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BLT Humanized Mice as a Small Animal Model of HIV Infection

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Abstract

Humanized mice are valuable models for the research and development of vaccine strategies and therapeutic interventions to control or eradicate HIV. The BLT humanized mouse model is particularly promising because the combination of transplantation of human fetal pluripotent hematopoietic stem cells with surgical engraftment of human fetal thymic tissue results in improved T cell reconstitution, maturation, and selection. To date, the BLT humanized mouse model has been used to study many aspects of HIV infection including prevention, mucosal transmission, HIV-specific innate and adaptive immunity, viral latency, and novel antiretroviral and immune-based therapies for suppression and reservoir eradication. Here we describe recent advances and applications of the BLT humanized mouse model of HIV infection and discuss opportunities to further improve this valuable small animal model.

The BLT Humanized Mouse Model

The goal of humanized mouse models is to generate a small animal model with a functioning human immune system capable of accurately modeling the human immune response to pathogens. A variation on this theme, BLT (**b**one marrow, **l**iver, **t**hymus) humanized mice are generated by surgical transplantation of human fetal liver and thymus tissue fragments into immunodeficient mice — typically NOD/SCID (NS), NOD/SCID *Il2y*^{-/-} (NSG) or C57BL/6 *Rag2*^{-/-} *Il2y*^{-/-} — followed by intravenous injection of human fetal liver-derived CD34⁺ hematopoietic stem cells (HSCs) [1-6]. The fetal human HSCs engraft in the mouse bone marrow and serve as progenitor cells to populate the mouse with human lymphoid and myeloid cell compartments, and the transplanted human thymus tissue provides for the active education of human T cells during the 13-18 weeks necessary for human immune reconstitution in the mice [1-5]. The degree of human chimerism can vary between batches, and may reflect factors such as time elapsed since donor tissue collection, purity of CD34⁺ cell isolations, frequency of long-term HSCs present within the CD34⁺

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population, and non-standardized chimerism standards between laboratories. However, human B cells, T cells, and myeloid cells are found in various different combinations in both the human and murine thymus, as well as murine liver, bone marrow, thymus, spleen, lymph nodes, lung, female reproductive tract, and gut [3,5,7-10].

Human Immune Reconstitution and Functionality

The functional human cellular immune response in the BLT mouse makes it a valuable system for the *in vivo* study of HIV-specific cellular immunity. HIV infection in BLT humanized mice is associated with HIV-specific CD8⁺ T cell activation, the level of which correlates with plasma viral load [5,11]. The magnitude, breadth, and specificity of human HIV-specific CD8⁺ T cell responses in BLT humanized mice also closely resembles that observed in HIV-infected humans, including the targeting of epitopes across the viral proteome [12], the rapid development of responses during the acute phase of infection, and the recapitulation of general HLA class I immunodominance hierarchies [13]. The functionality of the cellular immune response against HIV in BLT mice is supported by the observation of viral escape from early, dominant CD8⁺ T cell responses with kinetics similar to those of natural HIV infection. Notably BLT mice constructed with tissue expressing the HIV-protective HLA-B*57 allele also exhibited CD8⁺ T cell responses against highly conserved HLA-B*57-restricted epitopes in Gag and enhanced control of HIV viremia [13].

Another critical aspect of T cell immunity in HIV infection is the phenomenon of T cell exhaustion, whereby continuous exposure to high levels of antigen leads to functional defects in antiviral activity and proliferative capacity [14-18]. In rhesus macaque models of SIV infection, blockade of the co-inhibitory receptor programmed death-1 (PD-1) pathway associated with T cell exhaustion leads to enhanced T cell immunity and viral control [19], effectively reversing immune exhaustion. Notably, similar blockade of the PD-1 pathway in BLT humanized mice resulted in improved CD8⁺ and CD4⁺ T cell responses and viral suppression, indicating that this critical pathway governing T cell control of HIV is also functional in BLT humanized mice [20]. Therefore, this small animal model of HIV infection is capable of mounting robust HIV-specific T cell immunity and recapitulating many of the key aspects by which HIV evades these responses in humans.

