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Mucosal HIV-1 transmission and prevention strategies in BLT humanized mice

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Abstract

Clinical trials testing microbicides and related biomedical interventions to block HIV transmissions have produced contradictory results and to date it is unclear why. Further elucidation of the molecular basis of mucosal HIV transmission and extensive pharmacokinetic and pharmacodynamic analyses are essential to implementing effective prevention strategies. Animal models are of critical importance to this effort and bone marrow-liver-thymus (or BLT) humanized mice have recently emerged as a powerful small animal research platform for *in vivo* efficacy evaluation of mucosal and parenteral HIV-1 prevention interventions. The availability of this validated system for the pre-clinical evaluation of HIV-1 prevention approaches will accelerate the implementation of the best candidate interventions into clinical trials.

Keywords

humanized mice; BLT; HIV-1 prevention; efficacy evaluation; microbicides; PrEP

Microbicides to prevent HIV: the ups and downs

The large number of HIV transmissions that occur each day (>7000) highlight the urgency to prevent new infections [1]. Towards this end, there is much enthusiasm surrounding the potential of biomedical interventions such as topical or systemic pre-exposure prophylaxis (PrEP) to slow the spread of AIDS (Box 1) [2–12]. After years of frustrating failures [13–18], researchers investigating topical PrEP (also referred to as microbicides) to prevent HIV acquisition were energized in July 2010 by the report of the CAPRISA 004 clinical trial demonstrating that topical 1% tenofovir gel reduced the incidence of HIV transmission by 39% in South African women [19]. This report generated much optimism because it was the first demonstration that a microbicide could prevent HIV transmission in humans. However, this enthusiasm for the potential utility of microbicides was severely dampened again in November 2011. This is when the independent data safety and monitoring board (DSMB) discontinued the topical 1% tenofovir gel arm of MTN 003, the Microbicide Trials Network's clinical trial testing vaginal and oral interventions to control the epidemic (or VOICE) (http://clinicaltrials.gov/ct2/show/NCT00705679; last accessed on March 3, 2012). After a routine review of study data, the DSMB concluded that tenofovir gel was not effective in preventing HIV in the women enrolled in this trial (http://

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www.mtnstopsHIV.org/node/3909; last accessed on March 3, 2012). It was hoped that VOICE would confirm the CAPRISA 004 findings, but the data disagreed. These conflicting results serve to highlight the complexity of the interactions between HIV and the host at the mucosal surfaces during HIV acquisition and represent a clear need for suitable model systems that can elucidate the mechanisms responsible for such contradictory results.

HIV-1 tropism: one virus, two species

Understanding the mechanisms of HIV-1 transmission is critical to the design of effective prevention interventions. Unfortunately, HIV-1 replication is limited to two species: humans and chimpanzees. HIV-1's absolute requirement for human (or chimpanzee) cells as targets has severely limited *in vivo* HIV-1 research. For example, restriction factors present in the cells of the non-human primates (NHP) available for HIV-1 research restrict HIV-1 replication and transmission in these species, necessitating the use of the surrogate SIV or SIV/HIV (SHIV) chimeric viruses when HIV-1 is modeled in these systems [20–23]. Furthermore, the lack of proper receptor/co-receptor expression combined with numerous translational and post-translational blocks to HIV-1 replication in rodent cells prevent their use for HIV-1 research including transmission studies [24]. Thus from a modeling perspective, it is implicit that the target cells for *in vivo* infection and dissemination must be human (or chimpanzee) in origin for HIV-1 transmissions to occur. In particular this means that regardless of the route of exposure, appropriate HIV-1 target cells must be present in the vicinity of the site of inoculation for a transmission to occur. HIV-1 target cells include $CD4^+$ T cells, macrophages and Langerhan's (dendritic) cells.

BLT mice have a bona fide human thymic organ for the generation of human T cells

To overcome the major obstacle that is the limited species tropism of HIV-1, individually bioengineered human-mouse chimeras were developed with the aspiration that they would recapitulate key aspects of the human condition. Since the 1980s, many variants of `humanized mice' have been described and these models have been reviewed extensively elsewhere [25–34]. After many iterations, humanized mice were finally developed that are susceptible to vaginal, rectal and intravenous HIV-1 transmission [35–45]. In this regard, humanized bone marrow-liver-thymus (or BLT) mice (Box 2) have been shown to be especially well suited for mucosal HIV-1 transmission and prevention studies. Even though the focus of this review is on BLT mice other humanized mice models can serve as alternatives (Box 3).

All BLT mice have key common characteristics. Specifically, all BLT mice are individually made via a two-step protocol [46, 47]. The first step is the surgical implantation under the kidney capsule of an immunodeficient mouse of a small (1–2 mm^3) piece of human liver sandwiched between two small (1–2 mm^3) pieces of thymic tissue that together grow into an organ that in all respects represents a *bona fide* human thymus. This thymic organoid provides the microenvironment needed for the development of human T cells in the context of human thymic epithelium that results in the production of human leukocyte antigen (HLA)-restricted T cells [48–50]. In order to accomplish systemic reconstitution with all kinds of human hematopoietic cells derived from the bone marrow, including the production of T cell progenitors, these implanted animals are also receive a bone marrow transplant of human $CD34^+$ cells isolated from the same liver tissue [49, 51].

