SUPPLEMENT ARTICLE

HIV-Specific CD8$^+$ T-Cell Immunity in Humanized Bone Marrow–Liver–Thymus Mice

Timothy E. Dudek and Todd M. Allen

Ragon Institute of MGH, MIT, and Harvard, Boston, Massachusetts

CD8$^+$ T-cell responses play a critical role in the control of human immunodeficiency virus (HIV) infection, and recent vaccine studies in nonhuman primates now demonstrate the ability of T cells to prevent the early dissemination of simian immunodeficiency virus and perhaps clear residual infection. Recent advances in humanized mouse models, in particular the humanized bone marrow–liver–thymus (BLT) mouse model, show promise in their ability not only to support sustained infection with HIV, but also to recapitulate human HIV-specific immunity. The availability of a small-animal model with which to study human-specific immune responses to HIV would greatly facilitate the elucidation of mechanisms of immune control, as well as accelerate the iterative testing of promising vaccine candidates. Here we discuss data from our recent study detailing the composition and efficacy of HIV-specific CD8$^+$ T-cell responses in humanized BLT mice that was recently presented at a Harvard Center for AIDS Research symposium on humanized mouse models for HIV vaccine design.

Keywords. HIV; CD8; T cell; epitope; escape; humanized mice; vaccine.

ANIMAL MODELS FOR STUDYING HIV INFECTION

Animal models have greatly advanced our understanding of the pathogenesis and immunity of human infections and further accelerated drug and vaccine development. The simian immunodeficiency virus (SIV)–infected rhesus macaque model has, since its introduction in the mid-1980s, proven invaluable for the study of human immunodeficiency virus (HIV) and, most notably, for HIV vaccine research and design [1–4]. This model closely mimics critical facets of natural HIV infection, including the pathogenesis, host immunity, and disease progression seen in humans, albeit with an accelerated rate of disease progression. One limitation of the model, however, is the sequence differences between human and macaque genomes, most notably in the genetic loci related to host immunity, such as those of the major histocompatibility complex, T-cell receptor (TCR), and B-cell receptor (BCR) [5–7]. Another limitation of the model is the viral sequence differences that exist between HIV and SIV. Combined, these differences alter the targeting and efficacy of immune responses against different regions of HIV and SIV. Given our growing understanding of the important role that the specificity of both cellular and humoral immune responses has on the successful control of HIV, with some regions of the virus being much more susceptible than others to immune targeting, these differences in host and viral genetics have impeded efforts to identify critical correlates of vaccine-medicated immune protection against HIV.

The sequence diversity of HIV and its ability to rapidly mutate represent additional hurdles to engendering effective, population-wide, vaccine-induced immunity. Globally, multiple genetically distinct clades of HIV exist, and therefore a vaccine predicated on a single HIV strain may insufficiently elicit cross-reactive immunity to a geographically distinct infecting HIV strain. This diversity is partially due to the error-prone replicative nature of HIV that enables it to rapidly mutate to evade host immune responses, which, as mentioned, are predicated by the genetics of the resident human population. It may be important to develop vaccines that elicit responses against precise, vulnerable, and highly conserved regions of HIV to overcome this high degree of sequence diversity and rapid mutability and thereby attain broad and protective immunity [8–14]. Collectively, the critical

Correspondence: Todd M. Allen, PhD, Ragon Institute of MGH, MIT, and Harvard, 400 Technology Sq, Cambridge, MA 02139 (tallen2@partners.org).

The Journal of Infectious Diseases 2013;208(S2):S150–4

DOI: 10.1093/infdis/jit320

differences between host and viral genetics between humans and macaques, and HIV and SIV, impede our ability to translate important vaccine concepts that might overcome the inherent traits of HIV's sequence diversity and mutability. As such, it would be advantageous to be able to test HIV vaccine concepts by using a human immune system directly against HIV, rather than a macaque immune system against SIV.

