

BLT Humanized Mice as Model to Study HIV Vaginal Transmission

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The majority of human immunodeficiency virus type 1 (HIV-1) infections occur by sexual exposure, and vaginal transmission accounts for more than half of all newly acquired infections. Studies of vaginal transmission of simian immunodeficiency virus to nonhuman primates (NHPs) have suggested an important role for immune cell trafficking in the establishment of infection as well as in the process of viral dissemination. However, NHP models do not permit the study of HIV transmission and dissemination. The improvement of humanized mouse models with robust human immune cell reconstitution of the female genital tract renders these mice susceptible to intravaginal HIV infection. Thus humanized mouse models of HIV vaginal infection will allow the study of the mechanisms involved in HIV transmission and dissemination *in vivo*.

Keywords. HIV; humanized mice; mucosal infection.

Thirty-four million people are currently chronically infected with human immunodeficiency virus (HIV), and 90% of these infections have resulted from heterosexual intercourse. Women are particularly vulnerable during heterosexual transmission because of mucosal exposure to seminal fluids, and heterosexual women account for more than half of all individuals living with the virus [1, 2].

Sexual transmission of HIV is a relatively inefficient process estimated to be 0.1% for receptive penile–vaginal intercourse compared with 90% for a blood transfusion [3–6]. This suggests that HIV must overcome specific host barriers to achieve transmission and that these barriers are fairly robust and provide a bottleneck for HIV transmission. A clear understanding of HIV acquisition and dissemination in the female genital tract might provide critical insights in the vulnerabilities of HIV to be exploited for the development of therapeutics. In this article, we will review the current knowledge of HIV and SIV acquisition after intravaginal exposure and describe how humanized mouse models,

in particular the humanized bone marrow–liver–thymus (BLT) mice [7, 8], could play an important role in understanding HIV dissemination *in vivo*.

HIV Acquisition at the Cervicovaginal Mucosa

HIV transmission across epithelial surfaces is intrinsically inefficient because of inherent properties of the virus in the context of local host defenses. In fact, even though R5- and X4-tropic HIV variants are present in semen, only R5-tropic viruses are transmitted intravaginally [9], and recent data suggest that a single “founder” virus with particular genetic and phenotypic characteristics is capable of effectively transmitting and establishing systemic infection [10, 11]. To enter the genital mucosa and encounter a target cell, the virus has to overcome the physical barrier formed by the genital fluid, mucus, and epithelium, as well as defenses present in the mucus, such as antimicrobials and chemokines [12–14]. Data using human cervical explants and the nonhuman primate (NHP) model of simian immunodeficiency virus (SIV) infection suggest that HIV preferentially enters the genital mucosa at the level of the cervix or vagina, even though a role of the upper genital tract cannot be excluded [15, 16]. It is believed that the transformation zone where the

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stratified squamous epithelium covering the vagina and the ectocervix transforms into the single columnar epithelium covering the endocervix and the uterus is particularly susceptible to HIV entry. In addition to a thinner epithelium, the endocervix and transformation zone contain a higher number of potential target cells for HIV [17, 18].

Cell-associated and/or cell-free virus has been shown to traverse the epithelium through different mechanisms to access target resident immune cells. Virus can cross the epithelial barrier through breaches induced by repeated penile entry [19], by the use of microbicides causing disruption of the epithelium [20, 21], or by infection with previous sexually transmitted diseases causing genital ulcers [22, 23]. Another potential mechanism by which HIV can cross the epithelium is paracellular movement between epithelial cells. In the cervicovaginal epithelium, the superficial cells are not bound together by tight junctions [24], and it is believed that HIV can pass through narrow gaps or pores between cells [14].

