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Mucosal Immune Regulation: Does it Help Thwart HIV?

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Abstract

The impact of HIV on the mucosal compartment is now well recognized and contributes to driving immune activation, a characteristic of HIV pathogenesis. The mucosal immune system is greatly affected by HIV from the first days of the infection. A massive depletion of the mucosal CD4$^+$ T cells, over production of chemokines and cytokines and dysregulation of the epithelial barrier are some of the mucosal hallmarks of HIV infection. Individuals who control HIV replication, such as elite controllers (EC), or who maintain normal CD4$^+$ T cell counts, such as long-term non-progessors (LTNP), seem to maintain a balance within their mucosal environment that contributes to the delayed progression. At the female genital tract, which is the main route of entry of the virus in male to female heterosexual transmission, sexual activities have been associated with changes in mucosal immune activation that may influence the risk of HIV infection. However, not all exposures to HIV lead to infection and it seems that the immune mucosal milieu of the female genital tract is a critical determinant of HIV susceptibility. HIV Exposed Seronegatives (HESN) represents a group of individuals, such as female commercial sex workers, who are at high risk of infection but remain HIV uninfected. Repeated unprotected sex with many different partners makes them highly susceptible to HIV infection, yet it seems that they can prevent the establishment of HIV infection. What makes them "resistant" to HIV infection? In this review, we will focus on the regulation of the mucosal immune system during exposure and infection with HIV. What goes wrong in HIV infection and what goes right in HESN individuals? Lessons learned from HESN might help to have a better understanding of mucosal immune regulation and develop effective HIV preventive strategies.

Introduction

According to UNAIDS, women represent more than 50 percent of people living with HIV/AIDS Worldwide [1]. Despite the low probability of Human Immunodeficiency Virus (HIV) transmission during a single episode of unprotected vaginal sex (from 1/200 to 1/12000) [2], heterosexual transmission is the fastest growing phase of the HIV pandemic. Even if the transmission risk per exposure is lower, vaginal intercourse contributes more to the epidemic than anal intercourse or parenteral inoculation [2]. The mucosa-associated lymphoid tissue (MALT) is an important element in the interaction between pathogens and the host immune system. Since mucosal tissues of the female genital (FGT) and intestinal (gut-associated lymphoid tissue, GALT) tract are the main sites of HIV infection, HIV is considered a mucosal pathogen. The impact of HIV on the mucosal immune system is critical for disease outcome, and the state of the peripheral immune system does not necessarily reflect these profound mucosal dysregulations. Here, we will review alterations of the mucosal immune system caused by HIV infection and we will discuss how the impact of constant stimulation on mucosal immunity at the FGT affects HIV infection in commercial sex workers.

Impact of HIV on Mucosal Immunity

The mucosal immune system plays a critical role in protecting the host against many pathogens and environmental antigens while inducing tolerance to non-pathogenic commensal flora (reviewed by [3]). Mucosa associated lymphoid tissues (MALT) are specialized organized lymphoid systems that contain the majority of the lymphocytes of the body. The MALT is a non-sterile environment where lymphocytes and epithelial cells work together to maintain the balance between activation and tolerance. Mucosal immunology is increasingly gaining attention because of its important potential in the development of vaccines against mucosal pathogens such as influenza and HIV. The mucosal immune system is, in many ways, more complex than its systemic counterpart. Studies in macaques infected with simian immunodeficiency virus (SIV) and humans infected with HIV have shown that the SIV/HIV infection rapidly kills most CD4$^+$ T cells at mucosal surfaces [4-6]. The mucosal surface of the FGT is the main route of entry of HIV, and the mucosal immune system is rapidly perturbed by HIV infection more than any other immune compartment. The fundamental role played by mucosal surfaces in the pathogenesis of HIV infection has recently been a major focus of HIV research.

Irrespective of the route of entry, transmitted virus promptly crosses the epithelial barrier to reach target cells, resulting in an extensive, and possibly permanent, depletion of the resident mucosal memory CD4$^+$ T cells. This phenomenon is observed in the intestine as well as in other mucosal tissues including the FGT [5,7,8] (reviewed by [9]). The massive mucosal CD4$^+$ T cell depletion and the breakdown of essential immunoregulatory mechanisms leads to a severe impairment of mucosal defences, which results in a high rate of secondary opportunistic infections and contributes to the morbidity and mortality of HIV infected individuals [10]. Non-human primate (NHP) studies have demonstrated that during the first day following infection, many

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critical events occur at the mucosal level which affects the rate of disease progression. In humans, these events occur early in the course of the infection, prior to systemic alterations and sometimes months to years before the individual realizes his/her HIV status (reviewed by [11]).

The female genital tract (FGT)

The majority of female HIV infection occurs through the FGT. With a low pH, the presence of a natural microflora and many anti-viral proteins in the vaginal secretions/cervical mucus, the FGT environment is naturally considered to be a hostile environment for HIV transmission and replication (reviewed by [12]). Multiple factors influence this natural barrier against infection and an imbalance in mucosal regulation may provide an opportunity for HIV to penetrate the barrier and initiate a productive infection.

Hormones are important factors that can modify the mucosal immune compartment of the FGT. Throughout the menstrual cycle, hormone expression leads to changes in the thickness of the epithelial layer, frequencies and activation status of immune cell populations, antimicrobial peptide expression and cytokine/chemokine expression [13]. High levels of progesterone seem to be associated with an increased risk of HIV acquisition [14]. Recently, the use of hormonal contraception has also been associated with increased risk of HIV acquisition, showing the importance of hormone regulation on the mucosal immune response [15]. However, hormones are not the only factor that can influence the mucosal environment of the FGT; sexual activities and sexually transmitted infections (STI) are also influencing the mucosal milieu (reviewed by [16]), as discussed later in this review.

Although the mucosal tissues of the FGT are severely affected by the virus, the biological impact of HIV infection on the genital tissues and its implications for HIV replication and disease progression have still not been well characterized. Due to the challenges associated with obtaining fresh samples and the limited number of cells available with cytobrush sampling, the impact of HIV on the FGT immune system still remains unclear. The first steps of infection have been characterized in non-human primate models. Intravaginal inoculation of SIV in macaques showed that within 14 days post infection, there was an increase of CCL20 (MIP-3a), which was associated with an increase of $CD123^+$ plasmacytoid dendritic cells (pDC). Those pDC, in turn, produced CCL3 and CCL4, which attracted $CD4^+CCR5^+$ T cells to the site of infection and promoted the dissemination of the infection [17]. By inhibiting immune activation and cytokine/chemokine production, it could be possible to block systemic infection. Supporting this idea, Li et al. showed that applying glycerol monolaurate, a widely use anti-microbial agent that blocks T cell activation, at the cervico-vaginal mucosa can block the secretion of CCL20 and protects against SIV infection [17].

Knowledge on the interaction between HIV and human FGT is however limited. Even among healthy women, knowledge about mucosal immunity at the FGT is limited. Studies on vaginal and cervical tissue have gathered some pieces of information about the distribution of various immune subsets that could interact with HIV in these tissues. Immune cells from non-inflamed vaginal and cervical tissue derived from women undergoing hysterectomies showed a distinct lymphocyte distribution throughout the FGT. The lower vaginal mucosa contains few leucocytes with a majority of memory $CD8^+$ T lymphocytes, while ectocervix tissue contains similar lymphocyte subsets with a higher proportion of $CD4^+$ and $CD8^+$ lymphocytes [18]. The transformation zone represents the transition between the multilayer epithelium of the ectocervix and the single-layer columnar epithelium of the endocervix. The immune cell-rich transformation zone was shown to be the main point of weakness for SIV entry but infection is not restricted to this area (reviewed by [19]). It is the location with the highest proportion of immune cells (CD8+ and CD4+ T cells, granulocytes and macrophages) including a small accumulation of CD56+ natural killer cells (NK) [20]. This innate immune subset combats HIV replication by non-cytolytic mechanisms including the production of anti-viral cytokines or by direct lysis of infected cells (reviewed by [21]). While the predominant NK cell population in the lower reproductive tract display the cytolytic $CD56dimCD16^+$ phenotype, NK cells from the upper reproductive tract are mainly the cytokine-secreting CD56bright subset [22].

