differences remaining between human and mouse are significant enough to warrant the creation of chimeric mouse models bearing partial or intact human physiological systems. This is accomplished by engrafting human cells and/or human tissues into immunodeficient mouse strains incapable of rejecting these xenogeneic grafts.

The majority of the existing models are of mice that carry a partial or a potentially intact immune system. These models run a wide gamut, and include mice that express human genes in certain blood cell types, mice repopulated with human peripheral blood cells and mice given the complete complement of progenitor cells with the potential to reconstitute the entire immune system. In addition, experimental systems using other human tissue grafts have been created in order to study host/pathogen interactions, vascular regeneration, and transplantation tolerance. Clearly, combining these humanized tissue graft models with the models carrying a complete human immune system holds great promise for the development of improved vaccines and immunotherapeutics.

This workshop provided a general overview of the state of research in the development of a variety of humanized mouse models as outlined below.

Immune System Reconstitution

The inability to completely identify and characterize human hematopoietic stem cells *in vitro* has been a major impediment in hematopoietic stem cell research. Surrogate assay systems are therefore necessary to test the ability of candidate hematopoietic stem cell (HSC) populations to provide stable multilineage repopulation and differentiation into all blood cell types.

Dr. Leonard Shultz from the Jackson Laboratory gave a historical perspective on the development of immunodeficient mouse strains able to support the differentiation and maintenance of a complete human immune system. First reported in 1988 as supporting low levels of human lymphohematopoietic cell engraftment, the C.B17-*scid* (severe combined immunodeficiency) or SCID mouse strain has provided a solid starting point

for human immune reconstitution experiments. While the SCID defect in DNA repair allows some B and T cell leakiness with age, they show a severely impaired adaptive immune system for most of their lives and most importantly retain an intact innate immune system which causes xenogeneic graft rejection primarily through Natural Killer (NK) cell activity. Human HSC engraftment was greatly enhanced by breeding the SCID to non-obese diabetic (NOD) mice, which lack of hemolytic complement and have defects in macrophage, dendritic cells and NK cell function. Incremental improvements were further seen by placing the NOD-*scid* or *Rag1*^{null} mice (lacking B and T cells completely due to a mutation in the recombination activating gene 1) onto genetic backgrounds lacking functional β2 microglobulin *(β2m)* or perforin *(Prf1)* genes. These NOD-*scid* and NOD-*rag1*^{null} derived strains engraft HSCs at high levels and differentiate into multiple myeloid, erythroid and B cell lineages, but do not support T cell development, have residual NK activity and have a short lifespan due to lymphoma development. Dr. Shultz has finally overcome these issues by breeding NOD–*scid* animals to mice that have a null mutation in the IL-2 family common cytokine receptor γ chain gene (*IL2r*γ_c^{null}), encoding a functional subunit of the receptors for interleukin-2 (IL-2), IL-4, IL-7, IL-9, IL-15 and IL-21. This molecule plays an important role in lymphoid development resulting in abnormal thymopoiesis, dysfunctional peripheral T cells and, most importantly, a complete lack of NK cells. The NOD-*scid IL2r*γ_c^{null} mice survive beyond sixteen months of age and resist lymphoma development.

Experiments with these mice done in collaboration with **Dr. Rupert Handgretinger,** from St. Jude's Children's Hospital, and **Dr. Malak Kotb**, from the University of Tennessee, demonstrate a much enhanced engraftment of mobilized human peripheral blood HSCs into NOD-*scid IL2r*γ_c^{null} mice resulting in the differentiation of human B, NK, myeloid, and plasmacytoid dendritic cells in the bone marrow. The engrafted mice are capable of human antigen-specific humoral immune responses and, importantly, show *de novo* T cell development. The spleen, which displays normal human follicular architecture, contains functional mature CD4+ and CD8+ cells that reflect a varied use of V_β genes. Human T cell development was further enhanced by administrating human IL-7, clearly reflecting the dependency of thymopoiesis on epithelially-derived IL-7.

