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Immune Reconstitution of the Female Reproductive Tract of Humanized BLT Mice and their Susceptibility to Human Immunodeficiency Virus Infection

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Abstract

An HIV-1 vaccine capable of providing sterilizing immunity from vaginal infection would reduce the spread of HIV-1 to women. Unfortunately, only one of the four HIV-1 vaccine clinical trials has demonstrated any level of protection (31%) against HIV-1 transmission. Additionally, only one topical microbicide clinical trial has reported an overall reduction in HIV-1 transmission (39%). Developing even more effective vaccines and microbicides will require a better understanding of the key events involved in HIV-1 infection and dissemination at the site of exposure. Novel immunodeficient mice capable of being systemically reconstituted with human hematopoietic stem cells have provided new systems where HIV transmission studies can be performed. Specifically, a humanized mouse model of vaginal HIV transmission has been developed that utilizes the humanized bone marrow-liver-thymus (BLT) mouse. The female reproductive tract (FRT) of humanized BLT mice is reconstituted with functional human immune cells rendering them susceptible to vaginal HIV-1 infection. In this review we focus on four aspects of BLT mice for the study of vaginal HIV-1 transmission: 1) we discuss methods for creating humanized BLT mice and their reconstitution with human hematopoietic cells, 2) we describe reconstitution of the BLT mouse FRT with human immune cells, 3) we highlight the work done regarding vaginal HIV-1 transmission and 4) we summarize the efficacy of systemic pre-exposure prophylaxis (PrEP) to prevent vaginal HIV-1 transmission in BLT mice. BLT mice are a highly relevant small animal model for studying vaginal HIV-1 transmission, prevention and therapy.

1. Introduction

In 2008, UNAIDS and the World Health Organization estimated that worldwide there are 33.4 million people infected with human immunodeficiency virus 1 (HIV-1)[1].

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[1]Abbreviations: human immunodeficiency virus 1 (HIV-1); female reproductive tract (FRT); simian immunodeficiency virus (SIV); SIV/HIV chimeric virus (SHIV); bone marrow-liver-thymus (BLT); preexposure prophylaxis (PrEP); non-obese diabetic (NOD); severe combined immunodeficient (SCID); natural killer (NK); centi-Gray (cGy); dendritic cell (DC); gastrointestinal (GI); Langerhans cells (LC); acquired immune deficiency syndrome (AIDS); emtricitabine (FTC); tenofovir disoproxil fumarate (TDF).

Approximately half of those individuals are women. The prevalence of HIV-1 in women is even higher in areas like sub-Saharan Africa, the region with the highest incidence and prevalence of HIV-1, where social and economic conditions increase the probability that women will be exposed to HIV-1 (UNAIDS, 2009).

An HIV-1 vaccine capable of providing sterilizing immunity from vaginal infection would reduce the spread of HIV-1 to women. Unfortunately, only one of the four HIV-1 vaccine clinical trials, the ALVAC/AIDSVAX vaccine trial, has demonstrated any level of protection (31%) against HIV-1 transmission (Padian et al., 2010). In addition to the four HIV-1 vaccine clinical trials, several topical microbicides have been clinically tested for their ability to prevent vaginal HIV-1 transmission (Padian et al., 2010). The only successful microbicide clinical trial to date, CAPRISA 004, reported an overall reduction of 39% in HIV-1 transmission when a 1% gel formulation of the antiretroviral tenofovir was applied vaginally before and after vaginal intercourse. More impressively, a 54% decrease in HIV-1 infection was demonstrated among patients with >80% compliance (Abdool Karim et al., 2010). These successes demonstrate that HIV-1 vaccines and antiretroviral microbicides have the potential to reduce the global spread of HIV-1.

Developing even more effective vaccines and microbicides capable of fully protecting women from HIV-1 infection will require a better understanding of the key events involved in HIV-1 infection and dissemination at the site of exposure. *Ex vivo* tissue explants and epithelial sheets of the female reproductive tract (FRT) have provided valuable insight into three critical aspects of HIV-1 infection: 1) the anatomical sites of the FRT that support high levels of HIV-1 replication (Asin et al., 2009), 2) the early events of HIV-1 infection including the specific immune cell populations preferentially targeted by HIV-1 (Hladik et al., 2007, Gupta et al., 2002, Saba et al., 2010) and 3) the factors that influence HIV-1 transmission such as mucosal epithelium and inflammatory mediators (Asin et al., 2009, Greenhead et al., 2000). These *ex vivo* models of the FRT have also been used to screen potential antiretroviral microbicide candidates (Greenhead et al., 2000, Rohan et al., 2010). Although a wealth of knowledge has been gathered from these studies, *ex vivo* models cannot be used to test the efficacy of HIV-1 vaccines nor can they model key aspects of human biology that affect the *in vivo* infection process like hormonal regulation of the FRT, the mucosal immune response and viral dissemination into other tissues.