Distribution of antigen-presenting cell (APC) subsets localised in the FGT has been identified by immunostaining of ectocervical, endocervical and endometrial biopsies. Distinct populations expressing APC markers were present throughout these tissues, which harboured differing expression patterns of C-type lectins, important attachment factors for HIV (reviewed in [23]). Langherans cells (LC) expressing the langerin receptor were found within the epithelial layer while DC-SIGN+ and/or mannose receptor (MR)+ DCs and macrophages were localised in the submucosal compartment along with $CD123^+$ pDC and adjacent to $CD4^+CCR5^+$ T cell populations [24-26]. The role of LCs and submucosal DCs in HIV infection remains controversial. These cells are targets for HIV-infection and have the potential to disseminate the virus through C-type lectin-dependant and replication-dependant mechanisms. However, their function during *in vivo* infection remains to be clearly defined (reviewed by [2]). While well studied in the non-human primate model, the cascades of innate immune events that follow exposure to HIV and contribute to the spreading of the infection remain poorly described in the human FGT. Nonetheless, when viral dissemination occurs, it leaves profound immune alterations at the FGT.

Impact of HIV on FGT Immunity

Chronic HIV infection promotes a pro-inflammatory response at the genital tract resulting in higher mucosal levels of cytokine and chemokine expression that will, in turn, contribute to cell recruitment. MCP-1, MIP-1b, RANTES, MCP-3, MIG, TNF-a and IFN-g are overexpressed in HIV infected commercial sex workers (CSW) compared to HIV uninfected CSW and low risk women. In contrast, MIP-1a is underexpressed compared to HIV uninfected CSW [27,28]. These immunoregulatory molecules are involved in lymphocyte chemotaxis and the inflammatory response. Secreted by various cell types at the FGT such as epithelial, stromal or immune cells, MCP-1, MIP-1a, MIP-1b, RANTES and MCP-3 attract neutrophils, monocytes, macrophages, immature DCs, T cells and NK cells to the inflamed tissue, while MIG attracts activated T cells and NK cells (reviewed by [29]).

However, beta chemokines released by activated T cells such as RANTES, MIP-1a and MIP-1b may play a dual role during HIV infection. In addition to attract activated T cells, which increases the number of target cells for the viruses, they also inhibit cell infection. By competing with HIV for CCR5 receptor binding, these chemokines inhibit HIV infection *in vitro* [30-32] and their spontaneous or induced expression by PBMCs has shown to have a protective role against HIV acquisition among discordant couples (reviewed by [33]). Effect of beta-chemokines might depend on the timing of their secretion. Secreted prior to the infection, they could be protective by blocking the access to CCR5. However, their secretion following infection may increase the potential number of target cells and contribute to the establishment infection foci. Triggered during inflammatory responses, TNF-a drives

HIV-1 transcription through the activation of cellular transcription factors (review by [34]). HIV-induced secretion of TNF-a by epithelial cells contributes to epithelial barrier impairment by disrupting tight junctions, suggesting an important contribution of TNF-a in viral transcytosis and dissemination across the epithelial barrier [35]. Interestingly, cytokine concentrations in CVL during the acute phase of HIV infection did not differ from the concentrations observed prior to infection [36]. This suggests that cervical cytokine concentrations may increase over time in the course of HIV-infection. In fact, elevated levels of cytokines in women with early HIV infection were correlated with the presence of STIs [36].

Infections with other STIs such as Herpes simplex virus type 2 (HSV-2), human cytomegalovirus (CMV), Neisseria gonorrheae, Chalmydia trachomatis and many others also enhance cytokine and chemokine expression at the FGT (reviewed by [37]). STIs influence HIV susceptibility either by breaching the epithelial tissue, recruiting HIV target cells into the site of infection, or by generating a pro-inflammatory local immune milieu (reviewed by [37]). Elevated levels of pro-inflammatory cytokines are observed in CVL from symptomatic and asymptomatic women infected with a broad array of STIs [38,39] and higher concentration of inflammatory cytokines (IL-1β, IL-6, IL-8, and sCD40L) in CVL specimens correlate with an increased HIV acquisition among South African women, especially among Nesseria gonorrhea infected women [38]. HSV-2 infection negatively synergized with HIV in the mucosal compartment to favor viral replication. Indeed, HSV-2 infection increases the number of potential HIV target cells at the FGT. In turn, HIV infection impairs the HSV-2 mucosal immune control and local HSV-2 reactivation increases the risk of transmission of both viruses [40]. Women with cervico-vaginal inflammation due to bacterial vaginosis or cervicitis had higher level of pro-inflammatory cytokines such as IL-1b and IL-6 and this pro-inflammatory environment is likely to be important in susceptibility to HIV infection [41].

In addition to profound alterations of the cytokine/chemokine environment, the FGT of HIV-positive women exhibits fundamental changes in cells populations such as macrophage and lymphocyte populations and in immunoglobulin (Ig), even in the absence of symptoms [42]. Immunohistological studies of the ectocervix have shown an important redistribution of macrophage subsets during HIV-infection. The epithelium of HIV-infected women showed an increase in both the number of $CD68^+$ macrophages and the proportion of activated (HLA-DR+) subsets, with an increased proportion of macrophages harbouring a suppressive phenotype in the stroma [42]. CD1a+ LC subsets acquired a better capacity to present antigens and were depleted in the stroma of HIV-positive women, as observed during cervicitis [20]. This decline may be explained by a rapid LC emigration from the epithelium, similar to what is seen during infection of *ex vivo* organ culture (reviewed by [2]). These observations suggest the presence of a local mechanism for T cell activation, however the suppressive phenotype displayed by the local macrophages may impair the emergence of a local immune response [42].

An important gap in the knowledge is the impact of HIV-infection on innate cell populations of the FGT. Well characterized in the uterine compartment where they are abundant and play an important role in reproductive biology (reviewed by [43]), NK cells seem to be scarce in the lower FGT, i.e. the ectocervix compartment, of HIV-infected and uninfected women [42] and are poorly described in the FGT. While observed in the higher part of the FGT (endocervix and transformation zone) of uninfected women [20] and in vaginal biopsies of monkeys [44], no studies have addressed the impact of HIV-infection on NK phenotype and distribution in the human FGT. We have observed in cervico-vaginal mononuclear cell (CMC) samples that NK cells comprise about 10% of the CD3 negative lymphocytes population of the FGT, harbouring a more activated phenotype ($CD69^+$) among HIV-infected CSWs (Lajoie and Kirwan, Unpublished data).