After crossing the epithelial barrier, HIV virions need to encounter their immune target cells for viral replication. Susceptible target cells in the cervicovaginal mucosa include CD4⁺ T cells, dendritic cells (DCs), Langerhans cells, and macrophages. CD4⁺ T cells represent the main population of leukocytes in the cervicovaginal tissue (CVT) and are found in the lamina propria and the subepithelial stroma and within the epithelium [25–28]. The majority of these T cells harbor a memory phenotype and express high levels of CCR5 [29–32], which makes them very likely to be the initial and main target cell for HIV replication [33]. Findings using human explants show that HIV productively infects both activated and resting CD4⁺ T cells in the genital mucosa [29, 34, 35]. Whether DCs and Langerhans cells present in the cervicovaginal epithelium can support productive HIV infection *in vivo* is still controversial; however, both cell types can sample antigens at the mucosal surface and could then capture and transfer HIV to susceptible CD4⁺ T cells [29, 36–38]. Macrophages present and distributed among CVTs also express the HIV-1 receptors CD4 and CCR5, but their involvement in the initial events of HIV infection remains undefined [39–41].

NHP Model of SIV Infection – The Dissemination Paradigm

Ex vivo models cannot reproduce some key aspects of HIV transmission *in vivo*, such as the mucosal immune response and the requirements for viral dissemination into other tissues. To date, the main surrogate animal model used to study intravaginal HIV transmission is the NHP model of high-dose intravaginal SIV or SIV/HIV (SHIV) chimeric viruses [13, 42].

Studies of SIV sexual transmission to female macaques suggest that SIV crosses the mucosal epithelial barrier within hours to initiate infection [43]. Despite high-dose viral

innoculum, only low levels of viral RNA within a small cluster of infected resident memory CD4⁺CCR5⁺ cells are detected in the CVT in the first days after infection [13, 35, 44], consistent with the “genetic bottleneck” described for HIV transmission in humans [10, 11]. Also consistent with human studies, the foci of infected cells were consistently found in the endocervix and transformation zone [45, 46]. During the first week of infection, the infected population of T cells is restricted to the CVT, where it expands locally [13, 45]. Viral dissemination occurs when virus and virus-infected cells leave the tissue through afferent lymphatics and enter the draining lymph node, where they come in contact with a large number of densely packed CD4⁺CCR5⁺ T cells. Once in the draining lymphoid tissue, sufficient virus and infected cells are generated to disseminate and establish a systemic infection throughout the secondary lymphoid organs and the bloodstream [13, 45]. The time frame after intravaginal exposure during which the virus is not detectable in the peripheral blood is referred to as the eclipse phase. This phase has been estimated to last about 10 days for HIV acute infection in humans and about 7 days in SIV infection of NHPs [13, 47, 48]. During the second week of infection, replication explodes in the lymphatic tissues, where virus has access to many more susceptible target cells. Virus levels in blood and tissues peak at the end of this second week of infection, before declining to stable levels by 4 weeks. Once the systemic infection is established, infected tissues comprise a viral reservoir where virus is produced and stored and where proviruses are harbored in latently infected cells in SIV-infected rhesus macaques [49], just as in HIV-infected humans [50]. At that time, massive CD4⁺ T-cell depletion and epithelium damage induced by HIV or SIV infection has already begun in the gut [51–56].

NHP studies have uncovered a potentially important role for immune cell trafficking in establishing the local reservoir of HIV-infected cells as well as in the process of viral dissemination [45, 57]. Exposure to the virus induces an innate immune response at the mucosal site of entry, which might restrict HIV transmission and account in part for the small infected founder population. The expression of the chemokine CCL5, a CCR5 agonist, by the mucosa could potentially inhibit viral entry [58]. This is seen with Maraviroc, a small molecule antagonist of CCR5 [59–61]. SIV exposure also induces the release of CCL20 from epithelial cells, which serves to recruit CCR6⁺ plasmacytoid DCs (pDCs) into the region just beneath the epithelium. pDCs produce large quantities of interferon alpha (IFN- α), as well as the CCR5 ligand CCL4, which might also be expected to inhibit viral entry [45]. However, this response remains ineffective in clearing the virus. In contrast, the innate immune response might have the adverse effect of fueling SIV infection by providing new target cells [45, 57]. This theory is consistent with the observations that innate immune agonist treatments (eg, Toll-like receptor agonists) or previous mucosal infection

