Humanized Mice for Studying Human Immune Responses and Generating Human Monoclonal Antibodies

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ABSTRACT  The new-generation humanized (Hu) mouse models permit multilineage human hematopoiesis and generate T cells, B cells, macrophages, and dendritic cells required for a coordinated human immune response. Therefore, any desired antigen or human-specific pathogens that can infect humanized mice can be used to generate human antibody responses. Two leading humanized mouse models are currently being used. The Hu-HSC model uses the transplantation of human hematopoietic stem cells (HSCs), whereas the BLT mouse model is created by transplantation of human fetal liver, thymus, and HSC. A number of human pathogens such as HIV-1, dengue, Epstein-Barr virus, and hepatitis C virus have been studied in these systems. Responder antigen-specific B cells from these animals can be collected and used to generate human monoclonals by B-cell immortalization or by single-cell PCR methods to “rescue” antibody-producing genes for ectopic expression. Both models generate cellular and humoral immune responses. However, the antibodies generated are primarily of the IgM type because of the inefficient immunoglobulin class switch resulting in the suboptimal production of antigen-specific affinity-matured IgG. The current Hu mouse models thus far have permitted the analysis of human “antibodyome,” and recent reports demonstrated their utility in generating human monoclonal antibodies. Ongoing efforts at further refinements are expected to make these systems more efficient in the near future.

INTRODUCTION
The potential uses of human pathogen-specific antibodies are enormous in terms of both diagnostics and therapeutics. Early applications used polyclonal sera for prophylaxis and therapies, but problems such as allergic reactions, cost, and difficulty in their generation have led to the use of mouse-derived monoclonal antibodies that were humanized by various methods (1). These methods involved substituting part or all of the murine antibody backbone with its human equivalent to derive chimeric or fully humanized antibodies. Less labor-intensive methods used transgenic mice harboring human immunoglobulin genes for immunization to derive human antibodies (2). While this has hastened human antibody generation, some limitations exist, such as differences in the maturation processes between the mouse B cells expressing human antibodies and human B cells secreting human antibodies. Therefore, an ideal way to produce authentic affinity-matured human antibodies is to identify and harness the specific antibody-producing human B-cell clones themselves. Conventional methods involved immortalizing antigen-specific B cells from individuals who either recovered from a disease or were vaccinated with a desired antigen to derive stable antibody-producing cells. Alternatively, more recent high-throughput methods involved rescuing the specific antibody genes from either specific

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plasma cells or memory B cells (3). While these methods have now become routine, they both require collecting B cells from suitable human subjects. In addition to the paucity of specific pathogen-exposed human subjects when needed and the existence of low numbers of antigen-specific cells, there are other practical and ethical considerations. One such consideration is the derivation of antibodies against dangerous pathogens such as Ebola virus. These limitations pointed out the need for a more practical experimental system that permits isolation of large quantities of antigen-specific B cells against any pathogen or antigen of interest. In this regard, newer-generation humanized mice harboring a transplanted human immune system with a capacity to yield antigen-specific B and plasma cells are expected to fill this need (4, 5, 6) and are discussed here.

**IMMUNODEFICIENT MOUSE STRAINS FOR HUMAN CELL RECONSTITUTION**

A common denominator for all humanized immune system mouse models is the transplantation of mature or progenitor human hematopoietic cells by various routes into immunodeficient mice receptive to xenografts without graft rejection. In this regard, there has been a gradual evolution and improvements in the generation of immunodeficient mouse strains (7). Early strains such as nude mice, while lacking T cells and thus having defects in T-cell responses, still harbored mouse B cells and NK cells and therefore were not permissive for human cell reconstitution. The availability of severe combined immunodeficiency (SCID) mice that lacked both T and B cells and NK cells and therefore were not permissive for human cell reconstitution led to the creation of SCID-hu mouse models (3). While these methods of hu-peripheral blood lymphocyte (PBL)-SCID and SCID-hu mouse models (see below). Later improvements gave rise to nonobese diabetic (NOD)-SCID mice that have a lower level of NK cells and other innate immune defects that permit higher levels of human cell and tissue engraftments but were still less than ideal. A major advancement was the targeted disruption of the interleukin-2 (IL-2) receptor common gamma chain (IL2-Rcγ) coding for the common and essential signaling component for the action of cytokines IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 (7, 8). Impairment of IL-7 and IL-15 signaling blocks native mouse NK-cell development, thus permitting enhanced human cell engraftment. This mutation, when introduced together with the SCID, NOD, RAG1, or RAG2 gene mutations in different combinations by selective breeding, yielded a number of new-generation severely immunocompromised recipient mice for superior human cell engraftment (6). These include Rag2γ–/–, Rag1–/–γ–/– (RG), NOD/shi-scid/cγ–/– null (NOG), and NOD/SCID/cγ–/– (NSG) mice. Ongoing efforts are being directed toward introducing HLA class I and II human immune system and cytokine genes to generate new transgenic mice to permit more robust human antibody and cellular immune responses (see below).