Gamma-delta T cells are another important mediator of innate immunity that plays an important role bridging innate and acquired immunity. Activated by stress-induced molecules without the need for antigen processing and presentation, gamma-delta T cells also recognize target cells by a NK-like mechanism of activating and inhibiting receptors and participate to the local inflammatory response by secreting cytokines and chemokines (reviewed by [45]). During systemic HIV infection, gamma-delta T cell subsets are perturbed. Two subsets of gamma-delta T cells are commonly found in humans. The Vdelta1 subset expresses tissue regulation genes (IL-10 and IL-11) and is the predominant subset found in mucosal compartment [46]. The Vdelta2 subset is more prevalent in the circulation and expresses pro-inflammatory genes upon mitogen stimulation [46]. Inversion in the ratio of Vdelta2 to Vdelta1 is observed in HIV-infected subjects. Contraction of the circulating Vdelta2 subsets occurs concurrently with expansion of the Vdelta1 subset in the gut of HIV infected patients [47] (reviewed by [45]). Circulating Vdelta2 T cells become anergic in HIV-infected patients and are depleted by an apoptosis-dependant mechanism (reviewed by [45]). These alterations persist despite ARV therapy [47,48]. Despite early reports of gamma-delta T cells derived from the human cervix [49] recent studies have failed to identify a gamma-delta T cell population in the lower FGT during non-inflammatory conditions [42]. Monkey studies, however, have demonstrated an important homing of gamma-delta T cells to the cervico-vaginal mucosa of monkeys immunized by a gp120/p27/alum vaccine targeting the iliac lymph node [50]. Further analyses have demonstrated that gamma-delta T cells protected macaques against SIV infection by secreting beta-chemokines (RANTES, MIP1-a and MIP-1b) that block CCR5 binding and prevent infection of new target cells [50].

In addition to gamma-delta T cells, CD1d-restricted invariant NKT (iNKT) cells represent another rare lymphocyte subset that link innate and adaptive immunity. At this time, no reports of the presence or absence of iNKT populations at the human female genital tract have been published, despite the presence of CD1d expression in vaginal tissues [51,52]. In mice, however, iNKT activation is protective against genital tract *Chlamydia muridarum* infection [53], demonstrating a role for iNKT activation in mucosal immunity that has yet to be confirmed in humans. Data on innate cell subsets at the FGT during HIV-infection are scarce and we are far away to fully understand their contribution to HIV acquisition and pathogenesis. A better comprehension of the role of these subsets at the FGT is needed in order to harness them for vaccine and microbicide development.

Concurrent to the increased levels of cytokine and chemokine expression, elevated numbers of activated $CD8^+$ T cells can be found at the FGT of HIV-infected women, indicating active recruitment of non-resident $CD8^+$ lymphocytes [42]. Very few studies have characterized the HIV-specific T cell response in the FGT of HIV infected women. HIV-specific CTL responses are much less frequent in the cervical compartment than in the periphery [54], yet, following suppression of viremia by ART, CTL responses are preserved in the cervix while lost in the blood [55]. Gumbi et al. analysed the link between mucosal inflammation and HIV-specific $CD8^+$ T cell responses in the cervix

of HIV infected women. They found that genital HIV shedding was associated with an increased level of pro-inflammatory cytokines such as TNF-a, IL-1b, IL-6 and IL-8, although there was no association between HIV shedding and the cervical HIV-specific $CD8^+$ T cell response [56]. These results support the hypothesis that, during infection, release of viral particles at the genital tract is exacerbated by inflammatory cytokines and that the CTL response is not sufficient to contain HIV replication. $CD4^+$ T cells, preferentially IL-17 and IL-22 expressing T cell populations, are depleted in the cervix following the establishment of HIV infection [7,54]. While well described in the gut, the relevance of Th17 and Th22 cell depletion in the FGT is not understood. It has been suggested that depletion of these populations may contribute to the increased frequencies of candidiasis and others genital tract infections in HIV-infected women [7].

Deregulation of the mucosal immune system also influences systemic immunity. High genital levels of pro-inflammatory IL-1b, IL-6 and IL-8 were associated with lower systemic $CD4^+$ T cell counts while MCP-1 positively correlated with plasma viral load [57,58]. In early infection, elevated cervicovaginal lavage (CVL) pro-inflammatory cytokines were associated with lower blood $CD4^+$ count and higher blood viral load and genital HIV RNA concentrations [36]. These observations suggest that alterations of the mucosal compartment affect the systemic compartment. ARV therapy may restore $CD4^+$ count at the FGT since higher cervical $CD4^+$ T cell percentage was observed in treated women [55]. However, ART does not seem to entirely suppress viral production at the FGT, as low levels of virus can still be detected even if plasma viral load is undetectable [55]. To our knowledge, no studies have addressed the impact systemic cytokines/chemokines expression on the FGT immune system. Further studies need to be done in order to understand the relationship between both compartments.

Even if there is still an important lack of knowledge about the impact of HIV on the mucosal immune system of the FGT, it is quite certain that the impact of HIV on the mucosal environment is more important than previously realized. A growing body of studies have already begun to unravel the impact of HIV infection on the gastro-intestinal tract (GALT), the most studied compartment of the mucosal immune system.

The gastro-intestinal lymphoid tissue (GALT)

The gastro-intestinal tract contains the majority of the $CD4^+$ T cells in the body [59] and is an important site for early viral replication and severe $CD4^+$ T cell depletion during HIV infection. Compared to the FGT, we have a better comprehension of the impact of HIV/SIV infection on the GALT environment. The magnitude of T cells lost in the GALT of an HIV infected patient is not reflected in the peripheral blood. Studies have shown that early in infection there is a drastic loss of the memory T helper cell population. Data from multiple groups showed that, in the first days post infection, nearly 60% of the $CD4^+$ T cells are depleted [4-6], and that this depletion is largely irreversible. Indeed, HIV-infected individuals on anti-retroviral therapy show a slow and incomplete restoration of $CD4^+$ T cells in the GALT compared with the systemic compartment [60]. HIV-specific CTL response may, in part, protect against this rapid decline by limiting mucosal replication during chronic infection. The level of Gag-specific $CD8^+$ T cells is more elevated at the rectal mucosa than in the systemic compartment and is positively associated with CD4 counts and inversely with the plasma viral load [61].

Various T cell populations are differentially affected by the presence of the virus. Th17 and Th22 populations are involved in the generation of immune responses to microbial pathogens at mucosal surfaces and play a critical role in normal barrier homeostasis [18,62] (reviewed by [63]. In HIV infection, Th17 and Th22 cells are depleted more severely than the bulk $CD4^+$ T cell population in the intestinal compartment [64,65]. The strong depletion of these cells is associated with the development of malabsorption and nutriment complications in rhesus macaques [66] and with a decreased expression of markers regulating barrier integrity and intestinal function [67,68]. This change also coincides with increased inflammation and expression of immune activation genes. Damage at the mucosal barrier seems to increase absorption of microbial products at the systemic level. The lost of barrier integrity is associated with elevated plasma levels of LPS (and sCD14), which in turn contributes to chronic immune activation and the eventual loss of $CD4^+$ T cell-mediated immunoregulation [67].

In the gut, gamma-delta1 T cells are important players in maintaining mucosal homeostasis, and represent about 50% of the intrepeithelial lymphocytes and 10% of the lamina propria lymphocytes (reviewed by [69]). In HIV-infected patients, high levels of immune activation, as measured by elevated levels of B2-microglobulins and neoprepin, inversely correlated with duodenal gamma-delta intraepithelial lymphocytes, suggesting a role for these cells in limiting the immune activation in patients [70]. Underscoring this hypothesis, a decrease in the mucosal gamma-delta T cell count was observed toward the end of life in AIDS patients [70] .

HIV-infection also alters mucosal NK cell subsets. Mounting evidence suggests an important role of NK cells in the control of HIV replication. Control of HIV replication has been associated with certain HLA-Bw4 alleles, including HLA-B57 and HLA-B27, and their inhibitory and putative activating NK receptors, killer immunoglobulin-like (KIR) 3DL1 and KIR3DS1 respectively. Combinations of KIR and HLA alleles have been associated with HIV disease outcome, with enhanced NK cell function delaying AIDS progression (reviewed by [71]). A recent study has observed the presence of two distinct NK cell subsets residing in the gut with altered frequencies during HIV-infection [72]. Using immunochemistry of gut biopsies, Sip et al. [72] demonstrated that intraepithelial and lamina propria NK cells express two different isoforms of Nkp46, a natural cytotoxicity receptor expressed on all NK cells. In addition to harbouring a more terminally differentiated phenotype ($CD57^+$), intraepithelial NK subset were observed more frequently among individuals with the protective KIR/HLA genotype associated with spontaneous control of HIV replication, implying a potential implication for intraepithelial NK in the control of viremia. Moreover, the fact that both subsets expanded in the gastro-intestinal tract of HAART treated HIV-positive subsets with incomplete $CD4^+$ reconstitution has led to the suggestion that these cells accumulate in absence of viral replication to potentially compensate for the GALT compromised immunity [72].