increases HIV susceptibility [62, 63]. CCL4 produced by pDCs could also promote the recruitment of CCR5⁺CD4⁺ T cells, increasing the number of potential virus target cells [45]. In addition, IFN- α produced by recruited pDCs could also activate tissue stromal cells to produce CXCL10 (IP-10), which might also result in additional immune cell recruitment, including activated CD4⁺ T cells and pDCs bearing CXCR3, providing more susceptible cells for HIV amplification [64]. It is hoped that the development of the humanized mouse model of HIV vaginal transmission will allow these different possible roles of the innate immune response to be tested functionally in the context of HIV infection after intravaginal exposure.

The adaptive immune response to HIV and SIV has been described as “too late and too little” [46]. The response is too late because it is detectable only at the end of the second week of infection and, therefore, too late to clear the virus locally to prevent the spread. And the response is too little because even though the response may reduce viral load and slow the rate of disease progression [10, 47], it is insufficient to prevent the massive CD4⁺ T-cell depletion in gut and the formation of a latent viral reservoir. In the vast majority of cases, once the virus leaves the CVT and establishes a systemic infection, the immune system will not be able to clear the infection. Therefore, the best opportunity to prevent HIV disease lies at the site of mucosal entry, where the vulnerabilities of HIV can be exploited, and before dissemination to the lymphoid compartment, where the latent reservoir is established. In this regard, a robust SIV-specific CD8⁺ T-cell response is detected in the CVT [46]. Therefore, a vaccine strategy that could elicit a similarly robust and stable CD8⁺ T cell response in the CVT could potentially restrict HIV infection, and this, too, will hopefully be amenable to study in humanized bone marrow–liver–thymus (BLT) mice.

Humanized BLT Mice to Study HIV Dissemination In Vivo

Studies using the NHP model have elegantly established a transmission and dissemination paradigm for SIV vaginal transmission. Given the strict species tropism of HIV, the NHP model, and other animal models for that matter, cannot be used to study HIV transmission and dissemination in vivo [65–70]. Although the analogy to the SIV infection paradigm has been useful, it remains unclear if this paradigm will accurately reflect the events required for HIV vaginal infection. Investigators have therefore turned to humanized mouse models to study HIV infection in vivo. Humanized BLT mice are generated by implanting human fetal thymic and liver tissues and transferring autologous human fetal liver CD34⁺ hematopoietic stem cells into NOD/SCID or NOD/SCID γ ^{-/-} recipients. These mice are reconstituted with the major human hematopoietic lineages, including T, B, monocyte/macrophage,

dendritic, and natural killer cells, and human T cells become educated in the human thymic organoid. The development of the humanized BLT model has improved the human immune system reconstitution of immunodeficient mice in mucosal tissues, allowing them to sustain HIV vaginal and rectal transmission and therefore represent an attractive model to study the events required for mucosal transmission [71–74].

To be suitable for HIV transmission and dissemination studies after intravaginal challenge, the CVT of the BLT mice needs to show anatomic similarities at the proposed site of HIV entry and a sufficient reconstitution with human hematopoietic cells to HIV transmission. The anatomy of the mouse female genital tract is very similar to the human despite its smaller size and the presence of a double uterus. The uterine horns merge caudally to form an undivided corpus uteri and a single cervical canal, which projects into the upper vagina [75]. The murine vagina and endocervix are covered with a stratified squamous epithelium, whereas the endocervix and uterus consist of a simple columnar epithelium [75]. The physical barrier HIV would encounter is therefore similar to that in humans [76]. After reconstitution with human hematopoietic cells, the vagina, ectocervix, endocervix, and uterus of the BLT mice are repopulated with human T cells, macrophages, and DCs [72]. Human T cells are present within the epithelial layer and the lamina propria, and macrophages and DCs are present in the lamina propria throughout the genital tract, reproducing the distribution of these cells in human genital tract [17, 25, 72].