**TABLE 1**

<table>
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<tr>
<th>Model</th>
<th>Generation/mice used</th>
<th>Advantages</th>
<th>Disadvantages</th>
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**Hu-PBL Mice**

Hu-PBL mice are generated by the transplantation of human mature peripheral blood mononuclear cells (PBMCs) via the intraperitoneal (i.p.) route into SCID mice (9). More recent versions use either NSG or RG mice. The transplanted human immune cells persist for several weeks and show effector functions. These mice can be productively infected by viruses such as HIV-1 that target cells of the human hematopoietic system. Human memory B cells continue to produce antibodies that target cells of the human hematopoietic system. Can be productively infected by viruses such as HIV-1 xeno-GVHD.

**SCID-Hu MICE**

Coimplantation of human fetal thymus and liver (containing hematopoietic stem cells) fragments under the SCID mouse renal capsule generate mice that harbor a functional human thymus (called the thy/liv organoid) (10). These mice primarily produce human thymocytes and naive T cells. These T cells predominantly reside in the thy/liv organoid, and there is poor peripheral T-cell circulation. Because of the insufficient generation of a full spectrum of immune cells, they lack the capacity to generate a human immune response. Nevertheless, these models have been instrumental in the study of some key aspects of viral pathogenesis with viruses such as HIV and HTLV and laid the foundation for the generation of new humanized mouse models (11).

**Hu-HSC Mice**

This model involves transplantation of hematopoietic stem cells (HSCs) into a variety of immunodeficient mice and has evolved substantially over the years (7, 12, 13). Early versions involved the injection of hematopoietic progenitor cells (CD34+ cells) (also termed SCID repopulating cells) into conditioned adult SCID mice by intravenous (i.v.) or intrafemoral routes. While there was de novo lymphopoiesis, T-cell development was poor. Use of new-generation IL-2 γc−/− that encompasses RG, NOG, or NSG mice led to better engraftment. There are two versions of these with important differences. One is the injection of HSCs into adult irradiated NSG/NOG mice. While multiple hematopoietic lineages are generated, there is a poor yield of T cells. The second is intrahepatic injection of HSC into conditioned newborn RG, NSG, or NOG mice that results in superior human cell engraftment and the generation of T cells, B cells, macrophages, NK cells, and dendritic cells (6, 13). Infection of both versions of these mice with different pathogens or immunization with different antigens gives rise to human immune responses (see below). There is also mucosal human cell engraftment permitting HIV-1 infection by mucosal routes (14).

**BLT Mice**

This model is a slight modification and consequent improvement of the earlier SCID-hu mouse model. The name derives from transplantation of bone marrow, liver, and thymus (BLT); the main difference from SCID-hu mouse model is the additional transplantation of autologous HSCs purified from fetal liver (13, 15, 16). The original BLT version used NOD-SCID mice, whereas the new improved versions use NSG, NOG, or RG mice (17, 18). Superior human cell engraftment with the generation of T cells, B cells, macrophages, NK cells, and dendritic cells is seen. The presence of autologous human thymus permits appropriate T-cell education and human T-cell restriction.

**Hu-Liver-HSC Mice**

The models described above are restricted to human immune system transplantation with the generation of human immune cell subsets. As noted, human pathogens that infect the human hematopoietic system can be studied in addition to generating human immune responses against a variety of antigens. To further broaden their application in infectious disease research, a recent development is the derivation of Hu-HSC mice that permits infection with other human-specific pathogens with a predilection to infect other human organ systems such as the liver. Transgenic mice that simultaneously permit human hepatocyte and HSC engraftment were recently developed (19). This permitted creation of Hu-liver-HSC mice susceptible to the hepatitis C virus and with a capacity for human immune response.