BLT model strengths and limitations

Even though all BLT mice have the same components, several variations in the humanization protocols have been utilized. Variations in the protocols used to generate BLT mice include: (i) either transplanting the $CD34^+$ cells the same day as implanting the liver and thymus or delaying the transplant by several weeks; (ii) humanizing one of several immunodeficient mouse strains such as NOD/SCID, NOD/SCID IL2Rγc−/− (NSG), NOD/Rag1 IL2Rγc−/−, or Rag2−/− IL2Rγc−/−; (iii) implanting the liver and thymus under only one kidney or performing bilateral implants under both kidneys; and (iv) supplementing the liver implant with $CD34^+$ cells in matrigel prior to implantation under the kidney capsule [37–39, 44, 48–61]. In general, these changes from the original BLT production protocol do not alter the systemic human reconstitution of BLT mice although the choices made when generating BLT mice can affect the number of mice that can be produced from a single human donor. For example, the pivotal protocol variable affecting the humanization levels in BLT mice is transplanting sufficient human $CD34^+$ cells into the immunodeficient mouse strain being humanized. Transplantation of relatively low numbers (hundreds of thousands) of human $CD34^+$ cells to generate NSG-BLT mice results in robust humanization [43]. Transplantation of higher numbers of cells (x10) are needed to generate NOD/SCID-BLT mice with robust levels of humanization which limits the number of animals that can be produced in NOD/SCID mice compared to NSG mice [37–39, 44, 49]. With sufficient numbers of transplanted cells, engraftment is similar in BLT mice generated with both of these immunodeficient backgrounds [48] and similar levels of human engraftment result in similar susceptibility to HIV-1 transmission [37–39, 45].

Shortly after the bone marrow transplant with human $CD34^+$ cells, BLT mice exhibit peripheral reconstitution with human hematopoietic cells [37–39, 44, 45, 48–52, 55, 56, 59–61]. One strength of the model is that engraftment of the mouse bone marrow with human stem cells results in the development and dissemination of T, B, natural killer, myeloid and dendritic cells throughout each BLT mouse including peripheral blood, human thymic organoid, bone marrow, spleen, lymph nodes, liver, lungs, small and large intestines and the female reproductive tract (FRT) [37, 44, 48–52, 60–63]. Thus, systemic dissemination of HIV-1 can be studied in BLT mice. Notable differences between humans and BLT mice include the fact that the exposed mucosal surfaces are comprised of mouse epithelial cells and the sub-mucosa is comprised of a mixture of mouse stromal cells and human leukocytes and the fact that the human cells in the FRT are subjected to an estrous cycle versus a menstrual cycle that could result in a more highly activated condition than would be found in NHP or young women [64]. Furthermore, the mechanisms governing the recruitment of human leukocytes into the BLT mouse FRT have yet to be elucidated.

Human immune responses in BLT mice also represent strengths and limitations of the model. One limitation of the model is that human B cell responses in BLT mice have not been optimized [65]. Nevertheless, a significant strength of the model relative to other humanized mouse models is that antigen-specific human antibody responses requiring T cell help have been documented by multiple research groups [44, 48, 60]. A further strength of the model is that human T cells in BLT mice have been shown to be able to generate HLA class I- and class II-restricted adaptive immune responses to viruses as well as delayed-type hypersensitivity responses to model antigens including tetanus toxoid and collagen type V [48–50]. In addition, human T cells in BLT mice have been shown to become activated by human dendritic cells and to mount a potent T-cell immune response to superantigens [49].

Mucosal HIV-1 transmission in BLT mice

It is important to emphasize that one key to the rapid success and acceptance of BLT mice as an HIV-1 transmission model is the fact that human hematopoietic cells efficiently populate the FRT and the rectum of these animals [37, 43, 44]. Specifically, detailed immunohistochemistry and flow cytometry analyses of the gut and the FRT of BLT mice have demonstrated the robust reconstitution of these organs with all the human cells that have been postulated to be important for mucosal HIV-1 transmission. Human $CD4^+$ T cells, macrophages and dendritic cells are all present throughout the gut and the FTR of BLT mice [37, 43, 44]. It is these human cells that render BLT mice susceptible to mucosal HIV-1 transmission by the same strains of virus (i.e. HIV-1 strains which utilize CCR5 as a co-receptor for viral entry) that infect the vast majority of humans and that are susceptible to the same drugs that are used for treatment and prevention in humans.

To provide clarity and context in the interpretation of the HIV-1 prevention data presented in the next section, it is also important to indicate the experimental conditions used in these studies. All publications on rectal HIV-1 transmission in BLT mice to date have incorporated simulated coitus to reproduce the abrasions known to occur in humans that participate in rectal anal intercourse [38, 44]. In addition, all publications evaluating vaginal HIV-1 transmission in BLT mice utilized atraumatic inoculation [37, 39, 45]. In two of these reports there was no artificial thinning of the vaginal epithelium with hormone pretreatment [37, 39] while in the third report pre-treatment with the hormone medroxyprogesterone prior to viral exposure was performed [45].