As expected by the repopulation of the CVT with human immune cells, intravaginal HIV transmission of humanized BLT mice has been successfully reported using a single atraumatic application of a high dose of cell-free CCR5-tropic primary isolate HIV-1_{JR-CSF} [72, 77–80]. This method ensures a highly reproducible infection rate with a detectable viremia in the peripheral blood as early as 2 weeks after infection as measured by plasma p24 or plasma HIV RNA [72, 77–80].

In the BLT mouse, as in humans, once HIV is detectable in plasma, systemic infection is established, and infected cells are already detectable in all organs of the mouse, including the gastrointestinal tract, and a viral reservoir is established [72, 81, 82]. Systemic infection is then followed by a progressive drop in the proportion of human CD4⁺ T cells in peripheral blood as well as in the tissues of infected mice, with the most dramatic loss encountered in the gut [72], which recapitulates human infection [52, 83]. The humanized BLT mice model is therefore a relevant candidate to study intravaginal HIV transmission.

Humanized Mice to Study HIV Prevention Strategies

Based on the current knowledge of SIV and HIV intravaginal infection and dissemination, the first events of infection at the mucosa provide a critical window of opportunity where the

vulnerabilities of HIV can be exploited. Several approaches to prevent HIV infection at this site have been studied in humanized mice and will be discussed below, including microbicides, antiretroviral drugs, RNAi knockdown, and neutralizing antibodies, demonstrating the utility of the humanized mouse model for protection studies against intravaginal acquisition of HIV.

Microbicides have been successful in protecting BLT mice from intravaginal HIV acquisition. In a study where 1% tenofovir was topically applied before and after HIV exposure to mirror the protocol of the CAPRISA 004 clinical trial, a protection of 88% of the exposed BLT mice was achieved [78] in comparison with 39% to 54% in women [84]. A novel approach to inhibit HIV infection and dissemination using topically applied CD4⁺ cell targeting aptamer-siRNA chimeras (CD4-AsiC) was evaluated using BLT mice. A mix of aptamers was used that induced the knockdown of either the HIV coreceptor CCR5, HIV gag, or HIV vif proteins by specific siRNA. The different aptamers were applied before and/or after HIV exposure. This regimen of CD4-AsiCs resulted in 50% protection from intravaginal HIV-1 transmission, highlighting the potential benefits of using this targeted approach as an active component of microbicide [79].

Another approach to inhibit HIV dissemination is to restrict the foci of infection and eliminate the virus at the local site of infection. One strategy proven successful is the use of systematically administered antiretroviral drugs to individuals at high risk (targeted systemic pre-exposure prophylaxis) [85, 86]. These results were reproduced in BLT mice in a study assessing the effect of the antiretrovirals found in Truvada (tenofovir disoproxil fumarate [TDF] and emtricitabine [FTC]) and following the approach used in one of the arms of the VOICE clinical trial [72]. A daily treatment starting 3 days before and continued until 4 days after intravaginal HIV challenge resulted in 100% protection of the BLT mice, demonstrating the ability of systemic pre-exposure prophylaxis to prevent intravaginal HIV-1 transmission [72].

The possibility to use neutralizing antibodies (NAbs) to block intravaginal HIV infection has also been tested in humanized mice [87, 88]. The use of NAbs has been shown to be a successful prophylaxis in the NHP model of SIV infection [89]. A similar result was obtained in a model of vaginal HIV transmission using Rag2^{-/-}γc^{-/-} engrafted with human fetal liver CD34⁺ hematopoietic progenitor cells (RAG-hu humanized mouse) where the topical application of the NAb VRC01 1 hour before intravaginal challenge protected 78% of the mice against HIV infection [87]. In the same study, a cocktail of 4 NAbs—b12, 4E10, 2F5, and 2G12—protected 100% of the mice [87]. The humanized mouse model offers the unique opportunity to test gene therapy approaches to block HIV infection by modifying the human hematopoietic stem cells before reconstitution of the mice [90–92]. Using this approach, humanized