**OVERVIEW OF Hu MOUSE PREPARATION, INFECTION, AND IMMUNIZATION**

A general outline of Hu mouse preparations is depicted in Fig. 1. Preparation of Hu-HSC mice is not technically intensive since no surgery is involved (13). Immunodeficient mouse strains, namely RG, NOG, and NSG, are commonly used (6, 20, 21, 22, 23). Injection of HSC into neonatal mice versus adult mice gives far superior engraftment with the generation of human immune-competent mice that harbor all four needed immune cell subsets, namely T cells, B cells, macrophages, and
dendritic cells. Newborn mice, preferably within 3 days of birth, are injected with CD34+ HSCs from different sources that include cord blood, fetal liver, or human bone marrow. Of these, the efficiency and duration of engraftment appear to be better with the fetal liver-derived CD34+ cells because of their more primitive lineage status in development. The following protocol is routinely used in our laboratory and yields well-engrafted mice. Single-cell suspension is prepared from fragments of human fetal liver (16 to 20 weeks gestation) by enzymatic digestion with collagenase, DNase, and hyaluronidase. The cells are incubated with CD34 antibody and later subjected to immunomagnetic bead-based positive section. The purity of CD34+ cells generally ranges between 90% and 99% after two successive cycles of selection. The purified cells are cultured overnight in a human cytokine media mix containing IL-3, IL-6, and stem cell factor (25 ng of each per ml). Preferably, fresh cells are injected into neonates, although previously frozen cells can also be used. The neonatal mice are irradiated at 350 rads 2 to 4 hours before cell injections. We routinely use 5 × 10^5 human fetal liver-derived CD34+ cells per mouse pup to ensure consistent engraftment, although lower numbers of cells can be used. Cells are injected via insulin syringe in a 25-μl volume intrahepatically by visualizing the dark area occupied by the liver under the relatively transparent skin (Fig. 2). Postinjection, pups are returned to their mothers and weaned 3 weeks later. Mice are housed in BSL-2 conditions. The engrafted mice are screened to determine the levels of human CD45+ cells in peripheral blood at approximately 12 weeks of age. In general, we obtain mice with 40% to 90% human cell engraftment. Human cell engraftment is seen in primary and secondary lymphoid organs. In addition, mucosal engraftment with human cells is also seen in the female reproductive tract as well as in the gut, permitting HIV-1 mucosal transmission. In general, 20 to 30 Hu-HSC mice can be made with a typical batch of fetal liver derived CD34+ cells.

Original preparations of BLT mice used NOD-SCID mice, and these have been effectively used in many experimental settings including HIV-1 mucosal viral transmission (15, 16, 24). Owing to far superior engraftment, more recent protocols use NSG mice (17, 25, 26). Male mice are preferred for surgical tissue implantation because of their larger kidney size. Adult mice are conditioned by sublethal whole body irradiation at 325 rads before transplantation. Human fetal thymic and liver tissues are dissected into 1-mm fragments and introduced together under the left kidney capsule of
anesthetized mice by the use of a trocar. Later, each of
the mice is injected (i.v., tail route) with autologous 2.5 ×
10^5 CD34+ HSC purified from the remaining fetal liver.
The reconstituted mice are evaluated for human cell
engraftment at 12 to 16 weeks posttransplantation be-
fore the use for various experiments. In general, 15 to 20
mice can be made with a typical set of fetal tissues.