Efficacy of antiretrovirals to prevent vaginal HIV-1 transmission in BLT mice

Several reports have investigated the efficacy of currently prescribed therapeutic and investigational antivirals for their ability to prevent vaginal HIV-1 transmission in BLT mice when administered systemically or topically. In the first report of its kind, a well known combination of antiretrovirals – tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC), the drugs found in Truvada® – was administered daily to BLT mice in a manner that would reproduce the approach used in one of the arms of the VOICE clinical trial being conducted in South Africa, Uganda and Zimbabwe [37]. After three days of systemic drug treatment, BLT mice were challenged once intravaginally with HIV-1. The treatment was then continued for another four days (equaling a total of seven days of treatment). After PrEP treatment and viral exposure, the mice were followed longitudinally to permit outgrowth of any transmitted viruses. Peripheral blood was sampled at routine intervals and multiple organs were sampled at harvest to detect the presence of HIV-1. Seroconversion was not used to determine infection as it is a less sensitive technique than the utilized molecular and cellular analyses which included: (i) plasma viral RNA (Amplicor); (ii) peripheral blood mononuclear cell (PBMC)-associated viral DNA (real time PCR); (iii) tissue cell-associated viral DNA (real time PCR) and (iv) virus rescue with activated allogeneic PBMC. Protection from infection in BLT mice was defined as a complete absence of any evidence of the presence of HIV-1 by any assay at any time point analyzed. Under these circumstances systemic administration of FTC and TDF resulted in 100% protection from HIV-1 infection (Table 1) [37]. These results demonstrated the ability of systemic PrEP to prevent vaginal HIV-1 transmission.

In a second report, a before-and-after protocol for the topical application of 1% tenofovir was evaluated for its ability to protect BLT mice from vaginal transmission [39]. This microbicide application approach in BLT mice was modeled after the CAPRISA 004 clinical trial where a dose of 1% tenofovir was applied <12 hr prior to exposure plus a

second dose was applied <12 hr following exposure [19]. Specifically, BLT mice received one vaginal application of 1% tenofovir four hours before exposure to HIV-1 and a second topical application of 1% tenofovir 4 hr after exposure. Mice were then longitudinally monitored for infection essentially as described above. In this case, topically applied tenofovir protected 88% of the exposed BLT mice from vaginal HIV-1 transmission (Table 1) [39].

In addition to topical tenofovir, in this same report six additional potential microbicides that target different stages of viral replication were evaluated [39]. These included: a C-peptide fusion inhibitor (C52L), a membrane-disrupting amphipathic peptide inhibitor (C5A), a trimeric D-peptide fusion inhibitor (PIE12-Trimer), a combination of reverse transcriptase inhibitors (FTC and TDF), a thioester zinc finger inhibitor that primarily affects virion maturation (TC247), and a small-molecule inhibitor of the small GTPase Rac that affects viral entry from within the target cell (NSC23766) [39]. For the study of these inhibitors, a more standard protocol for microbicide application was followed [13–18]. Specifically, vaginal application occurred 15 min prior to viral exposure. Peripheral blood was sampled longitudinally at routine intervals and multiple organs were sampled at harvest to detect the presence of HIV-1 and an extensive set of analyses similar to the one indicated above were performed to detect any presence of HIV-1. Protection from infection in BLT mice was again defined as a complete absence of any evidence of the presence of HIV-1 by any assay at any time point analyzed [39]. Protection levels from 0–100% were observed emphasizing how targeting the virus directly and earlier in replication yields better protection from transmission (Table 1). The peptide that destroyed virions prior to cell contact (C5A) and the peptides that inhibited virus-cell fusion (C52L and PIE-12 trimer) protected 100% of BLT mice. The reverse transcriptase inhibitors that act soon after cellular entry protected 89% of BLT mice. The inhibitor that primarily acts to prevent virion maturation following a round of replication (TC247) protected 57% of BLT mice and the RAC inhibitor that interferes with the structural modifications that occur within the cell during fusion (NSC23766) did not protect any BLT mice from vaginal HIV-1 transmission [39]. In summary, these data demonstrate that topical inhibitors targeting HIV-1 earlier in the replication cycle have higher levels of success in preventing vaginal HIV-1 transmission in BLT mice.

Evaluation of novel approaches to prevent vaginal HIV-1 transmission in BLT mice

A separate report described the evaluation of three topically applied $CD4^+$ cell targeting aptamer-siRNA chimeras (CD4-AsiC) for their ability to prevent vaginal HIV-1 transmission. CD4-AsiC were applied vaginally pre- and post-exposure to HIV-1 [45]. One type of CD4-AsiC carrying a siRNA against human CCR5 (cellular protein that functions as viral co-receptor) was applied 48 hr prior to viral exposure. This was followed by a second CD4-AsiC treatment at 24 hr prior to viral exposure, only this time there were three distinct CD4-AsiCs applied that carried siRNA targeting CCR5, *gag* (encodes viral structural proteins matrix and capsid) and *vif* (viral accessory protein that disrupts the innate immune activity of host APOBEC3 proteins). Finally, 4 hr post viral exposure two types of CD4-AsiC carrying siRNAs targeting *gag* and *vif* were applied. Exposed mice were then followed longitudinally and examined for the presence of plasma antigenemia (by ELISA), plasma viral RNA [by quantitative real time (qRT)-PCR] and peripheral blood CD4:CD8 ratios (by flow cytometry). This before-and-after application of three distinct CD4-AsiCs resulted in 50% protection from vaginal HIV-1 transmission in BLT mice highlighting the potential benefits of this targeted prevention approach (Table 1) [45].