HUMANIZED MOUSE MODELS

The opportunity to study HIV in a small-animal model was first realized in a mouse model in which human fetal liver and thymus engraftment into mice with severe combined immunodeficiency (SCID) was shown to support HIV infection and dissemination [15, 16]. Additional efforts explored the introduction of adult human peripheral blood mononuclear cells, fetal tissues, or hematopoietic stem cells into immunodeficient mice [17, 18]. Since these early studies, a number of improvements have been made that allow for a more complete development of the human immune response, reduced incidence of graft versus host disease, and improved functionality [19–25].

In the more recently developed humanized bone marrow–liver–thymus (BLT) mouse model, both human liver and thymus tissues are transplanted into sublethally irradiated nonobese diabetic (NOD)/SCID or NOD/SCID/*IL2Rγ*$^{-/-}$ mice prior to the injection of CD34$^+$ hematopoietic cells. This combined approach enables the transplanted human thymus tissue to become an active organ where human T cells can be educated within a proper human environment [26–30]. Numerous groups have used this model to explore the basic tenants of HIV infection and HIV-specific immunity, including sustained infection [30–35], mucosal transmission [31, 32, 34, 36–39], antiretroviral therapy and the establishment of viral reservoirs [32, 33, 36, 37, 40–43], and general T-cell and B-cell immunity [27–30, 44–54]. These initial studies have demonstrated great promise in the ability of this small-animal model to mimic the fundamental aspects of HIV infection and general human immunity. However, for such a model to be truly transformative to the field, it will be important to more stringently compare these results to the early events of human acute HIV infection, including viral dissemination; the influence of mucosal immune activation on HIV acquisition; the specificity, phenotype, and function of T-cell and B-cell responses; and the mechanisms of viral control, such as the induction of broadly neutralizing antibody responses, ADCC, and T-cell targeting of conserved regions of the virus. While many of these comparisons will be limited to peripheral blood, focused studies comparing these parameters in lymph nodes and mucosal tissues will also be critical. Ultimately, it will be important to explore the ability to induce vaccine-elicited responses capable of controlling or blocking HIV infection.

THE ROLE OF CD8$^+$ T-CELL IMMUNITY IN THE CONTROL OF HIV

CD8$^+$ cytotoxic T lymphocyte (CTL) responses play a critical role in the control of HIV. The temporal relationship between the initial development of HIV-specific CD8$^+$ T cells and the reduction of viral loads provided one of the first indications that these cells are important in controlling HIV replication [55, 56]. It has since been demonstrated in the SIV-infected macaque model that the development of CD8$^+$ T-cell responses also corresponds with the early reduction in peak viremia [57], whereas depletion of these cells results in uncontrollable viral replication and rebounding viral loads [58]. Further evidence of the significant impact of these responses on the virus includes the escape from these responses through mutations within targeted epitopes that can occur within the first few weeks of infection, undermining the ability of these responses to critically contain early infection. In some cases, viral escape from even a single CD8$^+$ T-cell response has been shown to result in a dramatic loss of viral control [59, 60]. Guiding the specificity of these CD8$^+$ T-cell responses is the array of the 6 classical HLA alleles (2 for each of the A, B, and C loci) expressed in each individual. As proteins, each HLA molecule functions to present on the surface of a cell unique 8–12-amino acid "epitope" fragments derived from both host and viral proteins. There exists a strong relationship between the expression of certain HLA alleles and the control of viremia, CD4$^+$ T-cell depletion, and disease protection [61–63]. Protective HLA alleles, such as B*57 and B*27, have been demonstrated to restrict early CD8$^+$ T-cell responses that dominantly target highly conserved regions of gag that form the viral capsid [64, 65]. Importantly, the ability of HIV to escape from such responses is impaired as mutations within these epitopes disrupt the proper functioning of gag and thereby impair the ability of the virus to replicate [66–70]. However, despite our understanding of the mechanisms by which some HLA alleles and CD8$^+$ T-cell responses successfully control HIV, designing vaccines to widely elicit potent cellular responses toward a potential Achilles' heel of HIV has proven challenging [10]. As a proof of concept, Mudd et al recently demonstrated in the SIV-infected macaque model that immune responses can be focused against particular regions of SIV to significantly enhance protection [71]. As such, vaccination has the potential to overcome the natural hierarchy of HIV-specific CD8$^+$ T-cell immunodominance patterns [64] to redirect immunity toward more-effective viral targets. However, the cost and time required to test HIV vaccines in humans is extremely prohibitive and only reserved for the most promising vaccine candidates, precluding the ability to iteratively test vaccine concepts that might successfully refocus HIV-specific immune responses against the most vulnerable regions of HIV.