1.1. Animal models of HIV-1 infection

Non-human primate models using simian immunodeficiency virus (SIV) have served as the primary *in vivo* animal model for evaluating the efficacy of potential vaccines and microbicides (Van Rompay, 2010, Uberla, 2005, Veazey, 2008). In addition, macaques have been used to study rectal and vaginal SIV transmission (Keele et al., 2009, Miller et al., 2005). Macaque models provide access to relevant anatomical compartments where the early events taking place in the FRT can be studied. Despite the utility of macaque models, these studies are limited by the inability of HIV-1 to productively infect macaque cells, an issue that has been partially overcome by the use of SIV/HIV chimeric viruses (SHIVs) (Harouse et al., 1999). Despite their current utility, SHIVs require further refinement before they can serve as faithful surrogates of HIV-1, particularly in the context of HIV-specific pharmacological interventions to prevent vaginal HIV-1 transmission (Van Rompay, 2010).

Novel strains of immunodeficient mice capable of being systemically reconstituted with human hematopoietic stem cells have provided new systems where *in vivo* HIV studies can be performed (Denton and Garcia, 2009). Specifically, a humanized mouse model of vaginal HIV transmission has been developed that utilizes the humanized bone marrow-liver-thymus (BLT) mouse. The FRT of humanized BLT mice is reconstituted with functional innate and adaptive human immune cells rendering them susceptible to vaginal HIV-1 infection

(Denton et al., 2008). In this review we focus on four aspects of BLT mice for the study of vaginal HIV-1 transmission: 1) we discuss methods for creating humanized BLT mice and their systemic reconstitution with human hematopoietic cells, 2) we describe the reconstitution of the BLT mouse FRT with human immune cells, 3) we highlight work done regarding vaginal HIV-1 transmission in this system and 4) we summarize the efficacy of systemic pre-exposure prophylaxis (PrEP) to prevent vaginal HIV-1 transmission in BLT mice.

2. The humanized BLT mouse model

2.1. Generation of BLT mice

Humanized BLT mice are individually created by transplanting human fetal liver-derived $CD34^+$ hematopoietic stem cells into mice implanted with autologous human fetal liver and thymus. A sandwich of 1–2 mm pieces of human fetal thymus-liver-thymus tissue is implanted under the kidney capsule of 6 to 8 week old NOD/SCID mice or NOD/SCID/$\gamma_c^{-/-}$ mice (stock number 00557, NSG mice; The Jackson Laboratory). In comparison to other immunodeficient strains of mice like SCID, both NOD/SCID and NOD/SCID/$\gamma_c^{-/-}$ mice have a lower level of endogenous mouse natural killer (NK) cell activity which facilitates more prolonged and robust human immune cell reconstitution (Greiner et al., 1998, Shultz et al., 2005). Mice are exposed to sublethal gamma irradiation (325 centi-Gray (cGy) for NOD/SCID mice and 300 cGy for NOD/SCID/$\gamma_c^{-/-}$ mice) 4–24 hours prior to transplantation of $CD34^+$ stem cells (1–2.5 × 10^6 and 2.5–8 × 10^5 $CD34^+$ cells for NOD/SCID and NOD/SCID/$\gamma_c^{-/-}$ mice, respectively) to facilitate engraftment. The irradiation and transplant can be performed the same day of implantation or three weeks after implantation with equivalent results. The transplanted $CD34^+$ cells engraft and some maintain their progenitor potential while others differentiate into different hematopoietic lineages (i.e. $CD3^+$, $CD19^+$, $CD14^+$ etc.) (Sun et al., 2007, Denton et al., 2008, Melkus et al., 2006, Brainard et al., 2009, Rajesh et al., 2010, Denton et al., 2010, Lan et al., 2006).

In BLT mice the implanted fetal thymus tissue develops into a human thymus (thymic organoid) where bone marrow derived human T lymphocyte progenitor cells can migrate and develop within the context of autologous human thymic epithelium into bona fide, fully functional, HLA-restricted peripheral T cells (Brainard et al., 2009, Melkus et al., 2006, Rajesh et al., 2010, Lan et al., 2006). Thus, BLT mice are characterized by their robust and sustained systemic repopulation with functional HLA-restricted human T cells.

2.2. Human immune cell reconstitution in peripheral blood of BLT mice

The utility of BLT mice as an animal model for HIV hinges on their sustained reconstitution with human hematopoietic $CD45^+$ cells. Human immune cell reconstitution in peripheral blood of BLT mice and the phenotype of the human immune cells present is routinely analyzed by flow cytometry (Melkus et al., 2006, Rajesh et al., 2010, Brainard et al., 2009, Lan et al., 2006) (Fig. 1). Human innate and adaptive immune cells can be detected in peripheral blood of BLT mice as early as four to eight weeks post-transplant in NOD/SCID/$\gamma_c^{-/-}$ and NOD/SCID BLT mice respectively, and is maintained for over nine months post-transplant (unpublished observations).

Flow cytometric analyses of peripheral blood from BLT mice demonstrated the presence of human $CD4^+$ and $CD8^+$ T cells, B cells, plasmacytoid and myeloid dendritic cells (DC) and monocytes. In addition, small populations of NK, NKT and gamma-delta T cells have also been reported (Melkus et al., 2006, Rajesh et al., 2010, Brainard et al., 2009, Lan et al., 2006). Comparison of the relative levels of the different lymphoid subsets present in the peripheral blood of humans and BLT mice demonstrates a striking similarity (Melkus et al., 2006). Furthermore, like in humans, the T cells of BLT mice display a highly diverse Vβ-T

cell receptor repertoire (Melkus et al., 2006). Thus, the peripheral blood of BLT mice mimics that of human peripheral blood both in its cellular composition and in the relative levels of human immune cell populations present.