Dr. Fumihiko Ishikawa from Kyushu University in Japan has also used these mice, but in his system the cord blood-derived HSCs are transplanted into the facial vein of neonatal NOD-*scid IL2r*γ_c^{null} mice. This protocol was chosen because it affords a larger transplanted cell dose per body weight than conventional adult transplantation and takes advantage of the greater permissiveness that neonatal systems have for xenogeneic stem cell engraftment and expansion. The transplanted HSCs give rise to the entire complement of human myeloid and lymphoid cells including erythrocytes, platelets, granulocytes and lymphocytes; their development appears to follow the conventional differentiation program, as evidenced by the presence in the recipients' bone marrow of normal hematopoietic intermediate progenitors such as common myeloid progenitors (CMPs), granulocyte/monocyte progenitors (GMPs), megakaryocyte/erythrocyte progenitors (MEPs), and common lymphoid progenitors (CLPs). In addition to showing a normal profile of T cell development and homing to peripheral lymphoid tissues, the engrafted human immune cells are capable of functional adaptive immunity demonstrated

by their ability to mount cytotoxic allogeneic responses in a HLA-dependent manner and to generate antibody in response to T-dependent antigens. Furthermore, the presence of IgA^+ plasma cells in the gastrointestinal mucosa suggests the successful reconstitution of human mucosal immunity. The engrafted human HSCs can support long term hematopoiesis and self renewal, as shown by efficient human immune system reconstitution of secondary recipients.

The NOD background is not essential for successful immune reconstitution of human HSCs, as long as the adaptive and innate arms of the murine immune system are defective. **Dr. Markus Manz** from the Institute for Research in Biomedicine in Germany has successfully transplanted human cord blood HSCs into the liver of huAIS-RG (human Adaptive Immune System *Rag2*^{null} *IL2r*γ_c^{null}) newborn mice which lack B and T cells because of the *Rag2*^{null} mutation and lack NK cells because of the *IL2r*γ_c^{null} defect. This strategy was chosen because, in addition to the permissiveness of the neonatal immune system to xenotransplantation, the liver, one of the major sites of hematopoiesis during fetal and neonatal life, is likely to provide a superior environment for the engraftment and expansion of HSCs. The transplanted animals develop primary and secondary lymphoid tissues that display a normal structure. Both T and B cells develop and are functionally competent, capable of mounting cellular allogeneic responses and antigen-specific immunoglobulin responses marked by the presence of IgM and IgG secreting cells in the blood. Human myeloid and plasmacytoid dendritic cells (mDC and pDC) are also present and show functional antigen presenting activity. As observed in the NOD-*scid IL2r*γ_c^{null} recipients, the HSCs transplanted in huAIS-RG mice support long–term hematopoiesis and give rise to a self renewing population that can engraft in secondary recipients.

Beyond their use to further characterize human HSCs to improve human immune reconstitution, mice that can produce all the components of the human immune system could potentially be very useful as a system to generate human immune cells for stockpiling and populating artificial lymph nodes. **Dr. Takeshi Watanabe**, from the Riken Yokohama Institute in Japan, presented his work on the creation of mouse artificial lymph node-like structures using a biocompatible collagen sponge seeded with mouse thymic stromal cells transfected with lymphotoxin alpha (LTα). These organoids are implanted under the renal capsule of mice and after three weeks they show high endothelial venule-like vessels and follicular dendritic cell networks along with germinal centers. The observation that B cells within the organoids can produce antigen–specific antibodies demonstrates that this artificial system is fully functional. Importantly, the repopulated artificial lymph nodes can be transplanted into syngeneic naive normal or SCID secondary hosts. The next step of this research will be to implant the artificial lymph node scaffolds into NOD-*scid IL2r*γ_c^{null} mice repopulated with human HSCs. This system will facilitate the study of secondary lymphoid organ development and the induction of adaptive immune responses, with possible future application in therapies for immune deficiencies and severe infections.