BLT mice were reconstituted with human hematopoietic stem cells transduced with a lentiviral construct encoding a class-switched immunoglobulin A Nab, b12. This transgene was expressed in plasma cells and B cells in lymphoid organs and mucosal sites [88]. Mice producing immunoglobulin A b12 were protected from CD4⁺ T-cell loss in peripheral blood and tissue and had fewer HIV p24⁺ T cells in the CVT and gut after intravaginal HIV challenge [88]. Both of these studies demonstrate the potential of HIV NAbs as prophylaxis against HIV intravaginal infection in vivo.

As previously described, the adaptive immune response against SIV in the NHP CVT is robust but comes too late to prevent transmission, dissemination, and the establishment of a systemic infection [46]. However, a vaccine strategy that could elicit a robust, stable, and long-lasting anti-HIV immune response within the CVT might be a successful strategy to restrict and stop the infection before it becomes systemic. In BLT mice, human T cells are educated in the transplanted autologous human thymic tissue, which allows them to mount a robust human cellular and humoral immune response against HIV [73]. In particular, the CD8⁺ T-cell response during acute phase of HIV infection in BLT mice closely reproduces the one described in humans in its kinetics, magnitude, and specificity [74]. Furthermore, viral sequencing data suggest that the immune pressure is strong enough to induce escape mutations of HIV. BLT mice reconstituted with tissue from the same donor showed comparable results and corresponded to the HIV mutations described in human acute infection in individuals with the same human leukocyte antigen (HLA) type [74]. Although there is no report to date directly describing the human immune response against HIV in the CVT of the BLT mice, it is likely that this model could be used to study the local response to infection and how to improve it to block HIV dissemination.

Finally, another opportunity to restrict the infection locally would be to stop cellular trafficking to inhibit the recruitment of target cells at the infection site or to block their dissemination from the CVT to the lymphoid compartment. This approach has been previously suggested in the NHP model of SIV infection by the use of glycerol monolaurate (GML) [45]. GML inhibits immune activation and chemokine and cytokine production by human vaginal epithelial cells [93, 94] and was found to decrease CCL20 levels in cervical/vaginal fluids of treated rhesus macaques [45, 95]. Topical treatment with GML was thought to prevent the initial mucosal epithelial signaling responsible for recruiting target cells into the CVT and protected animals against acute systemic infection [45]. The role of lymphocytes trafficking in HIV dissemination was recently shown in vivo using BLT mice [96]. In this study, T-cell trafficking was interrupted by blocking their egress from the lymph nodes using the sphingosine 1 phosphate receptor functional antagonist FTY720 (fingolimod). Treatment of mice at the time of subcutaneous HIV infection impacted HIV dissemination

and strongly reduced plasma viremia [96]. However, the dissemination of HIV within the lymphatics was not completely abrogated, and the treatment did not result in the clearance of the virus. When FTY720 treatment was withdrawn, systemic viral replication occurred, suggesting that HIV was sequestered within the lymph nodes [96]. This study demonstrated that in BLT mice the trafficking patterns of human immune cells could be altered in vivo, making it possible to study the role of immune cell trafficking in HIV dissemination.

Conclusion

Humanized mice offer the unique opportunity to study a model of HIV infection in vivo. The fact that these mice can be reliably infected through intravaginal exposure opens numerous possibilities to study novel strategies to prevent HIV sexual transmission. The vaginal infection model also provides an opportunity to deepen our knowledge regarding the mechanisms of virus dissemination after acquisition in the CVT and the involvement of immune cell trafficking in this process. Nevertheless, many aspects of the model still need to be defined to use this model for vaccine development targeting protection against heterosexual transmission.

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

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