Efficacy of systemic PrEP for the prevention of rectal and parenteral HIV-1 infection in BLT mice

As indicated above, BLT humanized mice have been used to evaluate prevention of vaginal HIV-1 infection via topical or systemic applications of antivirals. Beyond these studies, systemic PrEP with FTC and TDF has also been tested in this model for its ability to protect BLT mice against rectal HIV-1 exposure [37, 38]. These experiments were performed using the general approach employed in the iPrEx clinical trial [66]. Systemic PrEP consisted of seven daily FTC and TDF doses administered as indicated above. Three hours following the third dosing, the viral exposure occurred. Extensive longitudinal and post-mortem analyses were utilized to detect the presence of HIV-1 and protection from infection in BLT mice was again defined as a complete absence of any evidence of the presence of HIV-1 by any assay at any time point analyzed [38]. Systemic PrEP with FTC and TDF led to protection from rectal and intravenous HIV-1 transmission in BLT mice (Table 1) [38]. Specifically, this regimen protected 100% of treated BLT mice from rectal HIV-1 transmission. Remarkably, this regimen also prevented 88% of intravenous transmissions in this model. In addition to these results, the ability of this seven day FTC and TDF regimen to prevent intravenous HIV-1 transmission when started 24 hours post-exposure was also tested [38]. The post-exposure dosing of FTC and TDF delayed detection of viremia but did not protect any (n=4) of the treated animals [38].

Comparison of HIV-1 prevention in BLT mice, monkeys and humans

Collectively the observations made to date clearly demonstrate the flexibility of the BLT mouse system for evaluating both systemic and topical HIV-1 prevention interventions. However, one issue of utmost importance is how these data compare to similar evaluations performed in NHP and in ongoing or completed clinical trials of HIV prevention. For this reason we compared the results obtained in BLT mice and NHP to available clinical trial data to examine to what extent either model mimics the human situation.

Several of the eleven route and inhibitor combinations tested in BLT mice have also been evaluated in NHP and in humans (Tables 1, 2). In humans, two clinical trials have tested topical tenofovir to prevent vaginal HIV transmission (CAPRISA 004 and VOICE) [19]. CAPRISA 004 found topical tenofovir to be capable of reducing HIV incidence by 39% while the VOICE trial was halted for a lack of efficacy due to unresolved causes [19]. It remains unclear if the BAT24 protocol (applied <12 hr prior to exposure plus a second dose was applied <12 hr following exposure) for microbicide application in CAPRISA 004 is more efficacious in humans than the once daily administration of the microbicide in the VOICE trial. It is clear that in BLT mice and in the NHP models, where compliance is not an issue, topical tenofovir protected a high percentage of BLT mice (88%) and macaques (100%) [39, 67]. If taken at face value, these results suggest that this antiretroviral compound has the capacity to block vaginal HIV-1 infection if present at the appropriate time and in the appropriate amounts at the site of exposure. The lack of efficiency in humans may be attributable to poor adherence to the study drug leading to a lack (or suboptimal levels) of drug at the time and site of exposure or perhaps host-specific co-factors in the form of pre-existing conditions such as cervicitis or STDs that have not yet been modeled in the BLT system.

The remaining microbicide comparisons are between BLT mice and NHP as these inhibitors have not yet been tested in humans. The combination of tenofovir (applied at 1% in both BLT mice and macaques) plus FTC (applied at 0.7% in BLT mice and 5% in macaques) protected 89% of BLT mice and 100% of macaques [39, 67]. The topical formulation for the zinc finger inhibitor TC247 used in the NHP study were at a higher concentration compared

to the BLT studies, but the protections levels in the BLT mice (57%; 0.5 mM) and macaques (83%; 37.6 mM) were comparable [39, 68]. Finally, topical application of the peptide fusion inhibitor C52L was more effective at protecting from vaginal viral transmission in BLT mice (100%; 500 μM) compared to macaques however this apparent discrepancy might be due to the lower levels of drug used for some of the primate experiments (53%; 50–1500 μM) [39, 69].

In addition to the topical PrEP studies, four clinical trials have tested systemic PrEP with FTC and TDF for the prevention of vaginal and rectal HIV transmission (iPrEx, Partners PrEP, TDF2 and FEM-PrEP) [66, 70]. The iPrEx trial tested daily oral administration of the reverse transcriptase inhibitors FTC and TDF to prevent rectal HIV-1 transmission in men who have sex with men (MSM) and was conducted at 11 sites in 6 countries [66]. Similarly, the TDF2 and Partners PrEP tested daily oral administration of FTC and TDF to prevent HIV transmission in heterosexually active young adults in Botswana or serodiscordant heterosexual couples at 9 sites in Uganda and Kenya, respectively [70]. FEM-PrEP was recently halted for futility due to unresolved causes while the iPrEx, Partners PrEP, and TDF2 reported that systemic PrEP with FTC and TDF reduced the incidence of HIV transmission in the study populations by 44–73% (Table 2) [66, 70]. Similar to these three studies, systemic PrEP with FTC and TDF showed robust efficacy in both the BLT mouse and NHP models when tested for the prevention of rectal virus transmission. Specifically, there was 100% protection when FTC and TDF were administered intraperitoneally in BLT mice and 66% or 100% protection when the drugs were administered via oral or subcutaneous routes, respectively, in macaques [38, 71]. Taken together, these data demonstrate that human, BLT mice and NHP models can yield congruent protection levels for systemic PrEP with FTC and TDF, especially when consideration is given to the fact that protocol compliance is compulsory in the animal experiments.