HIV-SPECIFIC CD8$^+$ T-CELL RESPONSES IN HUMANIZED MICE

Various studies have now begun to explore cellular immune responses to HIV in humanized mice. Following HIV infection, virus-specific CD8$^+$ T-cell responses and increases in effector/memory CD8$^+$ T-cell responses have been observed [30, 72]. Furthermore, depletion of CD8$^+$ T-cell responses following HIV infection has been shown to result in significant increases in viral load [73, 74]. Virus-specific cellular responses to other infections, including those due to dengue virus, Epstein-Barr virus, and hepatitis C virus, have also now been demonstrated in humanized mice, demonstrating the broader usefulness of this model to the study of other infectious diseases [52, 75, 76]. However, in contrast to the detailed work in humans regarding the breadth, specificity, function, and effector functions of HIV-specific CD8$^+$ T-cell responses [77–79], a similar comprehensive analysis of CD8$^+$ T-cell responses to HIV in humanized mice is still lacking. Ideally, to be of specific value to HIV vaccine design, a small-animal model would need to recapitulate at least the basic attributes of these responses, including the vast breadth and magnitude of the response [78, 79], specificity for the same HIV epitope targets as in humans [77], and conservation of the basic mechanisms by which some HLA alleles and responses exhibit control over HIV through the targeting of conserved regions of the virus [66, 68, 80].

We recently characterized both CD8$^+$ T-cell responses and viral evolution during the acute phase of infection in humanized BLT mice to more comprehensively characterize HIV-specific cellular immunity in this model [45]. Here we found that humanized BLT mice not only develop multiple CD8$^+$ T-cell responses restricted by various HLA alleles from the human donor tissue, but that these responses were found to target the same CD8 epitopes that are targeted in humans during the acute phase of infection. Specifically, in one group of BLT mice responses were detected against the HLA-A*01 RY9 (RRGWEILKY) and the HLA-C*03 AL9 (AAIDLSHFL) epitopes in env and nef, responses previously shown to be immunodominant in humans expressing those HLA alleles [64, 65]. In 2 other independent groups of BLT humanized mice expressing HLA haplotypes that included HLA-B*57, CD8$^+$ T-cell responses were detected against the B*57-IW9 (ISPRTLNAW), B*57-KF11 (KAFSPEVIPMF), and B*57-TW10 (TSTLQEQIGW) epitopes in gag. Similar to the A*01 and C*03 epitopes, these epitopes also represent some of the most commonly targeted epitopes restricted by this protective HLA allele during the acute phase of HIV infection in humans [64, 65]. Since HLA-B*57 in humans appears to exert its protective immunity over HIV through the focused targeting of these very same conserved epitopes in gag [68–70], these data suggest that the fundamental mechanisms by which CD8$^+$ T-cell responses control HIV may also be preserved in this model. These data demonstrate that, during the first few weeks after infection, humanized BLT mice expressing various HLA haplotypes are capable of mounting multiple HIV-specific CD8$^+$ T-cell responses, with specificities similar to those of natural HIV infection, thus demonstrating maintenance of the processing and presentation pathways of human HIV CD8 epitopes in these chimeric mice.

