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BLT humanized mice as a small animal model of HIV infection

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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.

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The BLT humanized mouse model

One 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 (bone marrow, liver, thymus) 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 $Rag2^{-/-}$ $Il2\gamma^{-/-}$ — 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+ 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, 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 crucial 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 crucial 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 hypogammaglobulinemeia [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, that is, 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•,22••,23,24••,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