Concluding remarks

To date, clinical interventions to prevent mucosal HIV transmission have produced mixed results which are creating confusion in this field. Even in trials showing the highest levels of success at preventing mucosal HIV transmission, the successes are limited. Where success has been achieved there is a strong correlation between detectable drug levels and protection – a finding that implicates poor adherence to the study drug as contributing to the trials' limited successes [19, 66, 72, 73]. Encouragingly, currently available information highlights the predictive value of BLT humanized mice for HIV-1 transmission prevention approaches such as PrEP. Therefore, the BLT mouse model system may help elucidate mechanisms that lead to discordant clinical trial results such as those indicated in Table 2.

BLT mice are a reliable and reproducible small animal experimental system for HIV-1 prevention research and BLT mice could be broadly utilized to effectively screen the candidate HIV-1 prevention interventions, such as novel vaginal and rectal microbicides. In both topical and systemic PrEP studies, BLT mouse experiments yielded results that closely approximate those observed in the NHP experiments. Although it should be noted that in virtually all cases, the results obtained in both BLT mice and NHP show higher levels of protection than the human data. Understanding the reasons for these differences is an important area for future study. In addition, there are several other related research areas requiring further investigation in which BLT humanized mice are likely to play a pivotal role. Early events that occur during mucosal HIV-1 transmission are still unclear and these could be examined in BLT mice. With regard to HIV-1 prevention, the potential of drug resistant viruses developing in PrEP participants is a risk that must not be ignored [70]. This resistance problem leads into another critical area for future studies, namely the need for novel agents that are not currently used as front-line therapy such as FTC and TFV [70].

BLT mice are already being utilized in this effort (Table 1); however, more could be done in this model to accelerate the development pipeline of quality, novel HIV-1 prevention compounds. Lastly and perhaps most importantly will be the future use of BLT humanized mice to establish pharmacokinetic and pharmacodynamic correlates of protection that can be translated into human studies. In summary, BLT humanized mice have been and will continue to be instrumental in the pre-clinical evaluation of HIV-1 prevention strategies and for the analysis of the key aspects that govern HIV transmission.

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References

1. WHO-UNAIDS. 2010 Report on the Global AIDS Epidemic. 2010. http://www.unaids.org/globalreport/global_report.htm
2. Cohen MS, et al. Narrative review: antiretroviral therapy to prevent the sexual transmission of HIV-1. Ann Intern Med. 2007; 146:591–601. [PubMed: 17438318]
3. Derdelinckx I, et al. Criteria for drugs used in pre-exposure prophylaxis trials against HIV infection. PLoS Med. 2006; 3:e454. doi:410.1371/journal.pmed.0030454. [PubMed: 17090213]
4. Gay, CL., et al. Using antiretrovirals to prevent HIV transmission. In: Mayer, KH.; Pizer, HF., editors. HIV Prevention: A Comprehensive Approach. Academic Press; 2009. p. 107-145.
5. Grant RM, et al. AIDS. Promote HIV chemoprophylaxis research, don't prevent it. Science. 2005; 309:2170–2171. [PubMed: 16195446]
6. Landovitz RJ. Recent efforts in biomedical prevention of HIV. Top HIV Med 12(4):100–3. 2007; 15:99–103.
7. Liu AY, et al. Preexposure prophylaxis for HIV: unproven promise and potential pitfalls. JAMA. 2006; 296:863–865. [PubMed: 16905792]
8. Mascolini M, et al. XVII International AIDS Conference: From Evidence to Action - Clinical and biomedical prevention science. J Int AIDS Soc. 2009; 12(Suppl 1):S4. [PubMed: 19811670]
9. Paxton LA, et al. Pre-exposure prophylaxis for HIV infection: what if it works? Lancet. 2007; 370:89–93. [PubMed: 17617276]
10. Youle M, Wainberg MA. Could chemoprophylaxis be used as an HIV prevention strategy while we wait for an effective vaccine? AIDS. 2003; 17:937–938. [PubMed: 12660549]
11. Cutler B, Justman J. Vaginal microbicides and the prevention of HIV transmission. Lancet Infect Dis. 2008; 8:685–697. [PubMed: 18992405]
12. McGowan I. Microbicides for HIV prevention: reality or hope? Curr Opin Infect Dis. 2010; 23:26–31. [PubMed: 19935418]
13. Halpern V, et al. Effectiveness of cellulose sulfate vaginal gel for the prevention of HIV infection: results of a Phase III trial in Nigeria. PLoS ONE. 2008; 3:e3784. doi:3710.1371/journal.pone.0003784. [PubMed: 19023429]
14. Kilmarx PH, et al. A randomized, placebo-controlled trial to assess the safety and acceptability of use of carraguard vaginal gel by heterosexual couples in Thailand. Sex Transm Dis. 2008; 35:226–232. [PubMed: 18490865]
15. McCormack S, et al. PRO2000 vaginal gel for prevention of HIV-1 infection(Microbicides Development Programme 301): a phase 3, randomised, double-blind, parallel-group trial. Lancet. 2010; 376:1329–1337. [PubMed: 20851460]
16. Nunn A, et al. Microbicides Development Programme: design of a phase III trial to measure the efficacy of the vaginal microbicide PRO 2000/5 for HIV prevention. Trials. 2009; 10:99. [PubMed: 19860888]
17. Skoler-Karpoff S, et al. Efficacy of Carraguard for prevention of HIV infection in women in South Africa: a randomised, double-blind, placebo-controlled trial. Lancet. 2008; 372:1977–1987. [PubMed: 19059048]