Over the past few years, our understanding of the role of the mucosal immune system in HIV pathogenesis has improved. The challenge now is to understand the full implications of the deregulation of the mucosal environment in HIV pathogenesis as well as the impact of perturbations in mucosal immunity on the systemic immune system. The elucidation of mechanisms to prevent mucosal T cell depletion and the impairment of the mucosal immune system is critical to move forward in the fight against HIV infection.

Natural Hosts and Elite Controller/Long Term Non-progressors

Important insights on the pathogenesis of HIV infection come

from the studies of monkey models. Infection of the African natural hosts, like African Green Monkeys (AGM) and Sooty Mangabeys (SM), with Simian Immunodeficiency Virus (SIV) leads to a non-pathogenic SIV infection. In marked contrast, infection of rhesus macaques results in a rapid progression to AIDS that mimics human HIV infection. Unlike pathogenic models, natural primate hosts preserve a healthy level of systemic $CD4^+$ T cells and do not progress to AIDS despite high viremia [73] (reviewed by [74]). One of the main characteristics of this model is the limited immune activation associated with the presence of SIV [75]. Like HIV infection in humans, SIV infection of natural hosts is associated with a rapid and profound depletion of the mucosal memory $CD4^+$ T cell subset [76]. However, in natural hosts, the mucosal immune activation that characterizes pathogenic primate models and HIV infected individuals is not observed [76]. In the natural host model, the mucosal T cell depletion observed during the acute phase of infection does not progress and reaches a stable plateau (in SM) or is followed by a significant recovery of these cells (in AGM) during the chronic phase of the infection [77] . In the monkey pathogenic model, as well as in HIV infected rapid/normal progressors, an important loss of Th17 cells in the MALT occurs. This depletion of the Th17 population does not happen in the non-pathogenic model and might explain why the mucosal integrity remains intact [78]. Another rather important factor that distinguishes between the pathogenic and non-pathogenic monkey models is the rapid resolution of the innate immune response observed in non-pathogenic infection. Rhesus macaques and humans show a persistent type I interferon response throughout the chronic phase of infection while natural host resolve it within 4 to 8 weeks. Hence, it has been proposed that natural host of SIV actively down-regulate the innate and adaptive immune responses to the virus to avoid progression to AIDS (reviewed by [74]). Rapid resolution of innate response might contribute to the maintenance of the mucosal compartment and barrier integrity preventing uncontrolled immune activation and disease progression.

Among those who are HIV infected, some individuals have the incredible capacity to naturally control HIV replication or maintain normal $CD4^+$ T cell counts; these individuals are termed Elite Controllers (EC) and long term non progressors (LTNP) respectively. Like in the SIV natural host capable of maintaining a normal $CD4^+$ T cell count despite the presence of active SIV replication, long term non progressors (LTNP) present a normal $CD4^+$ count and lower expression of immune activation and mucosal inflammation genes than HIV-infected individuals (as measured in microarray analysis) [79,80]. Low levels of systemic immune activation are not, however, the only factor contributing to a lack of disease progression in EC/LTNP populations. Despite low viral loads and normal $CD4^+$ T cell counts, HIV infected ECs have higher generalized immune activation than HIV negative individuals [81,82] suggesting an important role in maintaining low mucosal immune activation for delayed disease progression. Interestingly, EC patients do not suffer from the mucosal $CD4^+$ T cell depletion observed among natural host NHPs or HIV progressors [4] and elevated mucosal $CD4^+$ T cell and Gag-specific CD8+ T cell responses have also been observed among LTNP patients [80]. These data seem to indicate that despite elevated immune activation, EC and LTNP individuals are able to maintain improved mucosal immune responses that might explain their slow progression to AIDS.

These findings are quite intriguing. How natural hosts, ECs and LTNPs succeed in maintaining normal mucosal immune function despite T cell depletion is an unanswered question. What drives mucosal immune activation and chronic inflammation in normal HIV infected individuals? Is mucosal immune activation responsible for disease progression? The capacity to regulate mucosal immunity seems to be important for protection against HIV progression, but can the same be said for HIV acquisition?

HESN and protection against HIV

Over the last 30 years, many groups have studied the correlates of susceptibility to HIV, but some of the most interesting findings are provided by studying HIV-exposed seronegative (HESN) individuals. Despite repeated exposure to HIV, those individuals exhibited a natural resistance to HIV infection. Many cohorts of HESN have been identified, including commercial sex workers (CSW), discordant couples, hemophiliac individuals and children born from HIV infected mothers. Studying HESN has been an important step in understanding determinants of natural protection against HIV acquisition.

Many observations arising from these cohorts have led to the identification of different factors involved in natural protection. Numerous studies have examined the immunological and genetic correlates of protection among HESNs (for more details see [83,84]). One of the most studied correlate of protection is a deletion of 32 base pairs in the CCR5 gene. This polymorphism, found in 10% of the European population, has not been observed in the majority of HESN cohorts and cannot explain all cases of natural protection [85]. The presence of systemic HIV-specific T cell responses [33,86], polymorphisms in the Interferon Regulatory Factor (IRF) -1 gene [87-89], carrying homozygous KIR3DS1 genotype or combined KIR3DL1 high expressor alleles and HLA-B*57 genotype [90,91] and increased mucosal levels of anti-proteases are among others factors that have been described to explain this natural protection against HIV infection [92]. Compared to the GALT, the female genital compartment harbours IgG as the dominant antibody isotype. In the FGT, IgG and IgA originate from blood circulation and local production (reviewed by [93]) and functional capacity of IgA to neutralize HIV-1 and block trancytosis across the epithelium has been demonstrated [94,95]. However, the protective role of cervical IgA in HIV acquisition remains controversial. While some studies have reported a higher proportion of cervical IgA [95-97] among HESN CSWs and discordant couples, others reported its absence [98,99] or correlated its frequency at FGT with higher daily exposure to the virus [95,100]. Hence, it is still unclear to what extent cervical IgA protects against HIV.

Protection against vaginal HIV acquisition has also been correlated with stronger interactions between NK and immature DCs. Immature DCs (iDC) from HESNs induced higher IFN-g production from NK cells that become more potent in eliminating autologous iDCs upon *in vitro* isolation or differentiation [101]. In addition to altered NK-DC cross-talk, several studies looking at KIR genotypes or NK activities have linked increased NK activity to protection against sexually and intravenously acquired HIV infection [102-104] (reviewed by [33]). Recently, we've developed a model of protection termed Immune Quiescence (IQ) to explain HIV resistance [105] among the Pumwani cohort of CSWs in Nairobi, Kenya. Decreased levels of T cell activation and host proteins required for HIV-1 replication were observed in HESN sex workers that limit available target cells for viral replication [105]. Factors that affect lymphocytes trafficking to the FGT and that regulate the inflammatory state of the genital mucosa could be key contributors to the induction of immune quiescence and, therefore, protection to HIV [106,107].

Impact of Sexual Activity on the FGT and Implications for HIV Protection

In addition to hormonal cycles, sexual activities influence the

immune environment of the FGT. Contact between seminal fluid and cells of the FGT triggers a cascade of events involved in clearance of seminal debris, selection of fertilizing sperm, and induction and maintenance of immunological tolerance toward paternal antigens (review by [16]). Following coitus, seminal fluid induces a pro-inflammatory response illustrated by the secretion of GM-CSF, IL-6, IL-8, IL-1A, MCP-1 and CCL20 by epithelial cells [108-111] (reviewed by [112]). This local increase in cytokines and chemokines correlates with the recruitment of neutrophils, macrophages, DCs and lymphocytes in the epithelial and stromal layers of the cervix [110,113]. The remodeling of cervical immune cell populations following coitus might, therefore, affect HIV acquisition at the mucosa.

