Humoral Immunity in Humanized Mice: A Work in Progress

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Humanized mice historically have not been good models of human humoral immunity induced by either infection or immunization. However, newer versions of humanized mice generated in severely immunodeficient mice with a targeted disruption of the IL2Rγc gene have recently been reported to produce antigen-specific class-switched human antibodies, with some demonstrating neutralizing activities. Here we review the growing ability of humanized mice to support the study of human humoral immune responses, discussing the current and future potential of these models as well as their current limitations.

Keywords: immunology; humanized mice; BLT mice; fetal tissue transplantation; humoral immunity; B cells; antibody.

For the past several decades, investigators have created “humanized” mice to bridge the gap between small animal models and human studies and to examine the human immune system in an experimental setting. Humanized mice were first generated by transplanting human cells and/or tissues into mice with severe deficiencies of their own immune systems [1–3], and this basic approach has continued to the present. Advances in these models have come from gradual improvements in the ability of the strains of immunodeficient mice to engraft donor human cells and tissues, and from refinements in the procedures used to engraft those cells and tissues, as has recently been reviewed [4–6]. One of the major recent advances in this field came from the development of immunodeficient mice with a null mutation in the interleukin-2 (IL-2) receptor common γ-chain locus (IL2Rγc), the use of which has resulted in far higher levels of human cell engraftment than previously possible [7]. This review will focus on the capacity for modeling human humoral immunity in the newest generation of humanized mice that utilize IL2Rγc-null immunodeficient mice, particularly BLT (bone marrow-liver-thymus) and Hu-HSC (human hematopoietic stem cell) mice. BLT mice are generated by co-transplantation of human fetal liver and thymus fragments under the mouse renal capsule followed by intravenous injection of CD34+ HSCs isolated from the same fetal liver [8, 9]. Hu-HSC mice, in a current version also referred to as HIS (human immune system) mice, are generated by injecting newborn mice with human HSCs isolated from umbilical cord vein blood [10, 11], granulocyte-colony-stimulating factor-mobilized adult blood [12], or fetal liver [13]. Both mouse models are sublethally irradiated prior to HSC injection. Broad overviews of these new humanized mouse models have recently been published [7, 14]. This review will focus on their ability to model human humoral immune responses, describing their current capabilities and limitations and their potential for future improvement.

B-CELL DEVELOPMENT

Multiple investigators have documented incomplete B-cell development in both BLT and Hu-HSC mice [15–18], which most likely will need to be overcome to further improve the capacity of these mice to model human humoral immunity. We previously reported that even though mature human follicular B cells,
marginal-zone B cells, and plasmablasts were all present in BLT mice, a higher than expected proportion of the CD19+ B cells in the bone marrow and spleen were CD10+, suggesting some skewing toward immature cells [16]. The majority of human B cells in the peripheral blood of BLT mice express the CD5 antigen, also suggesting deviation of B-cell development in these mice from normal human B-cell development [15]. B-cell CD5 expression in humans is not as clearly characterized as it is in mice, and does not necessarily denote B-cell immaturity. Most mature B cells in human fetal circulation express CD5 [19], whereas most CD5-expressing B cells in adults are pre-naive or transitional B cells [20], which comprise only 9% of total CD19+ B cells in the peripheral circulation. B-cell development has similarly been reported to be incomplete in Hu-HSC mice. The human B cells in Hu-HSC mice are predominantly immature [18], and a larger than expected percentage of their CD19+ B cells express CD5 as well [21].