While different human pathogens and different im-
munogens are studied in Hu mice based on the need
of the investigator and the types of human tissues
transplanted, the following general scheme provides a
broad overview of these protocols. Viral pathogens such
as HIV-1, dengue, and Epstein-Barr virus (EBV) have
been studied more frequently to date in these new
models. With HIV-1, the most common route of ex-
perimental infection is via the i.p. route for pathogenesis
and therapeutic studies. A typical infection involves
either the CCR5-tropic viral strain BaL or the CXCR4-
tropic strain NL4-3. Injection with 1 × 10^5 IU gives rise
to viremia within a week and persistent life-long infec-
tion, based on the maintenance of human cell engraft-
ment that in turn depends on the quality of the HSC
injected (22, 27). For dengue viral infection, the viral
inoculum is delivered by i.p., subcutaneous, or intra-
dermal routes (28, 29). Infected mice develop acute vi-
remia generally lasting for 3 weeks. With EBV 1 × 10^5 to
1 × 10^6 RIU are injected i.p., and immune responses are
assayed at 4 to 10 weeks. With HIV-1, antibody re-
sponses are monitored at approximately 4 to 6 weeks.
IgM responses can be seen with dengue viral infection
within 2 weeks at the earliest, whereas IgG responses
take much longer, usually about 8 weeks. For experi-
mental immunizations, a variety of antigens have been
used. These include tetanus toxoid (TT), hepatitis B virus
(HBV), HIV-1, and West Nile virus envelope (WNV-E)
antigens among others (7, 17, 21). With regard to hu-
mance vaccine preparations, three doses each of HBV or
TT vaccine (each corresponding to 1/10 the human dose)
are given intramuscularly (i.m.) 2 weeks apart to Hu-
HSC RG mice (21), and antibody responses are evalu-
ated starting 2 weeks later. In a recent protocol using
BLT mice, recombinant HIV-1 envelope or WNV-E
protein were mixed with a synthetic adjuvant IC31 and
injected i.m. in both quadriceps muscles (17). Three
doses were given on days 0, 21, and 45, and mice were
monitored for immune responses for 90 days.

HUMAN CELL RECONSTITUTION AND
ANTIBODY RESPONSES IN Hu MICE

A number of reports documented the generation of
antigen-specific human antibody responses in Hu-HSC
mice (29, 30, 31, 32, 33, 34, 35, 36). Both IgM and IgG

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responses were reported by different investigators, although, in general, IgG responses were found to be weak. Human antibody repertoire in Hu-HSC RG mice by analysis of the length of the CDR3 hypervariable regions revealed that the IgM B-cell repertoire was similar to that seen in normal healthy individuals, thus indicating no obvious limitations to generate a wide spectrum of human antibodies of various specificities (21). However, immunizations of Hu-HSC RG mice with TT and HBV vaccines gave a predominantly IgM response with limited antigen-specific IgG production, indicating a general failure to class switch. The paucity of antigen-specific IgG is puzzling given that these mice do accumulate total serum IgG efficiently. One study used PBMC transfected with a human T-cell receptor specific for influenza hemagglutinin (HA) peptide and bearing an HLA matched with that of the transplanted HSC used to prepare the Hu mice. When these T cells were transferred into the respective mice, anti-HA human IgG response was observed, suggesting that isotype switch deficiency is not due to an intrinsic defect in the B cells but rather to impairment in T-cell cooperation (37). This impairment may be due to human T-cell restriction by murine major histocompatibility complex (MHC) in addition to a potential T-cell dysfunction. When IgD+CD19+ naive B cells from Hu-HSC mice were treated in vitro with anti-CD40 antibodies, IL-2, and IL-21 in the presence of antigen, they became activated and secreted IgG, again confirming the functionality of the B cells in these mice. In evaluating T-cell help, another study using HLA-DR4 (MHC class II) transgenic mice and reconstitution with matching HSC reported improved immune responses that included IgG class switching and higher levels of IgG production (38). A more recent phenotypic analysis of B cells from Hu-HSC mice prepared by using adult NSG mice indicated a normal B-cell developmental pathway (39). However, molecular analysis of single B cells indicated that, while the overall distribution of Vh genes reflected a normal human antibody repertoire, mature B-cell subsets showed autoimmune characteristics (39). Overall, the wide variations seen in Hu-HSC mice with regard to antibody production and class switch could be attributed to a number of factors. These include lack of proper human T-cell restriction and help as well as differences in protocols, including utilizing neonatal mice versus adult mice for HSC transplantation and HSC sources, namely cord blood and fetal liver. In any case, additional improvements are necessary as discussed below. SCID-hu mice have been further improved to generate BLT mice that provide a more appropriate thymic environment for human T-cell development and improved T-B cell cooperation (15, 16). This resulted in a more robust T-cell development and multilineage hematopoiesis including the generation of B cells, macrophages, and dendritic cells. The lineage-specific differentiation, positive and negative selection of T cells is expected to occur in the autologous human thymus. T cells in these mice were shown to generate MHC class I and II restricted human immune responses and offer T-cell help to the antigen-stimulated B cells (16, 40). A number of early studies have shown both IgM and IgG antigen-specific responses, albeit with varied robustness. However, later studies failed to show antibody class switching despite repeated booster immunizations (41). A recent study evaluated BLT mice more thoroughly to determine the antigen-specific antibody responses by immunization with adjuvanted HIV-1 and WNV envelope antigens (17). Profound differences were noted both in terms of B-cell composition and antibody responses in comparison with healthy human immune responses. Even repeated booster immunizations did not result in secondary responses characterized by generation of IgG. Unlike in the human, there was an abundance of a “B-1 like” B-cell population (CD19+ CD5+). The predominant antibody response characterized by the IgM phenotype and lack of IgG is attributed to the CD5+ B-cell subset that is believed to produce “natural antibody” by using a T-cell-independent pathway.