Fluctuation in seminal components between individuals causes variations in the pattern of cytokine synthesis and leukocyte recruitment during this early inflammatory response [114]. The HIV status of the male partner also influences cytokine concentrations in seminal plasma, and the increased concentration of seminal TGF-b observed during the chronic phase of HIV infection correlates with lower secretion of pro-inflammatory mediators by semen-exposed genital epithelial cells [115]. In addition to TGF-b, sperm contains prostaglandins, IL-8 and many other regulatory factors [110,111,114-116]. TGF-b contributes to the early inflammatory response but also to the induction of the Treg population involved in tolerance to paternally derived antigens [117]. In addition, exposure to semen during unprotected sexual intercourse results in peripheral T cell proliferation in response to the partner's cells [118,119]. Nonetheless, this alloimmune response to a partner's semen appears to be subject to important inter-individual variations. While the majority of individuals develop an immune response against their partner's cells, a few become tolerized and respond to allostimulation with a pattern of immunoregulation that cannot be explained by the matching of HLA class II alleles [118,119].

Sex work results in constant immune stimulation as a consequence of exposure to seminal fluid from multiple partners rather than only one partner. Some studies have looked at the impact of commercial sex work on systemic immune responses and found that sex work results in suppression of the alloimmune response, decreased of CXCR4 expression on naïve $CD4^+$ T cells, and an elevated proportion of activated $CD8^+$ T lymphocytes [120,121]. Previous studies have compared cytokine expression patterns from CVL and blood to conclude that immune activation of the systemic compartment does not reflect the level of activation at the mucosal compartment [27,28]. But what happens in the vaginal compartment upon frequent sexual activities? So far, few studies have looked at the impact of sex work on immune activation at the FGT. Shedding light onto this question, our group has observed that the duration of sex work positively correlates with higher proportion of NK cells in CMCs and a reduction of cytokine/chemokine expression in CVL of CSWs from Nairobi, Kenya (Lajoie et al. In preparation). After two years of commercial sex work, HIV-uninfected women harbored lower levels of MIP-3a, ITAC, MIG, IL-1a, IL-1b, IL-Ra, IL-8, IL-10, IP-10, MCP-1, MDC, MIP1-a, MIP-1b and TNF-a in CVL compared to sexually active, HIV-negative, non-CSW women. This difference was even more pronounced among individuals with a history of >10 years of sex work. In a recent study, cervical and peripheral cellular responses to mitogen stimulation were compared between low-risk women and CSWs active in the field for 1 to 38 years. Following mitogen stimulation, PBMC and CMC from CSWs demonstrated a reduced production of Th17/Th22-related cytokines in both compartments, but no difference in the magnitude of the immune response was observed according to duration of sex work [107]. Immunostaining of endocervical biopsies demonstrated that active sex work alters DC phenotypes at the FGT. Despite no observed DC accumulation, DCs subsets from CSWs expressed higher levels of CLR receptors compared to women from the low-risk population [24]. This distinct pattern of CLR expression in women practicing active commercial sex work might be induced by the inflammatory response triggered upon frequent exposure to sex-derived antigens and could increase the HIV susceptibility of CSWs [24].

However, we have to keep in mind that most of these studies have compared HESN CSWs to sexually active low-risk women from the general population. Therefore, it is impossible to discriminate the effect of frequent exposure to sexual products of multiple partners to the pressure exerted by HIV upon the accumulating years of commercial sexual activities. These years of exposure may result in selection of women having low levels of baseline immune activation in the genital mucosa. Both factors can play a significant role in protection, and so far, none of them have been excluded. Any factors that are capable of influencing immune activation at FGT are likely to play an important role in HIV susceptibility. How sex work affects susceptibility to HIV infection is a question that remains to be understood.

Intriguing findings regarding this question comes from an observation from the Pumwani cohort in Nairobi. CSWs from this cohort experience many unprotected sexual exposures, more than 500 during their years of commercial sex work, and despite that, they remain uninfected [122,123]. However, this protection is not absolute and late seroconversions of previously resistant CSWs have occurred and were associated with a reduction of daily clients or interruption in commercial sexual activities. Retirement from sex work results in the loss of peripheral HIV-specific immune responses, but some late seroconversions cannot be entirely explained by a lack of preexisting HIV-specific CTL responses or by viral CTL escape [123]. Why the protection against HIV infection wanes during the arrest of sexual activities and what drives this protection has remained unanswered.

We therefore performed a preliminary study to assess the levels of mucosal and systemic immune activation in CSWs already planning a break from commercial sex work. We observed that an interruption of at least 3 weeks had a major impact on the immune system compared to CSWs that maintained their commercial sexual activities. Sex work is clearly a source of ongoing immune stimulation at the FGT, and an arrest in commercial sexual activities led to a decrease of the activation markers on peripheral and cervical lymphocytes, particularly among HIV-infected CSWs, as illustrated by CCR5 expression (Figure 1A). Surprisingly, even in the context of HIV-induced immune activation, interruption of sex work significantly reduces immune activation of HIV-positive CSWs, demonstrating the strong impact of allostimulation on peripheral and mucosal immune activation. Importantly, HIV-negative individuals experienced a dramatic increase in mucosal activation upon resumption of sex work (Figure 1B), and it is known that higher levels of cellular activation correlate with higher cellular susceptibility to HIV infection [124,125]. By increasing the number of potential HIV targets at the FGT, interruption of sex work may render HIV-negative women more susceptible to HIV acquisition. These observations may partly explain the previously observed late seroconversions of resistant women [123].

What contributes to the regulation of the levels of immune activation in the FGT and why sexual activity seems to influence this process are questions that require attention. As mentioned previously, it has been reported that HESNs present a quiescent phenotype at the FGT and periphery and this low level of activation is less suitable for the establishment of HIV infection and may contribute to protection