Although the BLT mouse exhibits substantial numbers of circulating human B cells at reconstitution, several studies have now demonstrated that this population is composed of high frequencies of pre-mature (pro, pre, immature, and transitional) B cells and reduced numbers of memory B cells [5,21,22], suggesting that the B cell compartment fails to fully recapitulate that of a typical adult human. However, HIV-specific human antibodies have been elicited in BLT mice by both immunization and infection. One study showed that during infection, HIV-specific antibodies detectable by Western blot typically arose six to ten weeks after infection [5], while another study showed that immunization with HIV-gp140 elicited HIV-specific IgM and IgG, detectable by ELISA, within 15 days [21]. Importantly, though total human IgM is overwhelmingly predominant in BLT immune responses, sometimes rising to nearly-human plasma concentrations, total human IgG is either very low or undetectable [22,23]. In fact, the defect in class-switched antibody

production has prompted the BLT mice to be proposed as a model for the study of hypogammaglobulinemia [22].

The HIV-specific humoral responses in these and other studies illustrates an interesting trend: only a small fraction of humoral immunity in the BLT model shows evidence of traditional, follicular, germinal center-based antibody production, i.e., class-switching, somatic hypermutation, affinity maturation, secondary immune responses, and post-GC cell types such as IgG+ memory B cells or long-lived bone marrow plasma cells [5,21-25]. This defect may be explained in part by the observation that the spleens and lymph nodes of BLT humanized mice do not develop B cell follicles. Rather, human T cells commonly form periarteriolar lymphatic sheath (PALS) regions around murine splenic arterioles, and are sometimes ringed by a halo of B cells; these structures have been reported in BLT mice in a number of studies, but these ring-like formations do not coalesce into node-like splenic follicles as seen in a normal human spleen (**Figure 1**). As depicted in **Figure 1**, larger T cell zones in BLT spleens tend to exhibit more extensive B cell rings, but despite the greater numbers of B cells, follicles remain unformed in BLT spleens.

The absence of B cell follicles capable of supporting germinal center reactions strongly suggests that the humoral immunity observed in BLT mice is not being driven by conventional post-germinal center B cells, but rather by extra-follicular B cells. A subset of these, innate-like B cells, have been described in humans as CD5+ B cells that produce —natural|| antibodies which are predominantly IgM, feature low rates of somatic hypermutation, and tend to be auto/poly-reactive, all characteristics that are consistent with observations in BLT mice [21,24]. The small amounts of IgG, as well as the CD27+ B cells occasionally found in the model [21,22,24] do superficially resemble post-germinal center products. However, IgM+ CD27+ B cells are also found in human cord blood and in the absence of traditional memory [26], and can be produced by a germinal center-independent pathway [27]. Additionally, small amounts of class-switched antibodies can be produced by known extra-follicular mechanisms [28]. Regardless of the specific etiology, however, the unusual humoral immunity seen in the BLT model does not fully recapitulate that of a healthy adult human.

Applications of Humanized Mice to HIV Research

The capacity of the humanized BLT mouse model to support mucosal infection, consistent and sustained viremia, and cellular immune responses [5,6,8,12,29-35] makes it an invaluable tool for the experimental, *in vivo* study of numerous aspects of both viral and host factors influencing HIV infection. For example, the BLT model has been used for the *in vivo* quantification of intrinsic differences in viral replication capacity which revealed that HIV strains isolated from HLA-B*57 elite and viremic controllers and those isolated from normal chronic progressors did not differ in terms of *in vivo* replication or CD4+ T cell depletion. These data demonstrated *in vivo* that elite control of HIV is unlikely to be mediated purely by defective virus [36]. Additionally, the susceptibility of the BLT mouse to both vaginal and rectal challenge with HIV [8,32,37-40] has facilitated the study of the dynamics of mucosal transmission and early viral dissemination [9]. Similarly, the capacity of the model to support live, *in vivo* multiphoton intravital microscopy during HIV infection revealed that

cell-to-cell virus spread may be enhanced by the ability of HIV to impair T cell motility in lymph nodes [41,42].