Autoimmunity

The development of various autoimmune diseases has been correlated to specific allelic variations of class I and class II HLA molecules, which are members the Major Histocompatibility Complex (MHC) gene family. MHC molecule expression in the thymus is essential in the selective processes that normally delete self-reactive T cells and allow only T cells capable of recognizing foreign antigen in the context of self-MHC to enter systemic circulation. Thus it is not surprising that some MHC variations might lead to defects in thymic selection and allow the escape of potentially autoreactive T cells. The HLA locus is the most polymorphic in the human genome, but in spite of the hundreds of existing alleles, only a handful of haplotypes seem to predispose to human autoimmune diseases. The strongest MHC association with an autoimmune disease is between the B27 class I allele and spondyloarthropathies. In addition class II DQ2/R3 DQ6/DR2 haplotypes are strongly associated with Multiple Sclerosis, Thyroiditis, Myasthenia Gravis, Uveitis, and Pemphigus Vulgaris. Class II DQ8/DR4 correlates with Rheumatoid Arthritis (RA), DQ2/R3 with Coeliac Disease, and both are associated with development of Diabetes.

The generation of transgenic mice expressing functional HLA molecules, pioneered by **Dr. Chella David**, from the Mayo Clinic in Rochester, has provided useful models to better understand the pathophysiological role of HLA genes in conferring susceptibility to and induction of autoimmune diseases. When human class II HLA transgenes are introduced in a mouse devoid of endogenous MHC II molecules, their pattern of expression matches the human system, including expression on T cells. The T cells developing in these transgenic mice follow the conventional patterns of differentiation and are functionally competent in mediating cellular and humoral responses. By creating mice transgenic for HLA class II haplotype molecules that predispose to distinct autoimmune diseases, Dr. David has established mouse models for RA, Relapsing Polychondritis, Experimental Autoimmune Encephalomyelitis, Coeliac Disease, and Type 1 Diabetes. These mice are superior to other existing experimental models of autoimmune diseases because they more closely reflect the human pathologies specific to each disease, such as the presence of rheumatoid factor in RA or development of Dermatitis Herpetiformis in Coeliac Disease. In addition to being a good experimental system for the study of autoimmunity, HLA class II transgenic mice have also proven useful for the study of toxic shock. This is a disease mediated by the interaction of staphylococcal and streptococcal enterotoxins with class II MHC. Because the interaction between the bacterial toxins and the mouse MHC class II is not nearly as good as with the human HLA class II, the few *in vivo* mouse models of toxic shock used heretofore have not been very informative. However, the HLA transgenic mice develop full-blown toxic shock syndrome after exposure to inhaled toxins, closely mimicking the human disease in symptoms and pathological effects. Therefore this model will also be very useful for studies of bacterial toxin pathogenesis in addition to toxic shock, including pathologies affecting the lung, skin, and gastrointestinal system. Finally, it should be noted that a very promising application of HLA transgenic mice is their use for the identification of new epitopes of infectious agents and the development of more effective vaccines.

Autoimmune skin diseases have a high prevalence in Western countries, but their complex pathophysiology and the significant differences between human and murine immunity render classical mouse models of such diseases inadequate. Humanized mouse models may be an answer to this problem because they maintain the advantages of small animal models while promising a better correlation with clinical outcome. Humanization in dermatology research is generally achieved either by injecting immune cells or by transplanting human skin onto immunodeficient mice, or by a combination of the two approaches. **Dr. Thomas Zollner**, from Berlex Biosciences, presented work that he and others have done using humanized mouse models of psoriasis for the discovery and testing of new treatment therapies. Psoriasis can be induced by intradermal injection of bacterial superantigens or autologous T cells into non-lesional skin grafts taken from psoriasis patients and grafted onto SCID/*beige* immunodeficient mice. This mouse strain has defective secretory lysosomes, impaired NK cell and granulocyte killing, and accepts adoptively transferred human lymphocytes and solid tissue grafts. The resulting dermatitis resembles psoriasis in some key features such as hyper-para-keratosis, akanthosis, papillomatosis, Munro's abscesses, T cell expansion, keratinocyte hyperproliferation and focal ICAM-1 expression. The gene profiling analyses that can be performed during disease induction and progression promise to be invaluable for the identification of potential drug targets and the design of novel therapeutics. Furthermore, all anti-psoriatic compounds currently used to treat humans have been successfully validated in the SCID/*beige* mice. Therefore, it is believed that efficacy testing data obtained in this humanized mouse model will allow the selection of the best new drug candidates to use in pre-clinical studies.