18. Van Damme L, et al. Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial. Lancet. 2002; 360:971–977. [PubMed: 12383665]
19. Abdool Karim Q, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science. 2010; 329:1168–1174. [PubMed: 20643915]
20. Bieniasz PD. Intrinsic immunity: a front-line defense against viral attack. Nat Immunol. 2004; 5:1109–1115. [PubMed: 15496950]
21. Hatziioannou T, et al. A macaque model of HIV-1 infection. Proc Natl Acad Sci U S A. 2009; 106:4425–4429. [PubMed: 19255423]
22. Pham QT, et al. Generation of human TRIM5alpha mutants with high HIV-1 restriction activity. Gene Ther. 2010; 17:859–871. [PubMed: 20357830]
23. Stremlau M, et al. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. Nature. 2004; 427:848–853. [PubMed: 14985764]
24. Bieniasz PD, Cullen BR. Multiple Blocks to Human Immunodeficiency Virus Type 1 Replication in Rodent Cells. J. Virol. 2000; 74:9868–9877. [PubMed: 11024113]
25. McCune JM, et al. The scid-hu mouse: current status and potential applications. Curr Top Microbiol Immunol. 1989; 152:183–193. [PubMed: 2680299]
26. McCune J, et al. The SCID-hu mouse: a small animal model for HIV infection and pathogenesis. Annu Rev Immunol. 1991; 9:399–429. [PubMed: 1910684]
27. Torbett BE, et al. hu-PBL-SCID mice: a model for human immune function, AIDS, and lymphomagenesis. Immunol Rev. 1991; 124:139–164. [PubMed: 1666627]
28. Jamieson BD, et al. The SCID-hu mouse: an in-vivo model for HIV-1 pathogenesis and stem cell gene therapy for AIDS. Semin Immunol. 1996; 8:215–221. [PubMed: 8883144]
29. Mosier DE. Viral pathogenesis in hu-PBL-SCID mice. Semin Immunol. 1996; 8:255–262. [PubMed: 8883149]
30. D'Cruz OJ, Uckun FM. Limitations of the human-PBL-SCID mouse model for vaginal transmission of HIV-1. Am J Reprod Immunol. 2007; 57:353–360. [PubMed: 17430499]
31. Shultz LD, et al. Humanized mice in translational biomedical research. Nat Rev Immunol. 2007; 7:118–130. [PubMed: 17259968]
32. Denton PW, Garcia JV. Novel humanized murine models for HIV research. Curr HIV/AIDS Rep. 2009; 6:13–19. [PubMed: 19149992]
33. Brehm MA, et al. Humanized mouse models to study human diseases. Curr Opin Endocrinol Diabetes Obes. 2010; 17:120–125. [PubMed: 20150806]
34. Denton PW, Garcia JV. Humanized Mouse Models of HIV Infection. AIDS Rev. 2011; 13:135–148. [PubMed: 21799532]
35. Akkina R, et al. Humanized Rag1−/− gammac−/− mice support multilineage hematopoiesis and are susceptible to HIV-1 infection via systemic and vaginal routes. PLoS ONE. 2011; 6:e20169. doi: 20110.21371/journal.pone.0020169. [PubMed: 21695116]
36. Berges BK, et al. Mucosal transmission of R5 and X4 tropic HIV-1 via vaginal and rectal routes in humanized Rag2−/− gammac −/− (RAG-hu) mice. Virology. 2008; 373:342–351. [PubMed: 18207484]
37. Denton PW, et al. Antiretroviral pre-exposure prophylaxis prevents vaginal transmission of HIV-1 in humanized BLT mice. PLoS Med. 2008; 5:e16. doi:10.1371/journal.pmed.0050016. [PubMed: 18198941]
38. Denton PW, et al. Systemic Administration of Antiretrovirals Prior to Exposure Prevents Rectal and Intravenous HIV-1 Transmission in Humanized BLT Mice. PLoS ONE. 2010; 5:e8829. doi: 8810.1371/journal.pone.0008829. [PubMed: 20098623]
39. Denton PW, et al. One Percent Tenofovir Applied Topically to Humanized BLT Mice and Used According to the CAPRISA 004 Experimental Design Demonstrates Partial Protection from Vaginal HIV Infection, Validating the BLT Model for Evaluation of New Microbicide Candidates. J Virol. 2011; 85:7582–7593. [PubMed: 21593172]