Several investigators have noted, however, that human B-cell maturity in humanized mice improves with increasing time following their human cell reconstitution. For example, we observed that the numbers of mature follicular type II (FO-II) cells present in BLT mice were greater at 22 weeks posttransplantation than at 20 or 16 weeks, suggesting that B-cell maturation increases in BLT mice with longer duration of reconstitution [16]. A recent study has suggested that human B cell development in Hu-HSC mice continues to improve with increased time as well [22]. Comparing B-cell maturity in these mice at different times after they were generated, most of the human B cells were immature in Hu-HSC mice assessed prior to 18 weeks, as others have previously shown. With increasing time postgeneration, however, most B cells were mature (CD20+CD10+) in an increasing percentage of mice, with this percentage surpassing 60% after 24 weeks [22]. An earlier study of Hu-HSC mice pre-conditioned with irradiation alone, irradiation plus busulfan, or busulfan alone noted that full maturation of the B cells (and T cells) of these mice occurred 5 to 6 months after these mice were generated [17]. Taken together, these studies suggest that human B-cell maturation in both BLT and Hu-HSC mice would be improved by delaying their use until 5 to 6 months postgeneration. In BLT mice as they are currently generated, however, the benefit of improved B-cell maturation at these later times is limited by the occurrence of xenogeneic graft-versus-host disease (GvHD). We and others have observed this form of GvHD, in which the donor human T cells target recipient mouse antigens [23], to occur with increasing frequency, morbidity, and mortality in BLT mice at increasing lengths of time postreconstitution [7, 24]. As investigators find ways to mitigate the problems caused by xenogeneic GvHD in humanized mice [25, 26], delaying their use for 5 to 6 months in order to improve human B-cell maturation may become more practical until better strategies are developed and standardized, some of which are discussed below.

### PRODUCTION OF BINDING ANTIBODIES

Multiple studies have measured circulating human antibody levels in BLT or Hu-HSC mice, either at baseline or in response to infection or vaccination, and have produced variable results. Table 1 shows the variability in total immunoglobulin G (IgG) and immunoglobulin M (IgM) blood levels serum levels in naive Hu-HSC and BLT mice, which overall are substantially lower than those found in humans, particularly human adults. In the case of infection, Epstein–Barr virus (EBV)–infected Hu-HSC mice were reported to have no detectable EBV-specific IgM or IgG responses to viral capsid antigen [30], and several studies of human immunodeficiency virus (HIV)–1-infected Hu-HSC found little or no production of HIV-specific IgM or IgG antibodies between 3 to 10 weeks postinfection [17, 31, 32]. However, HIV-specific class-switched human antibodies have now been detected in HIV-infected Hu-HSC mice both by Western blot [33] and by enzyme-linked immunosorbent assay [34]. In BLT mice, Sun et al [35] detected HIV-specific IgG antibodies against at least 2 HIV proteins by 8 weeks postinfection in 3 of 4 mice. We found that all mice infected with HIV for 12 weeks or longer demonstrated human antibodies to at least 7 HIV proteins by Western blot analyses [27]. In unpublished studies presented at the Recent Advances in Humanized Mice: Accelerating the Development of an HIV Vaccine workshop (Harvard Medical School, Boston, MA, 5 November 2012), we found titers of HIV-specific class-switched human IgG antibodies comparable to those in infected humans. These antibody titers often took until 20 weeks postinfection to develop in BLT mice (that were 13–18 weeks post-human fetal tissue

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline IgG and IgM Levels in Humans and Humanized Mice</th>
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<tr>
<td>Age</td>
<td>IgG (mcg/mL)</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
</tr>
<tr>
<td>&gt;16 y</td>
<td>11580 ± 3050</td>
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<tr>
<td>Infants</td>
<td></td>
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<tr>
<td>1–3 mo</td>
<td>4300 ± 1190</td>
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<tr>
<td>4–6 mo</td>
<td>4270 ± 1860</td>
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<tr>
<td>Hu-HSC</td>
<td></td>
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<tr>
<td>2.5–3 mo p.t.</td>
<td>1.1</td>
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<tr>
<td>2.5–3.5 mo p.t.</td>
<td>143 ± 12</td>
</tr>
<tr>
<td>3 mo p.t.</td>
<td>257 ± 76</td>
</tr>
<tr>
<td>6 mo p.t.</td>
<td>175a</td>
</tr>
<tr>
<td>6 mo p.t.</td>
<td>1000-3000a</td>
</tr>
<tr>
<td>BLT</td>
<td></td>
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<tr>
<td>3–3.5 mo p.t.</td>
<td>165</td>
</tr>
<tr>
<td>4 mo p.t.</td>
<td>140a</td>
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<tr>
<td>5 mo p.t.</td>
<td>ND</td>
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Abbreviations: BLT, bone marrow-liver-thymus; Hu-HSC, human hematopoietic stem cell; Ig, immunoglobulin; ND, no data; p.t., posttransplantation. * Approximate values gathered from figures.
transplantation) though, possibly consistent with the prolonged times required for improved human B-cell maturation in these mice as discussed above. The occurrence of Ig class-switching in these mice suggests that activation-induced cytidine deaminase (AID) is present and functional in their human B cells, as this enzyme is required for Ig diversification by both class-switch recombination and somatic hypermutation [36, 37]. B cells isolated from Hu-HSC mice have also been shown to undergo somatic hypermutation [38], further suggesting the induction of AID activity.