Microbial Infection and Antimicrobial Therapeutics Development

The original impetus for developing humanized mouse models almost twenty ago was the need to establish a small animal model for HIV pathogenesis. Mice do not have naturally occurring lentiviruses that could be used as a model system nor are they susceptible to HIV infection. The development of a SCID mouse model engrafted with human fetal liver and fetal thymic tissue has provided an invaluable experimental system for evaluating the efficacy and toxicity of new HIV anti-viral therapies. Dengue is another viral pathogen for which there is no vaccine or cure. Dengue virus is a mosquito-borne NIAID Category A pathogen which comprises four serotypes (DEN1-4) and for which there is no *in vivo* disease model. This pathogen is endemic in over one hundred countries, putting over two billion people at risk. There are fifty million cases reported each year and one percent of these cases progress to fatal hemorrhagic fever, which has no effective therapy.

Dengue virus infects peripheral blood monocytes, dendritic cells as well as erythrocytic and liver cell lines. Therefore, most protocols used to study Dengue virus pathogenesis *in vivo* involve infecting SCID mice engrafted with human PBLs or transplantable human erythroleukemia or hepatocarcinoma cell lines. **Dr. Joseph Blaney**, from the National Institute of Allergy and Infectious Diseases, presented his work aimed at developing a tetravalent vaccine able to protect against all four Dengue serotypes. As an alternative to non-human primate models, he and his colleagues generated a SCID mouse xenograft model with the human hepatoma cell line HuH-7 which forms a tumor in these mice. This

inexpensive model has been used very effectively to screen a large number of attenuated Dengue virus vaccine candidates. Infection of SCID-HuH-7 mice by intratumor injection results in levels of viremia approximating those observed in humans and thus provides a realistic setting in which to test vaccine efficacy. The screening of many monovalent vaccines candidates has identified several that prevent normal virus replication in the mice and has led to the current assessment of tetravalent formulations. Importantly, the viral attenuation phenotypes in SCID-HuH-7 mice were confirmed in pre-clinical vaccine testing in rhesus monkeys. Vaccine candidates for DEN1, DEN2, and DEN4, evaluated in both SCID-HuH-7 mice and rhesus monkeys, are presently in clinical trials, with a DEN3 candidate to follow shortly.

Although the SCID-HuH-7 model provides a very effective system for the testing and identification of novel vaccines, it has the disadvantage of not being useful for the study of pathogen-immune interaction since the immune system is of mouse origin. Dr. Shultz presented work done in collaboration with **Drs. Allan Rothman** and **Dale Greiner**, from the University of Massachusetts, in which NOD-*scid IL2r*γ_c^{null} mice engrafted with human cord blood cells were used to study human immunity to Dengue virus. Preliminary feasibility experiments, done with these mice after Dengue infection, show the presence of viral RNA in serum and spleen and Dengue antigen in the bone marrow. Spleen cells also produce Dengue antigen-specific IFNγ responses, suggesting that it will be possible to evaluate infectivity and human immune responses in this infection model.

Dr. J. Victor Garcia-Martinez, from the University of Texas Southwestern Medical Center, also reported on his preliminary studies with Dengue virus infection of NOD-*scid* mice reconstituted with human HSCs. Like what is seen in infected humans, mice receiving a small dose of the virus develop erythremia and thrombocytopenia; furthermore, there are sustained high levels of virus in peripheral blood as well as viral replication in the spleen, liver, and skin.

Taking advantage of the lack of T cells in NOD-*scid* mice reconstituted with human HSCs, Dr. Garcia-Martinez has also used this system as a model of Epstein-Barr virus (EBV) infection of immunocompromised individuals who lack T cells. EBV is one of the most common human viruses for which there is no cure or vaccine. It is a member of the herpes virus family and infects most people sometime during their lives. EBV can establish a lifelong dormant infection within the immune system, which in a small percentage of immunocompetent carriers contributes to the development of Non-Hodgkin's and Burkitt's lymphomas and nasopharyngeal carcinoma in some carriers. However, individuals with compromised cellular immunity such as AIDS patients are particularly prone to developing EBV-associated lymphomas. NOD-*scid* mice engrafted with human HSCs from EBV^+ donors can be easily re-infected with EBV. Interestingly, within a few weeks of infection they develop fulminating lymphoproliferative tumors such as large B cell lymphomas. Thus, this model could prove very useful for the study of EBV infection and tumor promotion and the development of vaccines and antiviral therapies.