40. Hofer U, et al. RAG2−/− gamma(c)−/− mice transplanted with CD34+ cells from human cord blood show low levels of intestinal engraftment and are resistant to rectal transmission of human immunodeficiency virus. J Virol. 2008; 82:12145–12153. [PubMed: 18842716]
41. Neff CP, et al. A topical microbicide gel formulation of CCR5 antagonist maraviroc prevents HIV-1 vaginal transmission in humanized RAG-hu mice. PLoS ONE. 2011; 6:e20209. doi: 20210.21371/journal.pone.0020209. [PubMed: 21673796]
42. Neff CP, et al. Oral pre-exposure prophylaxis by anti-retrovirals raltegravir and maraviroc protects against HIV-1 vaginal transmission in a humanized mouse model. PLoS ONE. 2010; 5:e15257. doi:15210.11371/journal.pone.0015257. [PubMed: 21203568]
43. Stoddart CA, et al. Superior human leukocyte reconstitution and susceptibility to vaginal HIV transmission in humanized NOD-scid IL-2Rgamma(−/−) (NSG) BLT mice. Virology. 2011; 417:154–160. [PubMed: 21684569]
44. Sun Z, et al. Intrarectal transmission, systemic infection, and CD4+ T cell depletion in humanized mice infected with HIV-1. J Exp Med. 2007; 204:705–714. [PubMed: 17389241]
45. Wheeler LA, et al. Inhibition of HIV transmission in human cervicovaginal explants and humanized mice using CD4 aptamer-siRNA chimeras. J Clin Invest. 2011; 121:2401–2412. [PubMed: 21576818]
46. Olesen R, et al. 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. [PubMed: 21256601]
47. Wege AK, et al. Functional and phenotypic characterization of the humanized BLT mouse model. Curr Top Microbiol Immunol. 2008; 324:149–165. [PubMed: 18481459]
48. Brainard DM, et al. Induction of robust cellular and humoral virus-specific adaptive immune responses in human immunodeficiency virus-infected humanized BLT mice. J Virol. 2009; 83:7305–7321. [PubMed: 19420076]
49. Melkus MW, et al. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. Nat Med. 2006; 12:1316–1322. [PubMed: 17057712]
50. Rajesh D, et al. Th1 and Th17 immunocompetence in humanized NOD/SCID/IL2rgammanull mice. Hum Immunol. 2010; 71:551–559. [PubMed: 20298731]
51. Lan P, et al. Reconstitution of a functional human immune system in immunodeficient mice through combined human fetal thymus/liver and CD34+ cell transplantation. Blood. 2006; 108:487–492. [PubMed: 16410443]
52. Shimizu S, et al. A highly efficient short hairpin RNA potently down-regulates CCR5 expression in systemic lymphoid organs in the hu-BLT mouse model. Blood. 2010; 115:1534–1544. [PubMed: 20018916]
53. Habiro K, et al. Induction of human T-cell tolerance to pig xenoantigens via thymus transplantation in mice with an established human immune system. Am J Transplant. 2009; 9:1324–1329. [PubMed: 19459808]
54. Hongo D, et al. Porcine thymic grafts protect human thymocytes from HIV-1-induced destruction. J Infect Dis. 2007; 196:900–910. [PubMed: 17703422]
55. Onoe T, et al. Homeostatic expansion and phenotypic conversion of human T cells depend on peripheral interactions with APCs. J Immunol. 2010; 184:6756–6765. [PubMed: 20483739]
56. Onoe T, et al. Human Natural Regulatory T Cell Development, Suppressive Function, and Postthymic Maturation in a Humanized Mouse Model. J Immunol. 2011; 187:3895–3903. [PubMed: 21876039]
57. Lockridge JL, et al. Analysis of the CD1 antigen presenting system in humanized SCID mice. PLoS ONE. 2011; 6:e21701. doi:21710.21371/journal.pone.0021701. [PubMed: 21738769]
58. Ma SD, et al. A new model of Epstein-Barr virus infection reveals an important role for early lytic viral protein expression in the development of lymphomas. J Virol. 2011; 85:165–177. [PubMed: 20980506]
59. Kim SS, et al. RNAi-mediated CCR5 silencing by LFA-1-targeted nanoparticles prevents HIV infection in BLT mice. Mol Ther. 2010; 18:370–376. [PubMed: 19997090]

60. Tonomura N, et al. Antigen-specific human T-cell responses and T cell-dependent production of human antibodies in a humanized mouse model. Blood. 2008; 111:4293–4296. [PubMed: 18270327]
61. Tonomura N, et al. Pig islet xenograft rejection in a mouse model with an established human immune system. Xenotransplantation. 2008; 15:129–135. [PubMed: 18447886]
62. Denton PW, et al. Generation of HIV Latency in BLT Humanized Mice. J Virol. 2012; 86:630–634. [PubMed: 22013053]
63. Marsden MD, et al. HIV latency in the humanized BLT mouse. J Virol. 2012; 86:339–347. [PubMed: 22072769]
64. Veazey RS. Microbicide safety/efficacy studies in animals: macaques and small animal models. Curr Opin HIV AIDS. 2008; 3:567–573. [PubMed: 19373023]
65. Biswas S, et al. 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; 134:419–433. [PubMed: 22044090]
66. Grant RM, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. N Engl J Med. 2010; 363:2587–2599. [PubMed: 21091279]
67. Parikh UM, et al. Complete protection from repeated vaginal simian-human immunodeficiency virus exposures in macaques by a topical gel containing tenofovir alone or with emtricitabine. J Virol. 2009; 83:10358–10365. [PubMed: 19656878]
68. Wallace GS, et al. Human immunodeficiency virus type 1 nucleocapsid inhibitors impede trans infection in cellular and explant models and protect nonhuman primates from infection. J Virol. 2009; 83:9175–9182. [PubMed: 19587055]
69. Veazey RS, et al. Protection of macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus-cell fusion. Nature. 2005; 438:99–102. [PubMed: 16258536]
70. Hurt CB, et al. Pre-Exposure Prophylaxis and Antiretroviral Resistance: HIV Prevention at a Cost? Clin Infect Dis. 2011; 53:1265–1270. [PubMed: 21976467]
71. Garcia-Lerma JG, et al. Prevention of rectal SHIV transmission in macaques by daily or intermittent prophylaxis with emtricitabine and tenofovir. PLoS Med. 2008; 5:e28. doi:10.1371/journal.pmed.0050028. [PubMed: 18254653]
72. Kashuba AD, et al. Pre-exposure prophylaxis for HIV prevention: how to predict success. Lancet. 2011 doi:10.1016/S0140-6736(11)61852-7.
73. Patterson KB, et al. Penetration of Tenofovir and Emtricitabine in Mucosal Tissues: Implications for Prevention of HIV-1 Transmission. Sci Transl Med. 2011; 3:112re114.