T-CELL RESPONSES AND HLA RESTRICTION IN Hu MICE

Initial reports showed induction of antigen-specific T-cell responses in Hu-HSC mice against various human pathogens (14, 42, 43). Immune control of HIV-1 and EBV infection was abolished by the depletion of CD8 T cells in Hu-HSC mice, thus providing additional indirect evidence for their role in protection (44, 45). However, other studies reported deficiencies in T-cell responses (37, 46, 47). While polyclonal stimulation of Hu-HSC mouse splenocytes by phytohemagglutinin, anti-CD3/anti-CD28 antibodies, and phorbol myristate acetate/ionomycin lead to cell proliferation and cytokine secretion, they were 10-fold less than what is seen with human PBMC, suggesting a functional defect. Moreover, human T cells responded poorly to in vivo immunizations as shown by the lack of interferon-γ or IL-4 secretion after specific antigen restimulation ex vivo. Specific CD4 and CD8 responses were measured by cytokine secretion assays, cell proliferation assays, or
cotoxic assays in vitro by restimulation of cells from mice infected with either HIV-1 or EBV. Even in reports showing immune activity, the responses are low and are attributed to several factors. The overall low level of T cells is believed to result from a potential lymphopenia-induced T-cell activation among other causes. Another reason for low levels of T cells in Hu-HSC mice is thought to be a lack of HLA restriction, since T-cell selection is happening in xenogenic mouse thymus in a H-2-restricted fashion, which might not be efficient for human cells. Furthermore, the low numbers of T cells generated also display poor survival in the periphery owing to their suboptimal interactions with mouse APCs and weak signaling (30). Supporting these possibilities, it was recently reported that when RG mice transgenic for HLA-DR4 were reconstituted with matching HLA-DR4 CD34 cord blood cells, the number of thymic and peripheral T cells was drastically increased. Shultz et al generated class I HLA-A2 transgenic mice and reconstituted them with matched HSC (6). These mice showed HLA restricted cellular immune responses to EBV. Based on these data, it has now become evident that enforced expression of HLA-A2 and HLA-DR4 enables HLA-restricted T-cell functions which also correlate with improved cytokine secretion and IgG production. Another desirable alteration in these mouse models is to knock out the murine MHC expression such that unwanted H-2-restricted responses can be avoided by the transplanted human immune cells. T-cell responses in BLT mice appear to be more normal because of reconstitution with autologous human thymus and HSC, thus permitting HLA restriction of T-cell responses and more efficient T- and B-cell interactions and cooperation. HIV-1 infection in BLT mice demonstrated epitope-specific T-cell responses to HIV antigens in a class I restricted manner in a recent study (48). Anti-HIV-1 CD8 responses were found to mimic those in humans in terms of their specificity, kinetics, and immunodominance. Furthermore, it was found that mice expressing the particular HLA class allele HLA-B 57 showed enhanced control of HIV infection as seen in humans that bear the same allele.