Dendritic cells (DCs) play an essential role in the host's immune response to pathogens and therefore are of key importance in achieving effective vaccination. **Dr. Karolina Palucka**, from the Baylor Research Institute, presented her work in vaccine development against the NIAID Category C influenza virus. She uses the HuMouse (Humanized Mouse) model consisting of NOD-*scid* $\beta 2m^{null}$ immunodeficient mice engrafted with human cord blood or peripheral blood mobilized HSCs. Like the NOD-*scid* mice reconstituted with HSCs, the HuMouse system is permissive for human B cell but not T cell development. Thus mature human T cells are adoptively transferred to these mice for functional assays. The studies focus on the development of pDCs and of myeloid dendritic cells (mDCs) because they are important in collectively orchestrating effector immune responses, and thus immunity to pathogens. In these mice, both pDCs and mDCs appear in the bone marrow as early as two weeks after transplant and then migrate to all host tissues, including the lung, spleen and lymph nodes, and display primarily an immature phenotype. Importantly, the ratios of pDCs to mDCs in blood, spleen and bone marrow are similar to what is observed in humans. The HuMouse human DCs show normal innate function, secreting expected levels of interferon alpha (IFNα) in response to *in vivo* intravenous infection with influenza virus (pDCs) or treatment with the adjuvant poly I:C (mDCs). Influenza infection by inhalation or poly I:C treatment also induces the maturation of human DCs in the draining lymph nodes. Above all, the clinical picture emerging from inhalation infection of the HuMouse mirrors human pathogenesis in the lung, with deep lung viremia and the production of a wide variety of inflammatory cytokines. Finally, preliminary data showing that the human dendritic cells can be targeted with DC-specific antibodies provide a solid base for the development of anti-DC antibody conjugates for the specific targeting of vaccine candidates to different DC subtypes.

Mice engrafted with human immune cells are not the only humanized models for the study of host pathogen interactions. In order to study human-specific enteric pathogens for which no good animal model exists, **Dr. Samuel Stanley,** from Washington University School of Medicine, created an experimental model by implanting human fetal intestinal xenografts into the subscapular region of SCID mice. The grafts in this SCID Mouse/Human Intestinal Xenograft (SCID-HU-INT) model develop into closed loop functional segments of human intestine, with appropriate tissue layers and mucin production. They display tissue morphology and cell types specific to the tissue of origin, and contain human epithelial cells, muscle cells, and fibroblasts along with some mouse endothelial cells and infiltrating inflammatory cells. Most xenografts do not contain human lymphocytes, with the exception of intraepithelial lymphocytes (IELs), depending on the age of the donor. However, if fetal liver and fetal thymus tissues from the same donor are cotransplanted, human T cells can be found in the lamina propria of the engrafted intestine. The SCID-HU-INT model thus provides a model similar to intact human intestinal tissue and has proved suitable for the study of several enteric pathogens, including NIAID Category B pathogens *Entamoeba histolytica*, *Shigella flexneri, Cryptosporidium parvum* and *Salmonella typhimurium.* The mice are infected by direct luminal inoculation of the pathogen into the graft and the progression of the disease is analyzed by quantifying parameters of inflammation such as neutrophil influx and damage to the intestinal permeability barrier in the graft. Studies of amebiasis in this

model have shown that intestinal epithelial cells play a critical role in the host innate response to *E. histolytica* trophozoites and in the induction of amoebic colitis that results from the infection; interfering with those responses reduces the ensuing inflammation-induced tissue damage. Similar results were obtained using *S. flexneri*. Importantly, the SCID-HU-INT model allows discrimination among different *Shigella* mutant strains on the basis of their virulence, thus providing a good model for the future evaluation of vaccine candidates. Furthermore, comparison between the human graft genes activated during infection with *E. histolytica* versus *S. flexneri* has led to the discovery of a large number of stereotypic genes along with several pathogen-specific genes. Preliminary studies of the *C. parvum* infection model have also shown clear similarities with the pathophysiology of the disease in humans.