Box 1. Pre-exposure prophylaxis (PrEP)

Currently prescribed therapeutic anti-HIV drugs, along with novel compounds, may have the potential to prevent new transmissions if administered prior to viral exposure. PrEP involves action by individuals at risk of HIV exposure and may offer mechanisms of protection from HIV to those unable to control circumstances that place them at risk of HIV transmission. Topical PrEP or microbicides typically involve a person directly applying an antiviral product to the vagina or rectum prior to and/or following sexual intercourse. Systemic PrEP typically involves a person engaged in risk-behavior taking antiretrovirals daily or intermittently for as long as they are at-risk. Information regarding PrEP clinical trials is available from AVAC: Global Advocacy for HIV Prevention at http://www.avac.org/ht/d/sp/i/354/pid/354.

Box 2. Generation of humanized BLT mice

All BLT mice are created in the same way by transplanting autologous $CD34^+$ cells into mice previously implanted with a piece of thymus and liver. This process is highly reproducible resulting robust and consistent levels of human cells in the periphery and organs. The BLT humanization procedure is very forgiving and it can be executed in a number of ways and with virtually any kind of immunodeficient mouse. For example, mice can be irradiated, implanted and transplanted in a single day or implanted one day and transplanted days or weeks later. NOD/SCID and NOD/SCID common γ chain null (NSG) mice have been most commonly used for BLT mouse preparation but other strains (e.g. Rag null/γ chain null (DKO) mice and NSG A2) can also be used to generate BLT humanized mice.

Box 3. Alternatives to humanized BLT mice

The focus of this review is on humanized BLT mice; however, other humanized mouse models are available and we have extensively reviewed their contributions to HIV research elsewhere (Denton and Garcia, 2011). These models include NSG-hu and $Rag2^{-/-}\gamma c^{-/-}$ (RAG-hu) mice that receive a transplant of $CD34^{+}$ cells without the thymus and liver implant inherent to BLT mice. Of these models, HIV-1 mucosal transmission has been reported in RAG-hu mice (Berges, et al. 2008). Furthermore, in RAG-hu mice (i) systemic administration of the integrase inhibitor raltegravir, (ii) systemic administration of the CCR5 inhibitor maraviroc, and (iii) topical administration of maraviroc were shown to prevent vaginal HIV-1 transmission (Neff, et al 2010; Neff, et al 2011).

Table 1

HIV-1 prevention studies in BLT mice and related studies in NHP

PrEP	Route of viral exposure	Inhibitor	Transmission in the treated group	Transmission in the control group(s)	Refs
Topical[a] in BLT mice	Vaginal	Tenofovir	1 of 8	3 of 4	[39]
		C52L	0 of 7	10 of 12	
		C5A	0 of 8		
		PIE12-Trimer	0 of 5		
		FTC-TDF	1 of 9		
		TC247	3 of 7		
		NSC23766	4 of 4		
		3 aptamer-siRNA chimeras	2 of 4	4 of 4 + 4 of 4	[45]
Systemic in BLT mice	Vaginal	FTC + TDF	0 of 5	7 of 8	[37]
	Rectal		0 of 9	12 of 19	[38]
	Intravenous		1 of 8	6 of 6	
Topical[b] in NHP	Vaginal (repeated exposures)	Tenofovir	0 of 6	8 of 9	[67]
		FTC + tenofovir	0 of 6	2 of 2 + 5 of 6	
	Vaginal	TC247	1 of 6	21 of 23	[68]
	Vaginal	C52L[c]	7 of 15	9 of 9	[69]
Systemic in NHP	Rectal (repeated exposures)	FTC + TDF	2 of 6 (oral) 0 of 6 (subcutaneous)	17 of 18	[71]

[a] Inhibitors applied as solutions.

[b] Inhibitors applied as gels.

[c] The concentration was not fixed, but covered a range.

Table 2

PrEP efficacy among BLT mice, NHP and humans

Subjects	Daily systemic PrEP with FTC and TDF[a]	Topical PrEP with 1% tenofovir	Refs
BLT mice (% protection)	• Rectal 100% (IP) • Vaginal 100% (IP)	• Vaginal 88% (BAT24)	[37−39]
NHP (% protection)	• Rectal 66% (oral), 100% (subcutaneous)	• Vaginal 100% (30 min prior)	[67,71]
Humans (% reduction in incidence)	• iPrEx 44% (oral) • Partners PrEP 73% (oral) • TDF2 63% (oral) • FEM-PrEP study discontinued due to futility (oral)	• CAPRISA 004 39% (vaginal, BAT24) • VOICE study arm discontinued due to futility[b] (vaginal once daily)	[19,66, 70]

[a] Abbreviations: IP, intraperitoneal; BAT24, a dose <12 hr before intercourse plus a dose <12 hr after intercourse.

[b] http://clinicaltrials.gov/ct2/show/NCT00705679; last accessed on March 3, 2012