**GENERATION OF HUMAN MONOCLONAL ANTIBODIES IN Hu MICE**

Since humanized mice are shown to harbor a normal human antibody repertoire, they can be exploited to generate a broad spectrum of antibodies, both neutralizing and nonneutralizing. The scope of their use is further broadened by the creation of new models, such as those harboring human liver, so that agents like hepatitis viruses can also be used to generate specific antibodies. A general schematic summarizing different methods of deriving human monoclonal antibodies in Hu mice is presented in Fig. 3. The broad scheme would involve either infection or vaccination with a desired antigen with or without an adjuvant. At the peak time of immune response, the splenocytes containing the B cells are harvested for later selection, expansion, and immortalization. Alternatively, antigen-specific individual B cells can be sorted postimmunization, and their antibody genes can be cloned and later incorporated into an expression system to yield large quantities of antibodies. Furthermore, the specific antibody gene sequences can also be class switched based on what type of antibody is desired for therapeutic purposes by the use of molecular techniques. Following some of these lines, one recent report described the entire process of generating human monoclonal antibodies by using Hu-HSC RG mice (21). This is summarized in brief here as a basis for broader applications. Mice were immunized with commercially available TT and HBc vaccines as described above. Postvaccination, memory B cells expressing surface immunoglobulins were fluorescence-activated cell sorter sorted from splenic and mesenteric lymph node single-cell suspensions. The sorted B cells were immortalized by transduction with a retroviral vector encoding BCL6 and BCL-XL genes and cultured in the presence of CD40L and IL-2. Another novel method that can be used is STAT5 overexpression to immortalize B cells (49). Supernatants from the immortalized B-cell pools cultured in microtiter plates were tested by enzyme-linked immunosorbent assay to identify specific antibody-producing wells. Cells from the desired antibody-positive wells were subjected to limiting dilution culturing to obtain monoclonal B-cell lines. In this study, only IgM antibody-secreting B-cell clones could be obtained, although IgG responses were seen in the immunized mice, albeit at lower levels. Thus, further improvements in the protocol are necessary to capture and immortalize these rare IgG-producing B cells. Other options available for efficient B-cell immortalization involve transformation of antibody-producing B cells activated by TLR9 agonists like CpG followed by high-efficiency electofusion with myeloma cells (50). Alternatively, using a molecular cloning approach, antigen-specific antibody genes can be rescued from the respective B cells for high-efficiency expression in a surrogate system, as has been accomplished with a number of antigens (51).
HUMAN PATHOGENS AND OTHER ANTIGENS STUDIED IN Hu MICE

A variety of human pathogens, particularly viruses, have been studied in new-generation Hu mice. HIV-1 is by far the most widely studied pathogen (22, 36, 47). Hu mice have also been used to study HTLV-1 proviral integration and the induction of T-cell lymphomas (52, 53).

Studies of other virus families have also been gaining momentum. A number of studies focusing on dengue viral infection showed viremia with concomitant humoral and cellular responses (28, 29). Hu mice with human hepatocyte reconstitution allowed infection with hepatotropic viruses such as hepatitis C virus and HBV inducing pathologies and immune responses (19, 54).

A variety of human herpes viruses have also been studied. These reports documented HLA-restricted adaptive T-cell immune responses to EBV, cytomegalovirus reactivation from latency, protective innate and adaptive immune responses against intravaginal HSV-2, and generation of an anti-Kaposi’s sarcoma-associated herpesvirus-antibody immune response (16, 43, 55, 56, 57). More recent studies have expanded the use of Hu mice to other nonviral pathogens. These encompass work with drug-resistant Salmonella enterica serovar Typhimurium (58, 59), persistent infection with the malaria parasite Plasmodium falciparum (60), and detection of Hu mouse adaptive and innate immune responses against Leishmania (61). In addition to live pathogens, a number of antigens and human vaccine preparations have also been tested to evaluate Hu mouse human immune responses. Immunization with DNP (23)-KLH antigen generated human T-cell proliferation and human IgG responses (40). Toxic shock syndrome toxin 1 caused an expansion of human T cells and activation of human dendritic cells (16). Administration of a variety of vaccines demonstrated adaptive immune responses including influenza-specific human CD8+ T cells, human IgM antibody responses to tetanus toxoid and HBV (21, 62). This list is only a partial representation of that reported in the current literature which is expected to grow because the use of these mouse models is expected to become more widespread.