Variable results have also been reported in studies investigating the ability of BLT or Hu-HSC mice to produce human antibodies in response to immunization. Antigen-specific human IgG has been detected in Hu-HSC vaccinated with tetanus toxoid [10, 17]. BLT mice immunized and boosted with tetanus toxoid generated little antigen-specific human IgM or IgG antibodies [29], however, and BLT mice immunized with recombinant HIVgp140 or West Nile virus–E protein complexed to IC31* adjuvant demonstrated weak antigen-binding human IgM and IgG responses [15]. In contrast, antigen-specific class-switched human antibodies were easily detectable in all BLT mice immunized with the T-dependent antigen, 2,4-dinitrophenyl hapten-keyhole limpet hemocyanin (DNP23-KLH) [39]. The majority of these mice demonstrated dinitrophenyl (DNP)–specific human IgG antibodies one week after DNP23-KLH immunization, with titers that increased over time, and with all immunized mice being positive by four weeks postimmunization. The development and distribution of DNP-specific human IgG subclasses in these mice were similar to that of antibody responses in humans after keyhole limpet hemocyanin immunization, with mainly IgG1 and IgG2 subclasses being produced [39].

DEVELOPMENT OF FUNCTIONAL ANTIBODIES

As noted above, multiple studies have demonstrated the production of antigen-specific human IgM and IgG antibodies in both Hu-HSC mice and BLT mice in response to infection or immunization [10, 11, 15–17, 22, 28, 33, 34, 39–41]. The ability of humanized mice to generate functional antibodies able to neutralize a pathogen has been demonstrated as well, in the case of dengue virus (DENV) infections of both Hu-HSC [40] and BLT mice [41]. In Hu-HSC mice, anti-DENV human IgG antibodies were produced by 6 weeks postinfection, and antibodies from almost one third of infected mice demonstrated the capacity to neutralize infection ex vivo [40]. In studies with BLT mice, human B cells stimulated ex vivo to a memory B-cell phenotype, after being collected from the spleens of mice immunized with a live-attenuated DENV vaccine strain, produced DENV-specific IgM antibodies with neutralizing activity against the virus [41]. In the case of antibodies to HIV infection, demonstration of the functionality of the virus-specific humoral responses detected has generally been lacking. This absence is in contrast to the recent demonstration that human virus-specific T cells expanding in HIV-infected BLT mice exert sufficient immune pressure to induce rapid viral evolution by selecting for escape mutations [42]. However, in unpublished studies presented at the Recent Advances in Humanized Mice workshop, we found that in addition to producing class-switched human IgG antibodies against the Env protein, HIV-1-infected BLT mice produce antibodies that have robust neutralizing activity against the infecting virus.

IMPROVING HUMORAL RESPONSES

The immunoglobulin gene repertoires of the human B cells in humanized mice have recently been shown to recapitulate those in humans quite faithfully, suggesting that these mice have the genetic potential to model diverse human antibody responses. High-throughput sequencing of the human B cells in Hu-HSC mice demonstrated extensively diverse repertoires that displayed a pattern of V, D, and J family usage in both heavy- and light-chain genes that was essentially indistinguishable from that of human peripheral B cells [43]. In a separate study, the IgM repertoires in Hu-HSC mice were also found to be indistinguishable from normal human peripheral blood B-cell IgM repertoires [28]. These results show that humanized mice have human B-cell genetic diversity that is adequate to generate effective humoral responses, by B cells bearing high-affinity B-cell receptors, after pathogen or antigen priming as in humans. While humanized mice therefore have the genetic potential to generate antibody responses as broad and as high affinity as humans, the models currently available still need to better meet the requirements for full human B-cell maturation in order to realize this potential.