2.3. Systemic human immune cell reconstitution of humanized BLT mice

The distribution of human cells in BLT mice is not limited to peripheral blood, rather it is systemic (Fig. 1). Analysis of the tissue distribution of human immune cell populations throughout BLT mice revealed an appropriate distribution of human lymphoid (B cells and T cells) and myeloid (monocytes/macrophages and DCs) cells in all tissues analyzed including the bone marrow, spleen, lymph nodes, liver, lung and the gastrointestinal (GI) tract (Brainard et al., 2009,Sun et al., 2007,Rajesh et al., 2010,Melkus et al., 2006).

In humans, the GI tract is a major site of HIV-1 replication and $CD4^+$ T cell depletion regardless of the route of transmission (Brenchley et al., 2004, Mehandru et al., 2004). In humanized BLT mice, human T, B and myeloid cells are found throughout the epithelium and lamina propria of the small and large intestines (Sun et al., 2007, Denton et al., 2008). Memory $CD4^+$ and $CD8^+$ T cells represent the majority of the T cells found in the intestines, only a few naïve T cells are present. The fact that human lymphoid reconstitution extends to the distal end of the lower GI tract renders BLT mice susceptible to rectal HIV-1 transmission that, like described above for humans, results in $CD4^+$ T cell depletion throughout the entire GI tract (Sun et al., 2007, Denton et al., 2008).

These analyses highlight the multi-lineage human immune cell reconstitution observed in lymphoid, non-lymphoid and mucosal tissues of BLT mice including relevant sites of HIV-1 replication and pathogenesis, such as the GI tract (Denton et al., 2008, Sun et al., 2007, Brenchley et al., 2004, Mehandru et al., 2004). Since the extensive and faithful human lymphoid reconstitution observed in the GI tract of BLT mice renders them susceptible to rectal HIV-1 transmission, a second mucosal site also important for HIV-1 transmission, the FRT, was examined for the presence of human lymphoid cells in BLT mice (Denton et al., 2008). These observations represent the focus of this review article.

3. Characterization of human immune cell reconstitution in the FRT of humanized BLT mice

3.1. Comparison of the human and mouse FRT

Worldwide the majority of new HIV-1 infections occur in women (UNAIDS, 2009). Even though rectal transmission does occur in women, vaginal exposure is the main route of HIV-1 transmission to women (UNAIDS, 2006). In this particular context, either cell-free or cell-associated virus present in the male ejaculate represents the infectious inoculum (Mermin et al., 1991, Pudney and Anderson, 1991, Vernazza et al., 1994). After vaginal exposure, the main possible portals of entry for HIV are the mucosal surfaces of the vagina, ectocervix, endocervix and uterus (Miller et al., 1992, Kell et al., 1992, Li et al., 2009, Howell et al., 1997). The gross anatomy of these key sites for HIV entry in the FRT is similar in mice and humans (Fig. 2A). In the mouse FRT, separate uterine horns merge caudally to form the corpus uteri which is internally divided by a midline septum (Leppi, 1964). The most caudal part of the uterus is the cervix consisting of a single endocervical canal and the ectocervix which protrudes into the cranial portion of the vagina (Leppi, 1964), similar to the anatomy of the human cervix and vagina. Therefore, despite some obvious anatomical differences (such as relative size and a duplex uterus), the mouse and human FRT show similarities at postulated key sites of HIV entry making humanized BLT mice a potentially relevant model for vaginal HIV transmission.

3.2. Identity and distribution of immune cells in the human FRT

The epithelial structure of the genital mucosa and the distribution of immune cells in the mucosa differ throughout the vagina, ectocervix, endocervix and uterus of the human FRT. The vagina and ectocervix are lined by stratified squamous epithelium, whereas the endocervix and uterus are lined by simple columnar epithelium. It is proposed that the stratified squamous epithelium of the ectocervix and vagina is a more resistant mechanical barrier to HIV entry than the simple columnar epithelium of the endocervix and uterus (Haase, 2010). Macrophages, plasma cells and B cells are dispersed throughout the lamina propria of the vagina, ectocervix, endocervix and uterus (Kamat and Isaacson, 1987, Hussain et al., 1992, Pudney et al., 2005, Yeaman et al., 1997). DCs are mainly present in the epithelium but are also found scattered throughout the lamina propria (Pudney et al., 2005).