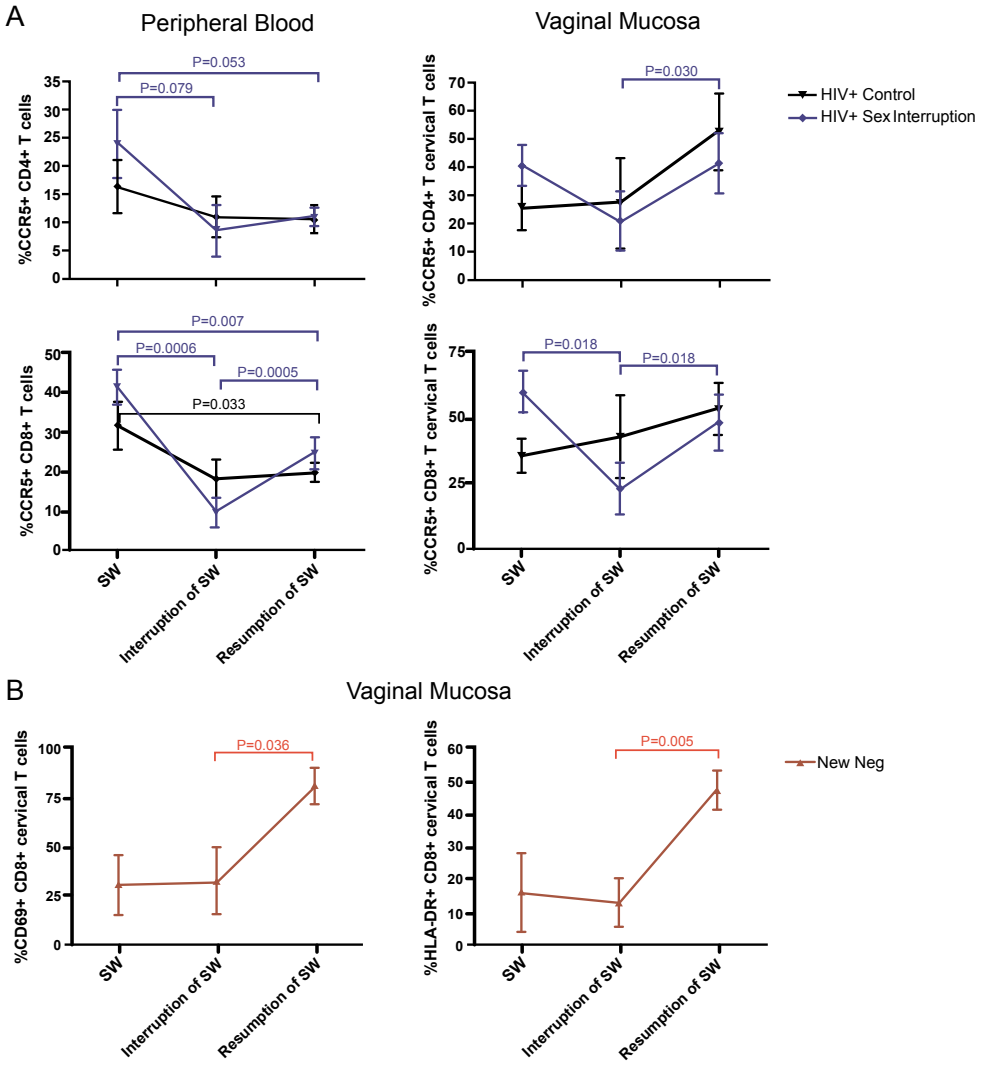

Figure 1: Impact of an interruption in commercial sex work among HIV infected and uninfected CSWs. (A) Proportion of CCR5 expressing peripheral and cervical CD4⁺ and CD8⁺ T cells among HIV-infected CSWs that interrupted their commercial sexual activities for 4 to 8 weeks (HIV+ Sex Interruption, blue line). Proportion of CCR5 expressing cells from women that remained active in sex work (HIV+ control, black line) is also represented. HIV-positive Controls (n=8), HIV-positive SWI (n=10). B) Frequency of CD69 and HLA-DR expression of cervical mononuclear cells in HIV-negative that were newly enrolled in the Pumwani Sex Worker Cohort (New Negative) during commercial sex work (SW), following an interruption in SW and a resumption to commercial SW. New Negative (n=5). Difference between time points was calculated by one-way Repeated Measures ANOVA, if significant, P-values were calculated by Paired t tests.

[105-107,125,126]. The way the mucosal immune system responds to commercial sexual activities and HIV pressure could contribute to this quiescent phenotype. And how HESN CSWs regulate their immune response to that constant immune pressure might be the key to their protection. What could drive this state of quiescence in HESN CSWs during their regular days of work and how does this protection wane when they interrupt commercial sexual activities? These questions need to be explored.

Regulatory T cells and Immunoregulation at the FGT

Pivotal in the regulation of the immune response, Treg subsets, that constitute 5-10% of CD4⁺ T cells [127], may play an important role in shaping immune balance at the FGT and protecting against HIV acquisition. Frequencies of peripheral Treg among heterosexual couples practicing unprotected intercourse negatively correlate with *in vitro* HIV infectivity of CD4⁺ T cells [119]. Emerging population of Treg limits allogenic stimulation and contributes to reduced immune activation and HIV replication [119,128]. Moreover, HESN CSWs harbor higher frequencies of peripheral Treg, which correlates with a lower proportion of CD69⁺ activated T cells and lower cellular infectivity [125,129]. By actively regulating levels of immune activation at periphery but also at mucosal sites, Treg may contribute to the protection of HESNs.

But what drives the emergence of Treg? Sex itself might be a part of the answer. In a mouse model, seminal fluid regulates the accumulation of Treg at the FGT [130]. Semen contains high concentrations of TGF-b [114,115,131] and exposure to seminal plasma converts naïve T cells into functional FOXP3-negative Treg that secrete TGF-b [132]. Constant exposure to antigen also influences the emergence of Treg. Mucosal sites, including the vagina, are responsible for maintaining the delicate balance between induction of tolerance to commensal microbiota and the capacity to mount an effective immune response against pathogens

[133] (reviewed by [134]). In the gut, repeated exposures to low-dose antigen will induce the development of regulatory T cell populations while high doses exposure induce anergy of T cells [135] (reviewed by [136]). In this way, it is reasonable to believe that frequent exposure to stimulants contained in semen (including HIV) might affect T cell responsiveness at the FGT of CSWs. Likewise, T cell receptor signaling pathways and antigen processing and presentation pathways are down-regulated in HESN CSWs compared to age-matched low-risk HIV negative control group [105,126]. Despite quiescent baseline cell activity, these women did not have a defect in their capacity to fight infection because they have the same ability to respond to recall antigen stimulation as controls [105]. Hence, constant exposure to semen could affect the threshold of T cell activation in HESNs, without impairing their capacity to mount an efficient immune response. To date, the frequency of Tregs in the FGT of HESN has poorly been explored.

Observations from a recent study that immunized rhesus macaques with a live-attenuated SHIV lentiviral vector producing continual low doses of virions support the idea that continuous exposure to low dose antigen might alter vaginal immune environment. Six to eight months following rhesus monkeys immunization, immunized monkeys experienced a decrease in immune activation levels, consistent with reduced levels of vaginal indoleamine 2,3-dioxygenase expressing DC, normally triggered by pro-inflammatory signals (reviewed by [137]), and a reduced number of blood pDCs with impaired capacity to produce IFN-a compared to non-immunized monkeys. Three days following SIV challenge, $CD4^+$ $FOXP3^+$ Treg populations expanded in the vaginal tract of immunized monkeys, and this expansion correlated with lower activation at the genital tract. These results suggest that SHIV-immunization had specific effects on innate immunity and immunoregulation that collectively increased the threshold for T cell activation resulting in avoidance or quick resolution of innate inflammatory responses in immunized monkeys after SIV challenge. The modest antiviral effector $CD8^+$ T cell response elicited by SHIV immunization could then participate in the elimination of the few HIV-infected cells at mucosal sites. Thus, a modest CTL response, insufficient to control HIV replication in other immunization models, in the context of mucosal immunoregulatory T cell populations and an anti-inflammatory environment was able to maintain control of SIV replication [138].

Conclusions

After more than 30 years of HIV research, numerous pieces of the puzzle are still missing. In the past few years we have begun to take a fresh look at the different compartments of the immune system and realized that immune dysregulation caused by HIV is even more striking in mucosal compartments. HIV infection alters homeostasis of the mucosal immune system at the GALT but also at the FGT. Conversely, we also came to understand that immune regulation of the mucosal compartments affects susceptibility to HIV acquisition. The fine balance between target cells and effectors cells at the FGT is likely to be an important determinant of HIV resistance. Understanding mechanisms of protection is not an easy task and few longitudinal studies have looked at the variation of immune parameters over time and sexual exposure in HESNs. Protection might rest on the capacity of HESNs to rapidly regulate pro-inflammatory responses triggered by commercial sexual activities or HIV exposure while maintaining innate and adaptive defenses that clear the infection, observations that are missed in the setting of a cross-sectional study. Many environmental or genetic factors may contribute to the emergence of protective cell populations. The maintenance or generation of a regulatory environment as indicated by fewer activated T cells may be an important factor in limiting HIV target cells at the FGT

Over the last few years, it became more obvious that mucosal immune system plays an important function in regulating the entire immune system activation, however, we are still facing an important lack of knowledge. There is an urgent need for future studies in order to have a better understanding of the role of mucosal immune regulation on the risk of HIV acquisition and/or progression to AIDS.