With humanized mice having comparable B-cell repertoires to humans, efforts to improve B-cell maturation in these mice, if successful, would allow them to become excellent model systems for studying human humoral immunity. Several interventional strategies have already been implemented and have demonstrated promise in improving the antibody responses of humanized mice. Effective B-cell responses to virus infection have been shown to be dependent on T-cell help. Improving T-cell help by adoptively transferring autologous mature human T cells isolated from the same umbilical cord blood source of HSCs was recently shown to enhance antigen-specific IgG responses in Hu-HSC mice. T-cell adoptive transfers in these mice led to significant improvements in B-cell maturation, whereas B-cell maturation declined with T-cell depletion [22]. In this study, the magnitude of antigen-specific IgG responses directly correlated with the numbers of mature B cells present. Improved human antibody responses have also been achieved by augmenting human cytokine levels in Hu-HSC mice. Hu-HSC mice supplemented with human granulocyte/macrophage...
colony-stimulating factor (GM-CSF) and IL-4 by hydrodynam-ic injection of cytokine-encoding plasmids produced increased levels of antigen-specific IgG upon immunization, including antibodies with neutralizing activity against H5N1 avian influenza virus [21]. The provision of human GM-CSF and IL-4 to these mice improved B-cell maturation, and resulted in the development of CD209 (DC-SIGN)+ human dendritic cells. Humanized mice generally have been noted to have poorly organized lymphoid structures in their secondary lymphoid organs, which likely contributes to their delayed B-cell maturation and reduced antibody responses. The addition of follicular dendritic cells may also improve humoral responses, as these cells are known to be important in selecting memory B cells during germinal center reactions. General immune stimulation itself may improve B-cell development, as indicated by experiments in which BLT mice were immunized with sheep red blood cells, and subsequently developed B-cell-containing follicle-like structures in the spleen and more organized T- and B-cell zones in the lymph nodes [8].

Genetic manipulation of immunodeficient mice used as recipients of HSC to generate humanized mice have also improved their antibody responses. Transgenic expression of the human signal regulatory protein alpha (SIRPα), a receptor that negatively regulates phagocytosis, in Rag2-null/IL2Rγc-null mice enhanced human HSC engraftment, and improved antigen-specific IgM titers and class-switching to IgG after immunization [44]. Transgenic expression of human leukocyte antigen (HLA) class II molecules in NOD/Rag1-null/IL2Rγc-null or NOD/SCID/IL2Rγc-null mice that were then reconstituted with HLA-DR-matched human HSC resulted in the production of all human immunoglobulin classes, and high titers of antigen-specific IgG antibodies upon immunization. In contrast, control mice reconstituted with the same HSCs but not expressing the HLA transgene produced only IgM [45, 46], demonstrating the critical role that human HLA molecules have in the thymus for the development of properly functioning T cells, which in turn play a critical role in the magnitude and class-switched nature of the humoral responses that subsequently develop in these animals.

CONCLUDING REMARKS

Substantial progress has been made in improving the ability of humanized mice to generate functional human antibody responses to both infections and immunizations. Incomplete human B-cell maturation in humanized mice stands out as a major impediment to improving these responses further. Several investigators have already reported strategies that can improve B-cell maturation in humanized mice, including increasing the length of time prior to their use [16, 17], improving T-cell help by adoptive transfer of autologous mature T cells [22], and providing additional human cytokine support [21].

Together with the recent demonstrations that the immunoglobulin gene repertoires of the human B cells in humanized mice recapitulate those in humans quite faithfully [28, 43], this recent progress creates optimism that the next generation of humanized mice will allow investigators to model human humoral immunity with increasing fidelity. Improving humoral responses in humanized mice will contribute greatly to the ultimate goal of creating a small animal system that can model the human immunity well enough to meaningfully assess candidate human vaccines and other immunotherapeutic strategies.

Notes

Financial support. This work was supported by a Harvard University Center for AIDS Research (HU CFAR, National Institutes of Health/National Institute of Allergy and Infectious Diseases [NIH/NIAID] P30-A1060354) Scholar Award to E. S.; and by NIH/NIAID P01-A1104715 (Project 2 and Core B), HU CFAR (NIH/NIAID P30-A1060354) Core support, and a Ragon Institute Initiative to A. T.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