T cells are the most abundant leukocytes in the vaginal, ectocervical, endocervical and uterine mucosa (Kamat and Isaacson, 1987, Givan et al., 1997, Pudney et al., 2005). The majority of T cells are concentrated in a band along the basement membrane just beneath the epithelium (Johansson et al., 1999, Pudney et al., 2005). T cells are also found dispersed throughout the epithelium and lamina propria (Johansson et al., 1999, Pudney et al., 2005). The abrupt transition from squamous to columnar epithelium at the ecto-endocervical border (the squamocolumnar junction) is termed the transformation zone. The density of T cells in the transformation zone is higher than in the vagina, ectocervix and endocervix (Pudney et al., 2005). The combination of abundant T cells, the appearance of columnar epithelium and the high cell turnover rate in the transformation zone has led to the hypothesis that it might be especially susceptible to HIV-1 infection as has been shown in the SIV/macaque models (Li et al., 2009, Pudney et al., 2005, Haase, 2010).

3.3. Cellular targets for HIV-1 infection in the human FRT

The mucosal epithelium of the FRT by itself is an effective barrier against infection (Bobardt et al., 2007) such that not all exposures to HIV-1 result in productive infection. The overall transmission rate for HIV-1 is 0.1 – 10% per penile-vaginal contact (Powers et al., 2008). There are three key scenarios that increase the likelihood of vaginal HIV-1 transmission: 1) when the index partner has early (primary infection) or late state disease (AIDS), times when viral shedding in genital secretions is high, 2) when the index partner has genital ulcers, which increases viral shedding in genital secretions and 3) when the recipient partner has genital ulcers, which create breaches in the epithelial layer and have an increased density of potential target cells due to local inflammation (Wawer et al., 2005, Gray et al., 2001, Quinn et al., 2000, Powers et al., 2008). After vaginal exposure HIV-1 may gain access to target cells by penetrating breaches in the epithelium caused by micro-trauma during sexual intercourse (Norvell et al., 1984). However, even in situations where there is no trauma or ulcers, HIV-1 is occasionally capable of overcoming these natural barriers and establishing a productive infection (Gupta et al., 2002, Maher et al., 2005, Nazli et al., 2010).

The identity and the location of the initial cells involved in HIV-1 transmission are a subject of great debate. Potential ways of establishing infection in the absence of mucosal disruption include epithelial transcytosis and viral translocation across the epithelium (Bobardt et al., 2007, Nazli et al., 2010). In addition, intra-epithelial Langerhans cells (LC) have long been considered as potentially playing an important role in HIV-1 transmission (Arrighi et al., 2004, McDonald et al., 2003, Geijtenbeek et al., 2000). More recent experiments have demonstrated that LCs can internalize HIV-1 by endocytosis, after which HIV-1 can survive in the cytoplasm and possibly trans-infect neighboring CD4+ T cells (Hladik et al., 2007).

Similarly, DCs have also been postulated as playing an important role in vaginal HIV-1 transmission and virus spread (Miller and Hu, 1999, Geijtenbeek et al., 2000).

However, the mucosa of the human FRT contains a large number of $CD4^+$ T cells (Hladik et al., 2007, Saba et al., 2010, Pudney et al., 2005, Johansson et al., 1999) and experiments in both non-human primates and human explant models suggest that the first productively infected cells are $CD4^+$ T cells (Gupta et al., 2002, Hladik et al., 2007, Zhang et al., 1999). Additional experimental evidence suggests that only a single or a few viruses "seed" the infection in one or a small founder population of regional $CD4^+$ T cells (Keele et al., 2008, Miller et al., 2005, Li et al., 2009, Maher et al., 2005) and that resting T cells account for almost 90% of the productively infected cells shortly after vaginal exposure in macaques (Zhang et al., 1999, Zhang et al., 2004).

3.4. Human immune cells in the FRT of BLT mice

In order for humanized BLT mice to serve as an adequate model to evaluate vaginal HIV-1 transmission, it is essential that the FRT of these mice are faithfully repopulated with the appropriate human immune cells. Immunohistochemical analyses of the FRT from BLT mice demonstrate an abundance of human hematopoietic cells located throughout the entire vagina, ectocervix, endocervix and uterus (Fig. 2B) (Denton et al., 2008). Specifically, analyses of the vagina, ectocervix, endocervix and uterus demonstrated the presence of human T cells, macrophages and DCs (Denton et al., 2008).

A more detailed microscopic analysis of the human T cells present in the FRT of BLT mice demonstrated that they are located within the epithelial layer, as a band at the epithelial-lamina propria junction along the basement membrane and throughout the lamina propria (Denton et al., 2008). This distribution of human T cells is very similar to the distribution of the same cells in the human vagina, ectocervix and endocervix (Johansson et al., 1999, Pudney et al., 2005). Furthermore, there are macrophages and DCs present in the lamina propria throughout the FRT of BLT mice also like in humans (Denton et al., 2008). In summary, the entire FRT of humanized BLT mice is well reconstituted with human hematopoietic cells. All of the cells postulated as playing an important role in HIV-1 transmission are present in the FRT of BLT mice and their distribution resembles the location of each specific cell type as normally found in humans.

Now that the human hematopoietic subsets considered to have an important role in HIV-1 transmission have been identified throughout the entire FRT of humanized BLT mice, the utility of BLT mice as a model for HIV-1 vaginal transmission can be expanded by performing a more detailed analysis of the human component of the FRT. Additional information regarding the following aspects of the FRT of BLT mice will be essential for future studies: 1) a comprehensive phenotypic characterization of the human immune cell subsets present in the FRT, 2) an analysis of the human immune cell populations present in vaginal fluids from BLT mice, 3) a detailed description of the cytokines present in the FRT and vaginal fluids and 4) a study of the changes occurring in the human immune cell populations and cytokine levels in the FRT during the different stages of the mouse estrus cycle.