VIRAL ESCAPE FROM CD8$^+$ T-CELL RESPONSES IN BLT HUMANIZED MICE

The propensity for HIV to escape from host immune responses through its error-prone replicative pathway represents a major mechanism of immune evasion and viral persistence. Conversely, viral escape can serve as a surrogate marker of the relative immune pressure exerted by a particular CD8$^+$ T-cell response. When we examined whether viral evolution was occurring during the first few weeks after HIV infection of humanized BLT mice, we observed reproducible patterns of viral adaptation within many of the targeted human CD8 epitopes. Some responses, such as those detected against the HLA-A*01 RY9 and the HLA-C*03 AL9 epitopes mentioned above, were capable of rapidly selecting for CTL escape mutations within the first 6 weeks of infection (Supplementary Figure 1*A*). The rapid kinetics of these mutations resembles that seen in humans and SIV-infected macaques, in which the most rapid onset of viral escape has been shown to occur 17–21 days after infection [81–85]. These data reveal that HIV-specific CD8$^+$ T-cell responses in humanized BLT mice are also functional in their ability to exert strong selection pressure against HIV during the acute phase of infection, with HIV in turn being capable of evading these responses through the same mutational escape pathways observed in humans.

EFFECTS OF THE PROTECTIVE HLA-B*57 ALLELE

As noted above, particular HLA class I alleles, such as B*27 and B*57, are associated with greater control of viral replication through the dominant targeting of conserved CD8 epitopes within the structurally constrained gag protein. To determine whether the clinical protective effect of HLA-B*57 translated to the BLT mouse model, we examined whether more potent suppression of viremia occurred in mice expressing this HLA allele. Humanized BLT mice heterologous for HLA-B*57 did in fact exhibit a similar level of control of HIV over non–B*57-expressing mice, with a 0.6 $\log_{10}$ viral RNA copies/mL reduction that was similar to the 0.7 $\log_{10}$ viral RNA copies/mL reduction observed in a large cohort of untreated, chronically infected individuals heterologous for HLA-B*57 [86]. Importantly, just as in natural HIV infection the B*57-restricted gag CD8 epitopes in these mice remained refractory to escape for at least the first 10 weeks of infection (Supplementary Figure 1*B*),

which was in contrast to viral escape from other HLA-B*57–restricted CD8$^+$ T-cell responses within the first few weeks of infection (Supplementary Figure 1*B*). The protective effect observed with the expression of HLA-B*57, coupled with the targeting of conserved CD8 epitopes in gag, suggest that the fundamental mechanisms by which some HLA alleles and their restricted CD8$^+$ T-cell responses control HIV may be intact in this small-animal model of HIV.

THE POTENTIAL FOR CD8$^+$ T-CELL RESPONSES TO BLOCK HIV ACQUISITION

Until only recently, the goal of an HIV vaccine was to induce a level of immunity capable of effectively containing an HIV infection, which would result in slower disease progression while blunting HIV transmission rates. Recent results from the RV144 HIV vaccine trial suggest, however, that partial protection against HIV infection is achievable and, in this case, correlated with levels of HIV-specific binding antibody titers [87, 88]. Antibodies have the capacity to neutralize the virus prior to cell entry; in contrast, cellular immune responses require viral infection and the subsequent expression of viral peptides on the cell surface before these responses can be activated. As such, cellular immune responses have historically been believed to be incapable of blocking HIV infection. However, recent studies of a cytomegalovirus vaccine vector illustrate that the induction of high frequencies of effector-memory CD8$^+$ T-cell responses at or near the site of infection can lead to the rapid clearance of SIV in infected rhesus macaques before viral dissemination, essentially neutralizing the infection immediately after exposure [89]. These data suggest that a T-cell–based vaccine could also engender "sterilizing" immunity. The humanized BLT mouse model, through its ability to accurately recapitulate human cellular immune responses to HIV, may therefore help facilitate the development of novel vaccine vectors capable of inducing similarly protective immune responses in humans.