Dr. Kim Barrett, from the University of California San Diego Medical Center, reported on her studies of *S. typhimurium* infection in the SCID-HU-INT model aimed at understanding the pathogenic mechanisms of these bacteria and thus providing a useful system for vaccine development. This non-typhoidal diarrheal pathogen, while not as deadly as *S. typhi*, can be fatal in immunologically vulnerable populations. *Ex vivo* experiments on fetal intestinal grafts developed in SCID mice demonstrate that *S. typhimurium* infection induces an upregulation of cyclooxygenase 2 (COX-2) in epithelial cells and an increase in basal ion transport, perhaps paralleling the diarrheal symptoms induced by this pathogen in human subjects. Importantly, as demonstrated in the models of *E. histolytica* and *S. flexneri*, these data suggest that at least part of the pathophysiology of *S. typhimurium* infection may be primarily due to the host response.

Transplantation

Immunodeficient mice reconstituted with a complete human immune system can be very useful for studies of transplantation tolerance, as long as the human T cell repertoire emerging from the recipient's mouse thymus reflects repertoires developing during normal human thymopoiesis. The lack of human MHC expression on thymic epithelia in these mice, however, prevents the development of a truly "human" repertoire profile. This obstacle can be overcome by expressing transgenic human class I and II HLA genes in mice transplanted with human HSCs. In proof of principle experiments that could be applied to various organ or tissue transplantation studies, Drs. Shultz and Greiner demonstrated that mouse β islets transgenic for human HLA can reverse the symptoms of chemically induced diabetes in NOD-*Rag1*^{null}*Prf1*^{null} recipient mice transplanted with HLA matched PBLs, but that they can be readily rejected by human PBLs of a disparate HLA haplotype. These experiments will be repeated by engrafting human β islets into HLA transgenic NOD-*scid IL2r*γ_c^{null} recipients reconstituted with a human immune system.

While complete human immune reconstitution of a mouse holds the greatest promise for transplantation research in general, there are specific instances where partial reconstitution has allowed significant progress. An example is the work by **Dr. Jordan Pober**, from Yale University School of Medicine, who developed a HuPBL-SCID/*beige* mouse model in which human vascularized skin, artery segments, or synthetic vascular beds consisting of human endothelial cells are engrafted to study what happens *in vivo* in

human immune-mediated vascular tissue injury. When human PBLs are transferred into the transplanted mice, T and B cells successfully engraft but other leukocytes do not. T cells enter the circulation within a week; by two weeks they infiltrate the allogeneic tissues and by three weeks they destroy human endothelial cells within the grafts. Treatment of the grafts with a variety of reagents has allowed dissection of the molecular events responsible for lymphocyte-mediated vascular tissue remodeling, dysfunction, and destruction, thus providing useful therapeutic targets for the induction of transplantation tolerance. More recent work using mice engrafted with HSCs coupled to future use of NOD-*scid IL2r*γ_c^{null} recipient mice will likely bring an even more physiological dimension to this work.

Limitations and Possible Solutions

The field of humanized mouse models has made tremendous progress since its inception over two decades ago. However, a number of practical limitations still prevent the current models from serving as more faithful paradigms of the human system in health and disease. With regards to immune system reconstitution models, more work is necessary to improve the quality of humoral and cellular immunity in terms of B cell affinity maturation and isotype switching, differentiation of broad and functional T cell repertoires, and regulatory mechanisms. To some extent, these issues could be addressed by introducing the transgenic expression of human HLA, and by engrafting the recipient mice with HSCs along with autologous human bone marrow stroma and thymic tissues to enhance plasma cell differentiation and positive and negative selection of thymocytes. Addition of human endothelium, growth factors and chemokines would also improve appropriate lymphocyte migration and organ vascularization.