4. Mucosal HIV-1 transmission in humanized BLT mice

4.1. Vaginal exposure of humanized BLT mice to HIV-1 results in efficient virus transmission

Based on the very encouraging results describing the substantial repopulation of the FRT of BLT mice with human cells, vaginal HIV-1 transmission experiments were performed to evaluate their susceptibility to infection via this highly relevant route of exposure in humans.

Vaginal transmission of HIV-1 in BLT mice was tested by placing a single cell-free dose of the CCR5-tropic primary isolate HIV-1_{JR-CSF} directly into the vagina using an atraumatic delivery technique (Denton et al., 2008). To ensure a highly reproducible exposure to the inoculum and to facilitate its retention in the vagina, mice are maintained under anesthesia during the inoculation procedure. Evidence of infection as determined by the presence of plasma p24 and/or plasma HIV-RNA can be detected as early as two weeks after vaginal exposure. Under these conditions, transmission has been documented in 88% of exposed mice (Denton et al., 2008). Hence, humanized BLT mice are highly susceptible to vaginal HIV-1 transmission, suggesting the potential utility of this system for a detailed *in vivo* evaluation of the sequence of events involved in vaginal HIV-1 transmission and subsequent dissemination.

4.2. Vaginal exposure to HIV-1 results in systemic infection and depletion of $CD4^+$ cells

Once systemic infection is established following vaginal HIV-1 exposure (as early as 2 weeks post-exposure) of BLT mice, there is a progressive drop in the proportion of human $CD4^+$ T cells in peripheral blood. This loss of $CD4^+$ T cells closely recapitulates the loss of these same cells observed during acute HIV-1 infection of humans where the acute infection phase is followed by a stabilization of $CD4^+$ T cell levels in peripheral blood. Subsequent to local transmission occurring at the FRT, HIV-1 is rapidly disseminated throughout all tissues in BLT mice. Evidence of productively infected cells as determined by *in situ* hybridization is clearly seen at primary/secondary and immunoregulatory sites (Fig. 3A). In addition, productively infected cells are clearly present in virtually all other mouse tissues like the heart and pancreas (unpublished data). All the tissues evaluated reflect the reduced levels of $CD4^+$ T cells observed in peripheral blood demonstrating the systemic depletion of these cells throughout the animal (Denton et al., 2008).

4.3. Vaginal HIV-1 infection results in human $CD4^+$ T cell depletion in the GI Tract

In humans, HIV-1 infection of the GI tract results in depletion of $CD4^+$ T cells (Brenchley et al., 2004, Mehandru et al., 2004). Therefore, the dissemination of HIV-1 to the GI tract of BLT mice after vaginal exposure is of particular importance and high relevance. As indicated above, the GI tract of humanized BLT mice is faithfully reconstituted with human lymphoid cells permitting the analysis of the sequela of vaginal HIV-1 transmission in both the small and large intestine. Vaginal HIV-1 transmission in BLT mice leads to disseminated infection of the entire GI tract. Infected cells can be clearly identified in the lamina propria and epithelium of the small intestine (Denton et al., 2008). Infection of the GI tract results in a dramatic loss of human $CD4^+$ T cells in this tissue (Fig. 3B). Furthermore, there is a specific and substantial loss of $CD45RA^{neg}CD27^{neg}CD4^+$effector memory cells in these tissues (Denton et al., 2008). This is a well-described characteristic of human HIV-1 infection and of macaque SIV/SHIV infection that is faithfully recapitulated in humanized BLT mice (Veazey et al., 2000, Mehandru et al., 2004).

5. Evaluation of approaches to prevent vaginal HIV-1 transmission in humanized BLT mice

As indicated above, in the absence of an effective vaccine or other successful preventive strategies, the AIDS epidemic continues to spread at an alarming rate. Vaginal transmission is responsible for the majority of new infections worldwide (UNAIDS, 2006). Therefore, there is a critical need for the development and implementation of strategies to prevent vaginal HIV-1 transmission. Beyond vaccines, there are two different preventative strategies currently being considered: topically applied microbicides and systemically administered antiretrovirals to individuals at high risk (targeted systemic PrEP) (Padian et al., 2010, Garcia-Lerma et al., 2010). The development and preclinical efficacy testing of topical

microbicides and other preventive strategies, such as targeted systemic antiretroviral PrEP, is dependent on the availability of adequate animal models. Until the development of humanized mice the most used surrogate animal model used to study vaginal HIV-1 transmission and prevention were non-human primates infected with SIV or SHIVs (Van Rompay, 2010, Uberla, 2005, Denton and Garcia, 2009, Garcia-Lerma et al., 2010). Using humanized mice to evaluate HIV-1 prevention approaches is of high significance because it allows the study of HIV-1 infection of human cells in the highly relevant context of the entire FRT *in vivo*.