In addition to investigations of the viral and host factors influencing HIV infection, the BLT mouse and other humanized mouse models are also proving to be valuable for evaluating therapeutic strategies for the prevention, treatment, and eradication of HIV infection. For example, both systemic and topical treatment of BLT mice with antiretroviral therapies has been demonstrated to dramatically reduce HIV infection through either intravaginal [6,43], intrarectal, [38], or oral exposure [34], including against a primary transmitted/founder virus [39]. Humanized mice have also been used to demonstrate the efficacy of novel approaches to limit viral replication such as CD4 aptamer siRNAs [30,44] and the recently described eCD4-Ig fusion molecule [45]. Importantly, HIV latency also appears to be supported by the model. Infectious virus has been produced from *ex vivo* stimulation of BLT splenocytes depleted of productively infected cells [29], and from CD4⁺ resting T cells after previous antiretroviral therapy had reduced viral loads to undetectable levels [46]. This characteristic of the BLT model can facilitate the study of novel therapeutic approaches to eradicate viral reservoirs through combined antiretroviral and immunity-based therapeutics. For example, Kitchen et al. recently demonstrated that transduction of HSCs with a Gag-specific TCR prior to engraftment in BLT mice resulted in improved suppression of HIV replication in multiple organs [7,47], and Halper-Stromberg et al. showed synergistic effects on HIV rebound when combining multiple inducers with broadly-neutralizing antibodies [48].

Finally, numerous groups have begun to use humanized mouse models, including the BLT humanized mouse, to deconstruct the mechanisms by which the Berlin Patient achieved long-term remission of HIV following allotransplantation of stem cells from a donor homozygous for a defective mutation in the HIV CCR5 co-receptor [49]. These groups are utilizing various technologies including zinc-finger nucleases [50,51], RNAi [52-55], and now CRISPR/Cas9 [56] to knock down CCR5 expression in the CD34⁺ HSC cells that give rise to the immune system of humanized mice. Although varying degrees of efficacy have been observed in terms of the ability to suppress HIV replication using these different approaches, humanized mice are serving as an important pre-clinical model to improve upon approaches already underway in patients that attempt to control HIV infection and ultimately eradicate HIV reservoirs [57,58].

Current Limitations of BLT Humanized Mice

The BLT humanized mouse model represents a valuable tool for the study of HIV infection, but there are nonetheless several limitations that remain to be improved upon. One limitation is the development of graft-versus-host disease (GvHD), which typically develops approximately six months after engraftment. This can limit the utility of the model for studies involving long-term chronic infection or prolonged immunization regimens. In addition, early development of GvHD may alter T and B cell activation states, which may impact the induction and efficacy of HIV-specific cellular and humoral immune responses. A recent report described an approach to reduce GvHD by altering the CD47/SIRP α anti-phagocytic signaling pathway in the mice. Genetic inactivation of CD47 in BLT mice of the

C57BL/6 *Rag2*^{-/-} *Il2y*^{-/-} background increased tolerance to transplanted human HSCs and significantly reduced GvHD [12].

Although the use of mice with an *Il2y*^{-/-} genetic background (which disrupts the common gamma chain cytokine receptor) increases tolerance for transplantation of human cells, it also has the side effect of limiting the development of secondary lymphoid tissue in these mice [59]. Given the critical role of secondary lymphoid tissue in the development of affinity-matured antibodies, alternative approaches to the use of the *Il2y*^{-/-} to reduce the number or efficacy of mouse NK cells may need to be explored.