All of these measures would also be invaluable in studies of autoimmunity, atopy, and transplantation. A case in point was provided by **Dr. Ronald Gill**, from the University of Colorado Health Sciences Center. Data from very elegant experiments of mouse β islet cell transplantation into *Rag*^{null} or NOD mice convincingly demonstrate that the current humanized models of allograft rejection may be biased toward rejection driven by restriction elements expressed by the human graft. Given the lack of human donor-derived antigen presenting cells, except in the graft, the current humanized mouse systems may underepresent pathways of T cell-dependent inflammation driven by the indirect presentation of graft antigens by recipient antigen-presenting cells. In addition to expressing autologous HLA molecules at the appropriate sites, this serious shortcoming might be remedied by expressing human chemokines and homing receptors to ensure the sufficient activation and appropriate homing of the engrafted human innate cells such as macrophages, NK, and NKT cells.

Studies of microbial infection, vaccine and antimicrobial drug development would be greatly enhanced by the expression of human MHC restriction elements in peripheral tissues, human viral receptors, microbial antigens in non-immune cells, as well as the presence of human epithelial grafts to better mirror human viral susceptibility and antigen presentation.

Certainly, implementing all the above strategies may bring humanized mice much closer to becoming "small" humans, thus mirroring more closely the human system in health and disease. However, in evaluating the potential research benefits and clinical applications of humanized mouse models one will have to consider the importance of human MHC diversity and the difficulty in modeling it in the mouse. Especially in the case of immune tolerance and transplantation, while providing important insights into specific immune mechanisms, the clinical translational capacity of even the most optimized humanized mouse models may be limited by lack of diversity in major and minor histocompatibility antigens and by different requirements of small short lived animals versus larger long lived humans. This point was clearly made by **Dr. George Georges**, from the Fred Hutchinson Cancer Research Center, who presented data from a well established canine model of bone marrow transplantation for the prevention of Graft Versus Host Disease (GVHD). The outbred dog model was chosen because the outcomes of canine hematopoietic cell transplantation are consistently similar to the human clinical experience and the relatively large litters provide a model for sibling transplants. Studies of this system have been very useful for developing therapeutic strategies that have improved the safety of human hematopoietic cell transplantation, including most GVHD prophylactic regimens that are currently used in patients. Furthermore, sequencing of the entire dog genome and state-of-the-art canine tissue typing, in conjunction with the availability of a broad array of canine-specific mAbs directed against lymphohematopoietic cells, provide powerful tools for developing new strategies for less-toxic conditioning regimens for hematopoietic cell transplantation. Thus it appears that if and when optimized mice are constructed in the future, it will still be prudent to validate the results in preclinical studies using large animal models such as the outbred dog or non-human primate. The humanized models should be useful in optimizing the likelihood that the most promising drugs or therapies will truly translate into human applications.

General recommendations

The workshop proceedings concluded with a panel discussion highlighting concerns and recommendations related to advancing the field of humanized mice. A major theme was the lack of any dedicated funding stream for the optimization of humanized mouse systems. Most panelists voiced the concern that in the absence of dedicated funds and review panels that understand the need for model development, the largely non-hypothesis-driven research necessary to optimize the models will continue to receive poor ranking in study sections, thus leading to delays in progress. Setting aside special funds for this research and establishing ad hoc reviews for grant proposals of this type might help move the field forward. Another issue raised during the discussion was the need for standardized humanized mouse systems for each general application, i.e. immune system reconstitution, autoimmunity, atopic diseases, microbial infection, vaccine and antimicrobial drug development, and transplantation tolerance. Thus far his goal has been elusive primarily because of the relative infancy of the field, but also because each investigator has had to be affiliated with some specific, hypothesis-driven project in order to fund the construction of each individual model. Again, most panelists agreed that a dedicated financial commitment to the field would aid in the quest for unified models. The very practical issue of model availability and distribution was also raised by a number of panelists. The idea of importation and propagation of existing and

future immunodeficient mouse strains within NIH-supported mouse repositories was advanced. Finally, a strong recommendation was made to organize meetings and symposia to inform the research community about the benefits that could be gained through the use of optimized mouse systems and to obtain additional scientific input on methods to optimize specific models.

A related article in *Journal of Experimental Medicine* to this summary can be found at: http://www.jem.org/cgi/content/abstract/202/10/1307?etoc.