5.1. Important considerations regarding the evaluation of PrEP approaches for the prevention of vaginal HIV-1 transmission in humanized BLT mice

The concept of targeted systemic PrEP involves the continuous treatment of HIV-1-negative individuals at high risk for acquiring HIV-1 with antiretroviral drugs in an effort to prevent HIV-1 transmission (Garcia-Lerma et al., 2010). However, there are major concerns regarding the potential side effects of the drugs used because systemic PrEP entails the administration of antiretroviral drugs to large numbers of otherwise healthy individuals (Derdelinckx et al., 2006). In addition, there is also the potential danger that drug resistant viruses may develop in individuals who unknowingly become infected with HIV-1 and continue to receive systemic PrEP, ultimately resulting in the spread of drug resistant viruses (Supervie et al., 2010). Therefore, there is significant interest in the development and implementation of animal models in which these concerns can be addressed experimentally.

For its clinical implementation, it is essential that any candidate drug be safe, highly efficacious and easy to use in order to maximize patient compliance (Derdelinckx et al., 2006). Experiments in BLT mice have successfully demonstrated the efficacy of systemic PrEP against vaginal HIV-1 infection using emtricitabine and tenofovir disoproxil fumarate (FTC/TDF, the combination of drugs present in Truvada®) (Denton et al., 2008). The combination of FTC/TDF was chosen because it meets important criteria for drugs included as potential candidates for clinical trials of systemic PrEP; high potency, favorable toxicity profiles, relatively high threshold for the development of viral resistance and its once daily administration without food restrictions (De Clercq, 2007, Derdelinckx et al., 2006).

5.2. Systemic administration of FTC/TDF efficiently prevents vaginal HIV-1 transmission in humanized BLT mice

The evaluation of PrEP in humanized mice was designed to reflect as closely as possible its implementation in humans. Specifically, humanized BLT mice were generated and carefully validated for their reconstitution with human cells prior to treatment. Treatment consisted of FTC/TDF administered systemically once per day for seven days. On the third day of treatment, mice were challenged intravaginally with a high dose of HIV-1. Mice were then monitored over time for the presence of any evidence of infection. Control (untreated) BLT mice were readily infected under these experimental conditions (Denton et al., 2008) as determined by the presence of readily detectable HIV in plasma. In contrast, daily treatment with FTC/TDF resulted in complete absence of any evidence of HIV infection in the peripheral blood of the treated mice (Denton et al., 2008). Complete protection from HIV infection was confirmed by systemic molecular and cellular analyses for the presence of any signs of infection. Specifically, in protected animals there was no evidence of viral antigenemia, viral RNA, viral DNA or productively infected cells in any tissue at any time point analyzed (Denton et al., 2008). These results demonstrate the strong protective effect of FTC/TDF against vaginal HIV-1 infection in BLT mice and also emphasize the applicability of BLT mice for preclinical evaluation of PrEP approaches to prevent vaginal HIV-1 transmission.

6. Conclusion

Vaginal infection is the predominant mode of HIV-1 transmission globally. The FRT is therefore one of the major locations for natural HIV-1 infection and any efforts aimed at preventing HIV-1 transmission to women (including vaccines) must successfully establish protection at this specific portal of virus entry. Despite advances obtained through studies using *ex vivo* tissue explants of the human FRT and non-human primates, vaginal HIV-1 transmission in humans is still not fully understood (Haase, 2010).

The availability of robust animal models such as BLT mice that recapitulate key aspects of HIV-1 transmission as they might occur in humans provides opportunities to further study details of vaginal transmission as well as the effects of acute and chronic HIV-1 infection. In addition, BLT mice allow for HIV-1 infection studies of human cell targets *in vivo* at the most relevant mucosal sites. The usefulness of BLT mice in testing HIV-1 prevention strategies has already been demonstrated by the efficient protection against vaginal HIV-1 transmission afforded by PrEP as described in this review. The susceptibility of BLT mice to mucosal HIV-1 transmission also allows the testing of different microbicide candidates. Importantly, the fact that thymocytes in BLT mice are educated in the context of HLA (in the developed human thymic organoid) makes this model highly relevant for evaluating human-specific immune responses to potential HIV-1 vaccines.

In conclusion, the recent development of humanized BLT mice and their validation as a highly relevant small animal model for the study of HIV-1 provides the field of HIV research with a powerful new tool. This model permits study of the earliest events of HIV-1 transmission and the pathogenic mechanisms of the virus during infection. Finally, BLT mice can be used to systematically analyze the efficacy of HIV-1 prevention, therapeutic and eradication interventions; key experiments aimed at stopping the spread of HIV-1.