CONCLUSIONS

A growing collection of studies now demonstrate that the basic tenants of HIV infection and replication, as well as HIV-specific immunity, are intact in the various humanized mouse models. Clearly, more-extensive studies are required to fully explore the strengths and weaknesses of these models, particularly in the BLT model, to determine the extent to which they can be used to explore vaccine-induced immunity to HIV. The ability to generate a number of identical BLT mice engrafted with the same human tissue will help to reduce the confounding effects of host genetic diversity inherent in most animal studies, enabling smaller group sizes during vaccine testing. This, combined with the reduced costs of a small-animal model, could greatly accelerate HIV vaccine design and testing. Nonetheless, future studies in these chimeric mice are required to define the true breadth and magnitude of immune responses to HIV, including the phenotype and mucosal/lymph-node-trafficking profiles of these responses and the breadth and plasticity of the TCR and BCR repertoires. Similarly, efforts are required to explore the ability of various vaccine regimens, such as those of adenovirus, modified vaccinia Ankara, cytomegalovirus, herpes simplex virus, and DNA vaccines, to induce effective cellular and humoral immunity to HIV. This model may additionally prove useful in helping to design and test vaccine adjuvants and antigens that are capable of redirecting responses not only toward mucosal tissues [89], but also, importantly, against regions of the virus that cannot readily escape [8–11]. Similarly, opportunities remain to continue to improve the model by including additional genetic modifications to further humanize the host strain, such as expression of human adhesion molecules to improve cell trafficking or human-specific cytokines required for immune maturation [24], as well as novel approaches to reduce the incidence graft versus host disease [46, 90, 91]. Thus, access now to an animal model that is capable of supporting HIV infection and reliably recapitulates human HIV CD8$^+$ T-cell–specific immunity should greatly accelerate such efforts, but careful consideration will need to be given to both the route of vaccination and the potential to induce both human and murine responses when assessing vaccine-induced immunity in this chimeric model.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Financial support. This work was supported by the National Institute of Allergy and Infectious Diseases (grants R01-AI090698 and HIVRAD P01-AI104715 to T. M. A.), an NIH training grant (5T32-AI07387-22 to T. E. D.), and an innovation award from the The Phillip T. and Susan M. Ragon Foundation.

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

1. Letvin NL, Daniel MD, Sehgal PK, et al. Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. Science **1985**; 230:71–3.
2. Daniel MD, Letvin NL, King NW, et al. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. Science **1985**; 228:1201–4.
3. Morgan C, Marthas M, Miller C, et al. The use of nonhuman primate models in HIV vaccine development. PLoS Med **2008**; 5:e173.