ADVANTAGES AND DISADVANTAGES OF DIFFERENT Hu MOUSE MODELS

From the standpoint of generating human monoclonal antibodies, there are advantages and disadvantages with each of the two new Hu-HSC and BLT mouse models. With regard to the ease of preparation, the Hu-HSC model is relatively easy to create since only a quick intrahepatic injection of HSC is needed and does not involve surgery. Additionally, human HSC from easily procurable sources such as cord blood can be used, and larger cohorts of mice from a single donor can be made. However, a disadvantage with this model is the lack of proper human T-cell restriction and ideal T- and B-cell cooperation because of the absence of an autologous human thymus. Coexpression of HLA class I and II genes by transgenesis will overcome this deficiency. A particular advantage with the BLT mouse model is the presence of transplanted human thymus, thus permitting proper T-cell education and T-cell restriction thus offering a better T- and B-cell cooperation. Both IgM and IgG responses are seen, although the IgG responses are found to be weaker, akin to that seen with the hu-HSC model. Major disadvantages are the complicated surgery required to implant human fetal tissues under the kidney capsule, and the number of mice that can be generated from a single fetal donor tissue is limited. Large-scale exploitation of this model also poses challenges owing to the requirement for fetal tissues that are limited in supply and often difficult to procure.

LIMITATIONS TO BE OVERCOME, CURRENT ADVANCES, AND FUTURE PROSPECTS

While the current human immunocompetent mouse models have come a long way since the description of the original human-mouse chimeras, there are several limitations that need to be overcome (9). These encompass (i) residual innate immunity in the immunodeficient mouse strains, thus requiring irradiation and prior conditioning; (ii) less than ideal T-cell numbers and lack of full maturation of B cells; (iii) lack of human HLA class I and class II restriction in hu-HSC mouse models and lack of appropriate levels of HLA APCs in the BLT mouse model; (iv) deficiencies in T- and B-cell cooperation resulting in low levels of antibody responses and inefficient immunoglobulin class switch; and, finally, (v) poorly cross-reactive native murine cytokines and required growth factors.

Some of these deficiencies are currently being tackled and rectified as detailed below. In addition to T cells, B cells, and NK cells, macrophages are also found to contribute to xenograft rejection in Hu mice. Mouse macrophages expressing native SIRPα receptor remove xenografted human cells that do not express the cognate ligand CD47 (“don’t eat me” marker of self) (63, 64). In this context, it has been recently shown that human SIRPα transgenic mice exhibit improved human cell reconstitution (65). Conversely, human HSC stably transduced with murine CD47 ligand encoding lentiviral vectors also showed increased engraftment (66). Thus, improvement of constitutive CD47-SIRPα interactions in Hu mice will enhance the survival of human cells in the mouse environment and will provide more robust and lasting human cell engraftment. As mentioned above, the expression of human HLA-DR4 in transgenic mice also leads to increased T-cell numbers in reconstituted mice. Coexpression of HLA class I and class II in doubly transgenic mice is likely to further improve human T-cell reconstitution and T-B cell cooperation, as well as help and mediate HLA-restricted immune responses (30). The knock-in of the human HLA class I and class II genes in mouse MHC loci will preclude the
unaltered mouse H-2-restricted human cell immune responses. Since many murine cytokines and growth factors are poorly cross-reactive with their corresponding human cell receptors, thus contributing to suboptimal human cell development and maintenance, supplying these in trans either by injection or by transgenesis overcomes these deficiencies. These cytokines include granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, macrophage colony-stimulating factor (M-CSF) for monocyte/macrophage, IL-7 for T cells, IL-15 for NK cells, and erythropoietin for erythrocytes (67). Knock-in replacement of mouse cytokine genes with their human equivalents in respective loci has an additional advantage of their constitutive expression governed by the mouse regulatory elements. Indeed, with the use of this approach, respective transgenic mouse strains have been developed. Expression of human thrombopoietin resulted in higher human cell engraftment and better HSC maintenance (68). Transgenic mice with human IL-3, GM-CSF (67), and M-CSF (69) knock-in genes exhibited improved myeloid differentiation and function, thus demonstrating the benefits of these enabling strategies. Thus, as can be seen from above, the current ongoing intensive work in different areas of generating Hu mice has identified several areas for improving the existing human immunocompetent models. However, these different strategies need to converge to yield a better mouse model. This will involve breeding a composite recipient mouse strain incorporating all desirable attributes. Such a mouse should permit ideal engraftment of human HSC and give rise to a Hu mouse capable of a robust human antibody response encompassing immunoglobulin class switching and high-affinity maturation. Given the recent rapid progress, such an ideal system will soon be available.

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