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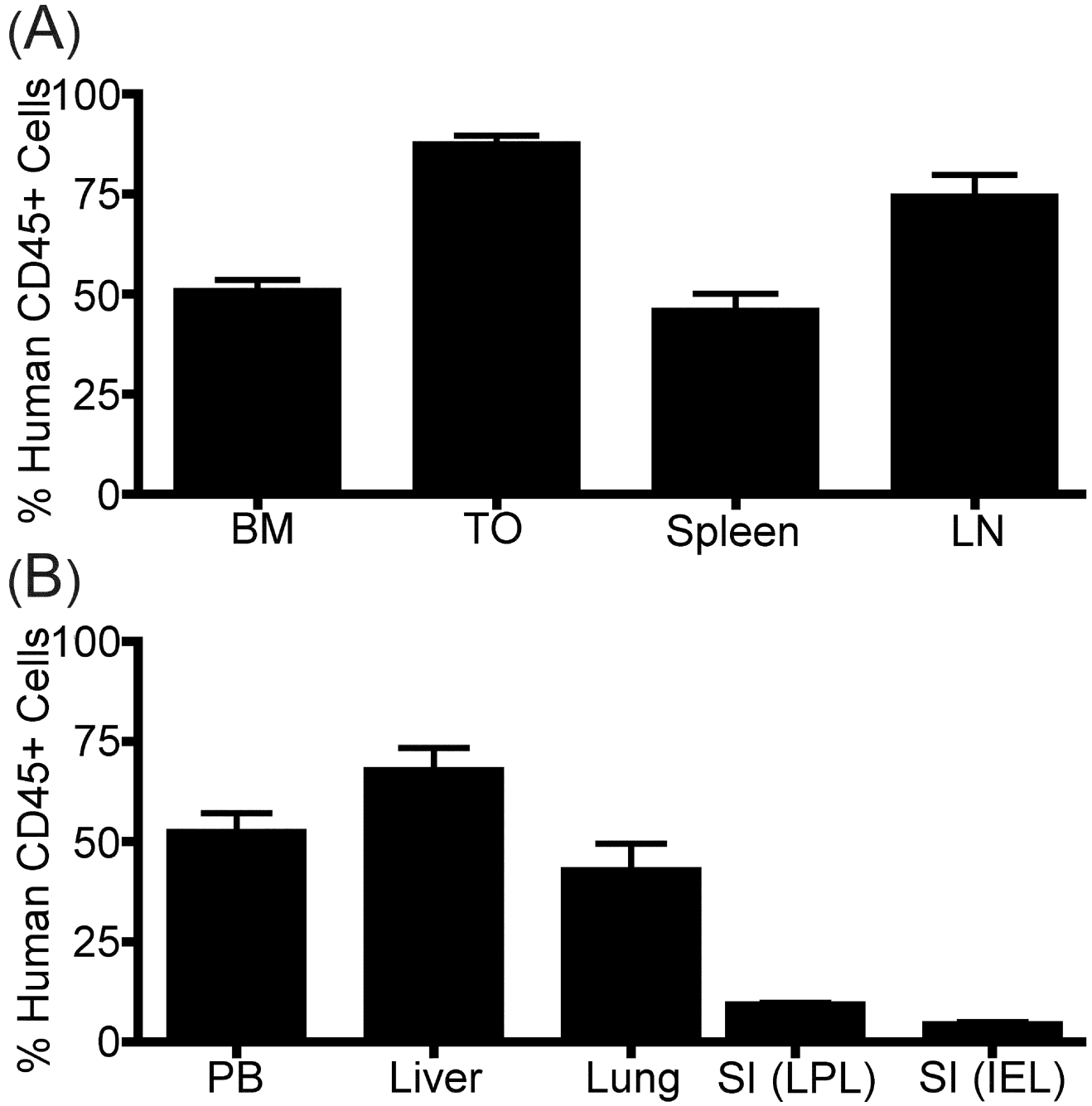

Fig. 1. Human immune cell reconstitution of BLT mice

(A) The primary and secondary lymphoid organs of BLT mice are reconstituted with human hematopoietic ($CD45^+$) cells. The percentage of human $CD45^+$ cells in the bone marrow (BM; n=26), thymic organoid (TO; n=26), spleen (n=26) and lymph nodes (LN; n=16) of BLT mice was determined with flow cytometric analyses. (B) Human hematopoietic cells were also found in peripheral blood (PB; n=22), the lung (n=9) and liver (n=7). In addition small intestine lamina propria lymphocytes (SI LPL; n=23) and small intestine intra-epithelial lymphocytes (SI IEL; n=23) have been described.