Conflict of Interest

The authors declare no conflicts of interest.

References

1. UNAIDS (2012) UNAIDS Report 2012.
2. Hladik F, McElrath MJ (2008) Setting the stage: host invasion by HIV. Nat Rev Immunol 8: 447-457.
3. Brandtzaeg P, Kiyono H, Pabst R, Russell MW (2008) Terminology: nomenclature of mucosa-associated lymphoid tissue. Mucosal Immunol 1: 31-37.
4. Guadalupe M, Reay E, Sankaran S, Prindiville T, Flamm J, et al. (2003) Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol 77: 11708-11717.
5. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, et al. (2004) CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med 200: 749-759.
6. Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, et al. (2004) Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med 200: 761-770.
7. McKinnon LR, Nyanga B, Chege D, Izulla P, Kimani M, et al. (2011) Characterization of a human cervical CD4+ T cell subset coexpressing multiple markers of HIV susceptibility. J Immunol 187: 6032-6042.
8. Mehandru S, Poles MA, Tenner-Racz K, Manuelli V, Jean-Pierre P, et al. (2007) Mechanisms of gastrointestinal CD4+ T-cell depletion during acute and early human immunodeficiency virus type 1 infection. J Virol 81: 599-612.
9. Hel Z, McGhee JR, Mestecky J (2006) HIV infection: first battle decides the war. Trends Immunol 27: 274-281.
10. Mestecky J, Moldoveanu Z, Smith PD, Hel Z, Alexander RC (2009) Mucosal immunology of the genital and gastrointestinal tracts and HIV-1 infection. J Reprod Immunol 83: 196-200.
11. Paiardini M, Frank I, Pandrea I, Apetrei C, Silvestri G (2008) Mucosal immune dysfunction in AIDS pathogenesis. AIDS Rev 10: 36-46.
12. Iqbal SM, Kaul R (2008) Mucosal innate immunity as a determinant of HIV susceptibility. Am J Reprod Immunol 59: 44-54.
13. Wira C, Fahey J, Wallace P, Yeaman G (2005) Effect of the menstrual cycle on immunological parameters in the human female reproductive tract. J Acquir Immune Defic Syndr 38 Suppl 1: S34-36.
14. Kaushic C, Roth KL, Anipindi V, Xiu F (2011) Increased prevalence of sexually transmitted viral infections in women: the role of female sex hormones in regulating susceptibility and immune responses. J Reprod Immunol 88: 204-209.
15. Heffron R, Donnell D, Rees H, Celum C, Mugo N, et al. (2012) Use of hormonal contraceptives and risk of HIV-1 transmission: a prospective cohort study. Lancet Infect Dis 12: 19-26.
16. Robertson SA (2005) Seminal plasma and male factor signalling in the female reproductive tract. Cell Tissue Res 322: 43-52.
17. Li Q, Estes JD, Schlievert PM, Duan L, Brosnahan AJ, et al. (2009) Glycerol monolaurate prevents mucosal SIV transmission. Nature 458: 1034-1038.
18. Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, et al. (2008) IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat Med 14: 275-281.

19. Haase AT (2011) Early events in sexual transmission of HIV and SIV and opportunities for interventions. Annu Rev Med 62: 127-139.

20. Pudney J, Quayle AJ, Anderson DJ (2005) Immunological microenvironments in the human vagina and cervix: mediators of cellular immunity are concentrated in the cervical transformation zone. Biol Reprod 73: 1253-1263.

21. Borrow P, Bhardwaj N (2008) Innate immune responses in primary HIV-1 infection. Curr Opin HIV AIDS 3: 36-44.

22. Mselle TF, Meadows SK, Eriksson M, Smith JM, Shen L, et al. (2007) Unique characteristics of NK cells throughout the human female reproductive tract. Clin Immunol 124: 69-76.

23. Turville S, Wilkinson J, Cameron P, Dable J, Cunningham AL (2003) The role of dendritic cell C-type lectin receptors in HIV pathogenesis. J Leukoc Biol 74: 710-718.

24. Hirbod T, Kaldensjö T, Lopalco L, Klareskog E, Andersson S, et al. (2009) Abundant and superficial expression of C-type lectin receptors in ectocervix of women at risk of HIV infection. J Acquir Immune Defic Syndr 51: 239-247.

25. Hirbod T, Kaldensjö T, Broliden K (2011) In situ distribution of HIV-binding CCR5 and C-type lectin receptors in the human endocervical mucosa. PLoS One 6: e25551.

26. Kaldensjö T, Petersson P, Tolf A, Morgan G, Broliden K, et al. (2011) Detection of intraepithelial and stromal Langerin and CCR5 positive cells in the human endometrium: potential targets for HIV infection. PLoS One 6: e21344.

27. Lajoie J, Poudrier J, Massinga-Loembe M, Guédou F, Agossa-Gbenafa C, et al. (2008) Differences in immunoregulatory cytokine expression patterns in the systemic and genital tract compartments of HIV-1-infected commercial sex workers in Benin. Mucosal Immunol 1: 309-316.

28. Lajoie J, Poudrier J, Massinga LM, Guedou F, Leblond F, et al. (2010) Chemokine expression patterns in the systemic and genital tract compartments are associated with HIV-1 infection in women from Benin. J Clin Immunol 30: 90-98.

29. Rancez M, Couëdel-Courteille A, Cheynier R (2012) Chemokines at mucosal barriers and their impact on HIV infection. Cytokine Growth Factor Rev 23: 233-243.

30. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, et al. (1995) Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. Science 270: 1811-1815.

31. Kaur G, Tuen M, Virland D, Cohen S, Mehra NK, et al. (2007) Antigen stimulation induces HIV envelope gp120-specific CD4(+) T cells to secrete CCR5 ligands and suppress HIV infection. Virology 369: 214-225.

32. Casazza JP, Brenchley JM, Hill BJ, Ayana R, Ambrozak D, et al. (2009) Autocrine production of beta-chemokines protects CMV-Specific CD4 T cells from HIV infection. PLoS Pathog 5: e1000646.

33. Tomescu C, Abdulhaqq S, Montaner LJ (2011) Evidence for the innate immune response as a correlate of protection in human immunodeficiency virus (HIV)-1 highly exposed seronegative subjects (HESN). Clin Exp Immunol 164: 158-169.

34. Williams SA, Greene WC (2007) Regulation of HIV-1 latency by T-cell activation. Cytokine 39: 63-74.

35. Nazli A, Chan O, Dobson-Belaire WN, Ouellet M, Tremblay MJ, et al. (2010) Exposure to HIV-1 directly impairs mucosal epithelial barrier integrity allowing microbial translocation. PLoS Pathog 6: e1000852.

36. Roberts L, Passmore JA, Mlisana K, Williamson C, Little F, et al. (2012) Genital tract inflammation during early HIV-1 infection predicts higher plasma viral load set point in women. J Infect Dis 205: 194-203.

37. Firoz Mian M, Ashkar AA (2011) Induction of innate immune responses in the female genital tract: friend or foe of HIV-1 infection? Am J Reprod Immunol 65: 344-351.

38. Mlisana K, Naicker N, Werner L, Roberts L, van Loggerenberg F, et al. (2012) Symptomatic vaginal discharge is a poor predictor of sexually transmitted infections and genital tract inflammation in high-risk women in South Africa. J Infect Dis 206: 6-14.

39. Spear GT, Kendrick SR, Chen HY, Thomas TT, Bahk M, et al. (2011) Multiplex immunoassay of lower genital tract mucosal fluid from women attending an urban STD clinic shows broadly increased IL1ß and lactoferrin. PLoS One 6: e19560.