4. Veazey R, DeMaria M, Chalifoux L, et al. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science **1998**; 280:427–31.
5. Scinicariello F, Attanasio R. Intraspecies heterogeneity of immunoglobulin alpha-chain constant region genes in rhesus macaques. Immunology **2001**; 103:441–8.
6. Jaeger EE, Bontrop RE, Lanchbury JS. Structure, diversity, and evolution of the T-cell receptor VB gene repertoire in primates. Immunogenetics **1994**; 40:184–91.
7. Boyson JE, Shufflebotham C, Cadavid LF, et al. The MHC class I genes of the rhesus monkey. Different evolutionary histories of MHC class I and II genes in primates. J Immunol **1996**; 156:4656–65.
8. Fischer W, Perkins S, Theiler J, et al. Polyvalent vaccines for optimal coverage of potential T-cell epitopes in global HIV-1 variants. Nat Med **2007**; 13:100–6.
9. Rolland M, Nickle DC, Mullins JI. HIV-1 group M conserved elements vaccine. PLoS Pathog **2007**; 3:e157.
10. Altfeld M, Allen TM. Hitting HIV where it hurts: an alternative approach to HIV vaccine design. Trends Immunol **2006**; 27:504–10.
11. Hanke T, McMichael AJ. HIV-1: from escapism to conservatism. Eur J Immunol **2011**; 41:3390–3.
12. Hessell AJ, Haigwood NL. Neutralizing antibodies and control of HIV: moves and countermoves. Curr HIV/AIDS Rep **2012**; 9:64–72.
13. Bonsignori M, Alam SM, Liao HX, et al. HIV-1 antibodies from infection and vaccination: insights for guiding vaccine design. Trends Microbiol **2012**; 20:532–9.
14. Burton DR, Ahmed R, Barouch DH, et al. A blueprint for HIV vaccine discovery. Cell Host Microbe **2012**; 12:396–407.
15. Namikawa R, Kaneshima H, Lieberman M, Weissman IL, McCune JM. Infection of the SCID-hu mouse by HIV-1. Science **1988**; 242:1684–6.
16. McCune JM, Namikawa R, Kaneshima H, Shultz LD, Lieberman M, Weissman IL. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. Science **1988**; 241:1632–9.
17. Mosier DE, Gulizia RJ, Baird SM, Wilson DB. Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature **1988**; 335:256–9.
18. Lapidot T, Pflumio F, Doedens M, Murdoch B, Williams DE, Dick JE. Cytokine stimulation of multilineage hematopoiesis from immature human cells engrafted in SCID mice. Science **1992**; 255:1137–41.
19. Zhang L, Su L. HIV-1 immunopathogenesis in humanized mouse models. Cell Mol Immunol **2012**; 9:237–44.
20. Legrand N, Ploss A, Balling R, et al. Humanized mice for modeling human infectious disease: challenges, progress, and outlook. Cell Host Microbe **2009**; 6:5–9.
21. Berges BK, Rowan MR. The utility of the new generation of humanized mice to study HIV-1 infection: transmission, prevention, pathogenesis, and treatment. Retrovirology **2011**; 8:65.
22. Duyne RV, Narayanan A, K-Hall K, Saifuddin M, Shultz L, Kashanchi F. Humanized mouse models of HIV-1 latency. Curr HIV Res **2011**; 9:595–605.
23. Denton PW, Garcia JV. Humanized mouse models of HIV infection. AIDS Rev **2011**; 13:135–48.
24. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. Nat Rev Immunol **2007**; 7:118–30.
25. Akkina R. New generation humanized mice for virus research: comparative aspects and future prospects. Virology **2013**; 435:14–28.
26. Lan P, Wang L, Diouf B, et al. Induction of human T-cell tolerance to porcine xenoantigens through mixed hematopoietic chimerism. Blood **2004**; 103:3964–9.
27. Lan P, Tonomura N, Shimizu A, Wang S, Yang YG. Reconstitution of a functional human immune system in immunodeficient mice through combined human fetal thymus/liver and CD34+ cell transplantation. Blood **2006**; 108:487–92.
28. Melkus MW, Estes JD, Padgett-Thomas A, et al. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. Nat Med **2006**; 12:1316–22.
29. Tonomura N, Habiro K, Shimizu A, Sykes M, Yang Y-G. Antigen-specific human T-cell responses and T cell-dependent production of human antibodies in a humanized mouse model. Blood **2008**; 111:4293–6.