The inability of BLT mice to develop high levels of hyper-mutated, class-switched IgG antibodies is likely the most widely encountered limitation of the model. Although HIV-specific human antibodies have been observed in BLT mice following either immunization or infection [5,21], the concentrations are typically quite low and the antibodies exhibit limited class-switch recombination and mutation rates [24]. Truly adaptive, robust, humoral immune responses are not commonly elicited in BLT mice through conventional prime-boost regimens. One explanation may be that although engrafting CD34⁺ fetal liver HSCs should enable reconstitution of the hematopoietic lineage, it fails to address cells of non-hematopoietic origin, including stromal cells critical to adaptive humoral immunity. Stromal cells, derived from mesenchymal rather than hematopoietic precursors, are critical immunomodulatory components of the immune system [60], and their absence in BLT humanized mice may be an important part of the observed lack of germinal centers and antibody dysfunction. Antibody production by the germinal center pathway is critically dependent on specific stromal cell subsets. Fibroblastic reticular cells, marginal reticular cells, and follicular dendritic cells (FDCs) are all mesenchymal cell types that play critical roles in the organization, maintenance, and function of lymphocytes within secondary lymphoid tissues [61]. FDCs in particular are crucial for the proper regulation of follicle formation, germinal center maintenance, affinity maturation, and the survival of activated B cells; selective ablation of FDCs in CD21-Cre⁺ROSA^{DTR}⁺ mice has been shown to result in follicle and germinal center collapse [62]. Interestingly, the splenic B cells in BLT mice form halo-like structures around T cell zones similar to those seen in the FDC-ablated CD21-Cre⁺ROSA^{DTR}⁺ mice, suggesting that similar mechanisms, possibly involving low-affinity attraction to T cell zones [63], may be responsible for the organization of splenic B cells in BLT mice. Therefore, the absence of human FDCs in BLT mice is likely contributing to the inability to generate and sustain intact B cell follicles. One possible solution to the lack of germinal center development may be to ensure sufficient inclusion of mesenchymal precursor cells along with the adoptively transferred HSCs, either in the form of expanded mesenchymal cells cultured from the donor liver tissue, or as an engrafted splenic organoid, similar to the thymic organoid.

Conclusions

Taken together, there have been numerous advances in the ability of humanized mice to recapitulate human immunity and host-pathogen interactions. In particular, the BLT humanized mouse model, which includes CD34⁺ HSCs as well as human liver and thymic tissue, demonstrates improved T cell immunity, and its ability to support productive HIV

infection has accelerated the study of HIV pathogenesis and novel approaches to harness anti-viral immunity to control HIV. Overcoming limitations in the BLT model, including a lack of human stromal cell populations and adaptive humoral memory, will continue to improve the utility of the model both for HIV as well as other human pathogens and disease states.

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Highlights

- BLT humanized mice are valuable models for the study of HIV infection.
- BLT mice recapitulate important aspects of human immunity, including T cell immunity.
- Various limitations in the model continue to exist.
- Recent approaches have improved engraftment and human immunity in the model

Figure 1. Absence of follicle formation and variable human lymphocyte retention in the spleen of BLT mice

Immunohistochemistry: human CD3 (*blue*), human CD35 (*brown*). (A) Human spleen, displaying arterioles, T cell zones, and distinct B cell follicles with FDC networks. (B-D) Spleens from three individual BLT mice, 11 weeks post-engraftment, displaying varying numbers of human lymphocytes. As in the human spleen, T cells form the periarteriolar lymphatic sheath, while B cells in the BLT mice appear as halos surrounding it. These cylindrical structures do not coalesce into node-like follicles, despite serial sectioning (not shown), and FDC networks are not present. Absence of follicle formation appears to be independent of the number of resident human lymphocytes. Arrows and embedded text: arterioles (**Ar**), T cell zones (**T**), follicles (**F**), follicular dendritic cells (**FDCs**). All mice were engrafted with tissue from the same human donor; B, C, and D display the largest, most-typical, and smallest numbers of human cells, respectively, of 12 similar mice imaged. The structures depicted are representative of those imaged in mice made from six different donor-tissue batches. Scale: top row bars equal 1mm, bottom row bars equal 200µm.