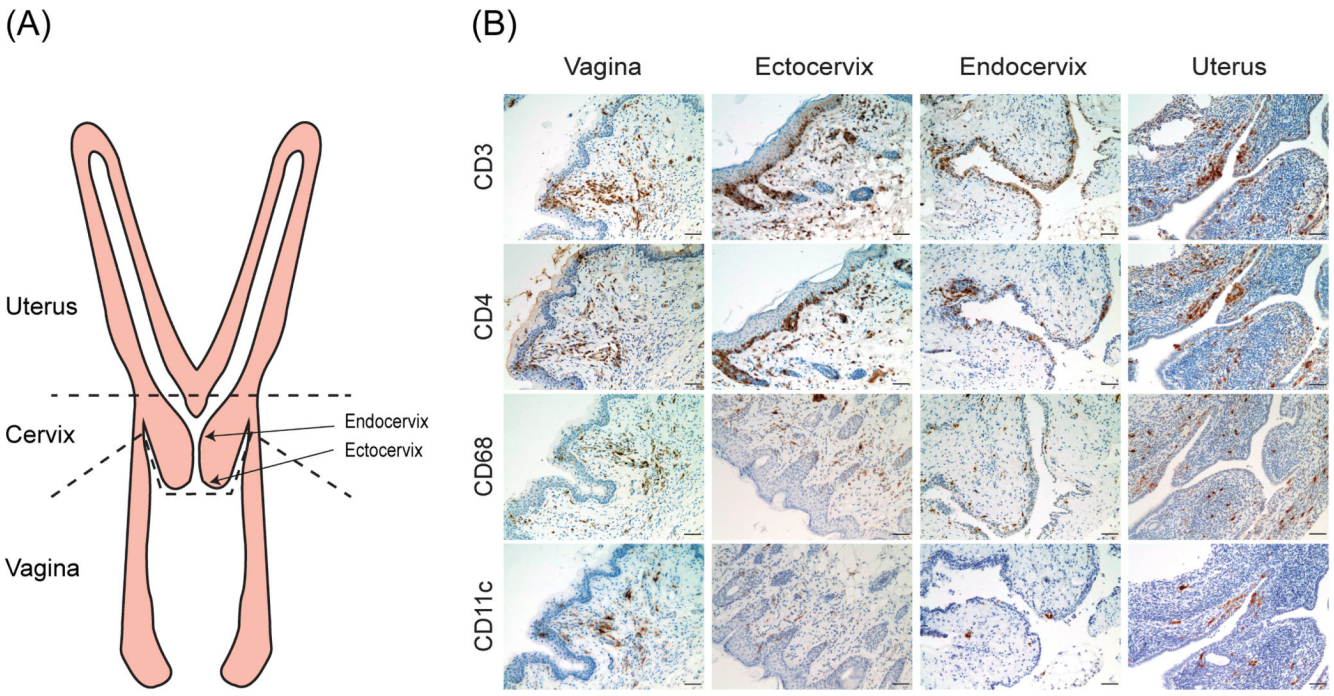

Fig. 2. Reconstitution of the FRT of BLT mice with human hematopoietic cells

(A) Diagram depicting the mouse uterus, cervix and vagina (separated by dashed lines in the figure). (B) Immunohistochemical analysis of the vagina, ectocervix, endocervix and uterus of female BLT mice demonstrating the presence of human hematopoietic cells (stained brown). Robust reconstitution with cells relevant to HIV infection, including human T cells ($CD3^+$), monocyte/macrophages ($CD68^+$) and dendritic cells ($CD11c^+$) is observed in each compartment of the FRT, demonstrating its efficient repopulation (bars indicate 25 μm) (Denton et al., 2008).

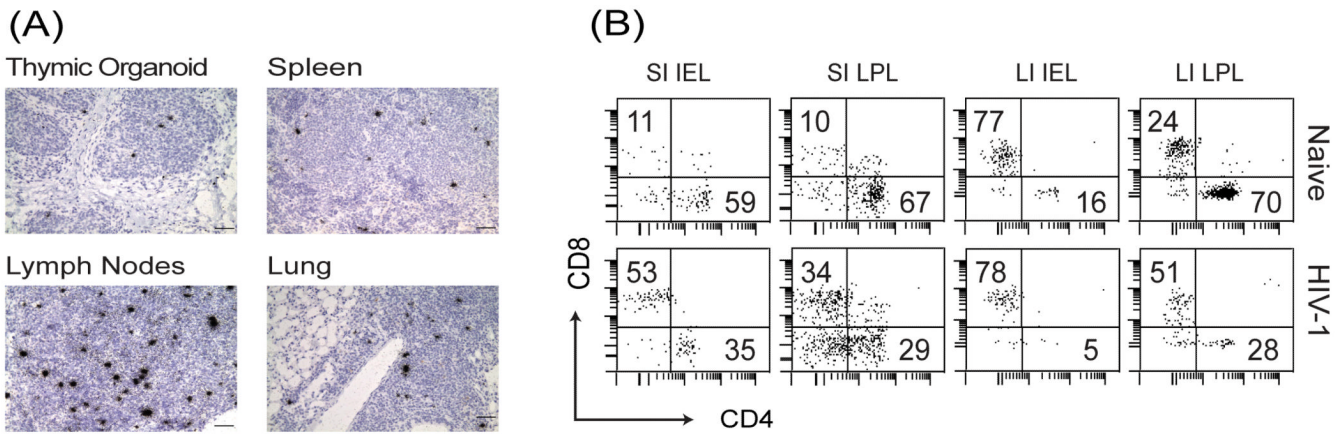

Fig. 3. Transmission after vaginal HIV-1 exposure results in disseminated HIV-1 infection and depletion of $CD4^+$ T cells in the GI tract

(A) *In situ* hybridization analysis for the presence of productively infected cells in multiple tissues from BLT mice vaginally exposed to HIV-1 (bars indicate 50μm). (B) Comparison of the $CD4^+$ and $CD8^+$ human T cells levels in the small intestine intra-epithelial lymphocytes (SI IEL) and lamina propria lymphocytes (SI LPL) and the large intestine intra-epithelial lymphocytes (LI IEL) and lamina propria lymphocytes (LI LPL) of naive and HIV-1 infected mice (Denton et al., 2008).