30. Brainard DM, Seung E, Frahm N, et al. Induction of robust cellular and humoral virus-specific adaptive immune responses in human immunodeficiency virus-infected humanized BLT mice. J Virol **2009**; 83: 7305–21.
31. Sun Z, Denton PW, Estes JD, et al. Intrarectal transmission, systemic infection, and CD4+ T cell depletion in humanized mice infected with HIV-1. J Exp Med **2007**; 204:705–14.
32. Denton PW, Estes JD, Sun Z, et al. Antiretroviral pre-exposure prophylaxis prevents vaginal transmission of HIV-1 in humanized BLT mice. PLoS Med **2008**; 5:e16.
33. Marsden MD, Kovochich M, Suree N, et al. HIV latency in the humanized BLT mouse. J Virol **2012**; 86:339–47.
34. Wheeler LA, Trifonova R, Vrbanac V, et al. Inhibition of HIV transmission in human cervicovaginal explants and humanized mice using CD4 aptamer-siRNA chimeras. J Clin Invest **2011**; 121:2401–12.
35. Zou W, Denton PW, Watkins RL, et al. Nef functions in BLT mice to enhance HIV-1 replication and deplete CD4+CD8+ thymocytes. Retrovirology **2012**; 9:44.
36. Olesen R, Wahl A, Denton PW, Garcia JV. Immune reconstitution of the female reproductive tract of humanized BLT mice and their susceptibility to human immunodeficiency virus infection. J Reprod Immunol **2011**; 88:195–203.
37. Stoddart CA, Maidji E, Galkina SA, et al. Superior human leukocyte reconstitution and susceptibility to vaginal HIV transmission in humanized NOD-scid IL-2Rgamma(-/-) (NSG) BLT mice. Virology **2011**; 417:154–60.
38. Hur EM, Patel SN, Shimizu S, et al. Inhibitory effect of HIV-specific neutralizing IgA on mucosal transmission of HIV in humanized mice. Blood **2012**; 120:4571–82.
39. Wahl A, Swanson MD, Nochi T, et al. Human breast milk and antiretrovirals dramatically reduce oral HIV-1 transmission in BLT humanized mice. PLoS Pathog **2012**; 8:e1002732.
40. Chateau M, Swanson MD, Garcia JV. Inefficient vaginal transmission of tenofovir-resistant HIV-1. J Virol **2013**; 87:1274–7.
41. Chateau ML, Denton PW, Swanson MD, McGowan I, Garcia JV. Rectal transmission of transmitted/founder HIV-1 is efficiently prevented by topical 1% tenofovir in BLT humanized mice. PLoS One **2013**; 8:e60024.
42. Dash PK, Gendelman HE, Roy U, et al. Long-acting nanoformulated antiretroviral therapy elicits potent antiretroviral and neuroprotective responses in HIV-1-infected humanized mice. AIDS **2012**; 26:2135–44.
43. Denton PW, Olesen R, Choudhary SK, et al. Generation of HIV latency in humanized BLT mice. J Virol **2012**; 86:630–4.
44. Onoe T, Kalscheuer H, Chittenden M, Zhao G, Yang YG, Sykes M. Homeostatic expansion and phenotypic conversion of human T cells depend on peripheral interactions with APCs. J Immunol **2010**; 184:6756–65.
45. Dudek TE, No DC, Seung E, et al. Rapid evolution of HIV-1 to functional CD8+ T cell responses in humanized BLT mice. Sci Transl Med **2012**; 4:143ra98.
46. Kalscheuer H, Danzl N, Onoe T, et al. A model for personalized in vivo analysis of human immune responsiveness. Sci Transl Med **2012**; 4:125ra30.
47. Biswas S, Chang H, Sarkis PT, Fikrig E, Zhu Q, Marasco WA. Humoral immune responses in humanized BLT mice immunized with West Nile virus and HIV-1 envelope proteins are largely mediated via human CD5+ B cells. Immunology **2011**; 134:419–33.
48. Tonomura N, Shimizu A, Wang S, et al. Pig islet xenograft rejection in a mouse model with an established human immune system. Xenotransplantation **2008**; 15:129–35.
49. Habiro K, Sykes M, Yang YG. Induction of human T-cell tolerance to pig xenoantigens via thymus transplantation in mice with an established human immune system. Am J Transplant **2009**; 9:1324–9.
50. Rajesh D, Zhou Y, Jankowska-Gan E, et al. Th1 and Th17 immunocompetence in humanized NOD/SCID/IL2rgammanull mice. Hum Immunol **2010**; 71:551–9.

51–91. See the Supplementary data online.