40. Rebbapragada A, Wachihi C, Pettengell C, Sunderji S, Huibner S, et al. (2007) Negative mucosal synergy between Herpes simplex type 2 and HIV in the female genital tract. AIDS 21: 589-598.

41. Hemalatha R, Ramalaxmi BA, KrishnaSwetha G, Kumar PU, Rao DM, et al. (2012) Cervicovaginal inflammatory cytokines and sphingomyelinase in women with and without bacterial vaginosis. Am J Med Sci 344: 35-39.

42. Ahmed SM, Al-Doujaily H, Johnson MA, Kitchen V, Reid WM, et al. (2001) Immunity in the female lower genital tract and the impact of HIV infection. Scand J Immunol 54: 225-238.

43. Wira CR, Fahey JV, Sentman CL, Pioli PA, Shen L (2005) Innate and adaptive immunity in female genital tract: cellular responses and interactions. Immunol Rev 206: 306-335.

44. Reeves RK, Gillis J, Wong FE, Yu Y, Connole M, et al. (2010) CD16- natural killer cells: enrichment in mucosal and secondary lymphoid tissues and altered function during chronic SIV infection. Blood 115: 4439-4446.

45. Agrati C, D'Offizi G, Gougeon ML, Malkovsky M, Sacchi A, et al. (2011) Innate gamma/delta T-cells during HIV infection: Terra relatively Incognita in novel vaccination strategies? AIDS Rev 13: 3-12.

46. Kress E, Hedges JF, Jutila MA (2006) Distinct gene expression in human Vdelta1 and Vdelta2 gammadelta T cells following non-TCR agonist stimulation. Mol Immunol 43: 2002-2011.

47. Nilssen DE, Brandtzaeg P (2012) Intraepithelial Î³Î´ T cells remain increased in the duodenum of AIDS patients despite antiretroviral treatment. PLoS One 7: e29066.

48. Poles MA, Barsoum S, Yu W, Yu J, Sun P, et al. (2003) Human immunodeficiency virus type 1 induces persistent changes in mucosal and blood gammadelta T cells despite suppressive therapy. J Virol 77: 10456-10467.

49. Christmas SE (1991) T-cell receptor gene expression by human gamma delta T-cell clones from peripheral blood and reproductive tissues in relation to non-MHC-restricted cytotoxic function. Scand J Immunol 33: 627-637.

50. Lehner T, Mitchell E, Bergmeier L, Singh M, Spallek R, et al. (2000) The role of gammadelta T cells in generating antiviral factors and beta-chemokines in protection against mucosal simian immunodeficiency virus infection. Eur J Immunol 30: 2245-2256.

51. Kawana K, Matsumoto J, Miura S, Shen L, Kawana Y, et al. (2008) Expression of CD1d and ligand-induced cytokine production are tissue specific in mucosal epithelia of the human lower reproductive tract. Infect Immun 76: 3011-3018.

52. Miura S, Kawana K, Schust DJ, Fujii T, Yokoyama T, et al. (2010) CD1d, a sentinel molecule bridging innate and adaptive immunity, is downregulated by the human papillomavirus (HPV) E5 protein: a possible mechanism for immune evasion by HPV. J Virol 84: 11614-11623.

53. Wang H, Zhao L, Peng Y, Liu J, Qi M, et al. (2012) Protective role of Î±-galactosylceramide-stimulated natural killer T cells in genital tract infection with Chlamydia muridarum. FEMS Immunol Med Microbiol 65: 43-54.

54. Gumbi PP, Jaumdally SZ, Salkinder AL, Burgers WA, Mkhize NN, et al. (2011) CD4 T cell depletion at the cervix during HIV infection is associated with accumulation of terminally differentiated T cells. J Virol 85: 13333-13341.

55. Mkhize NN, Gumbi PP, Liebenberg LJ, Ren Y, Smith P, et al. (2010) Persistence of genital tract T cell responses in HIV-infected women on highly active antiretroviral therapy. J Virol 84: 10765-10772.

56. Gumbi PP, Nkwanyana NN, Bere A, Burgers WA, Gray CM, et al. (2008) Impact of mucosal inflammation on cervical human immunodeficiency virus (HIV-1)-specific CD8 T-cell responses in the female genital tract during chronic HIV infection. J Virol 82: 8529-8536.

57. Campbell GR, Spector SA (2008) CCL2 increases X4-tropic HIV-1 entry into resting CD4+ T cells. J Biol Chem 283: 30745-30753.

58. Bebell LM, Passmore JA, Williamson C, Mlisana K, Iriogbe I, et al. (2008) Relationship between levels of inflammatory cytokines in the genital tract and CD4+ cell counts in women with acute HIV-1 infection. J Infect Dis 198: 710-714.

59. Hein WR (1999) Organization of mucosal lymphoid tissue. Curr Top Microbiol Immunol 236: 1-15.

60. Guadalupe M, Sankaran S, George MD, Reay E, Verhoeven D, et al. (2006) Viral suppression and immune restoration in the gastrointestinal mucosa of

human immunodeficiency virus type 1-infected patients initiating therapy during primary or chronic infection. J Virol 80: 8236-8247.

61. Critchfield JW, Young DH, Hayes TL, Braun JV, Garcia JC, et al. (2008) Magnitude and complexity of rectal mucosa HIV-1-specific CD8+ T-cell responses during chronic infection reflect clinical status. PLoS One 3: e3577.

62. Korn T, Bettelli E, Oukka M, Kuchroo VK (2009) IL-17 and Th17 Cells. Annu Rev Immunol 27: 485-517.

63. Sonnenberg GF, Fouser LA, Artis D (2011) Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. Nat Immunol 12: 383-390.

64. Brenchley JM, Paiardini M, Knox KS, Asher AI, Cervasi B, et al. (2008) Differential Th17 CD4 T-cell depletion in pathogenic and nonpathogenic lentiviral infections. Blood 112: 2826-2835.

65. Kim CJ, Nazli A, Rojas OL, Chege D, Alidina Z, et al. (2012) A role for mucosal IL-22 production and Th22 cells in HIV-associated mucosal immunopathogenesis. Mucosal Immunol 5: 670-680.

66. Stone JD, Heise CC, Miller CJ, Halsted CH, Dandekar S (1994) Development of malabsorption and nutritional complications in simian immunodeficiency virus-infected rhesus macaques. AIDS 8: 1245-1256.

67. Sankaran S, George MD, Reay E, Guadalupe M, Flamm J, et al. (2008) Rapid onset of intestinal epithelial barrier dysfunction in primary human immunodeficiency virus infection is driven by an imbalance between immune response and mucosal repair and regeneration. J Virol 82: 538-545.

68. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, et al. (2006) Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 12: 1365-1371.

69. Azizi A, Ghunaim H, Diaz-Mitoma F, Mestecky J (2010) Mucosal HIV vaccines: a holy grail or a dud? Vaccine 28: 4015-4026.

70. Nilssen DE, Müller F, Oktedalen O, Frøland SS, Fausa O, et al. (1996) Intraepithelial gamma/delta T cells in duodenal mucosa are related to the immune state and survival time in AIDS. J Virol 70: 3545-3550.

71. Altfeld M, Fadda L, Frleta D, Bhardwaj N (2011) DCs and NK cells: critical effectors in the immune response to HIV-1. Nat Rev Immunol 11: 176-186.

72. Sips M, Sciaranghella G, Diefenbach T, Dugast AS, Berger CT, et al. (2012) Altered distribution of mucosal NK cells during HIV infection. Mucosal Immunol 5: 30-40.

73. Silvestri G, Sodora DL, Koup RA, Paiardini M, O'Neil SP, et al. (2003) Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. Immunity 18: 441-452.

74. Chahroudi A, Bosinger SE, Vanderford TH, Paiardini M, Silvestri G (2012) Natural SIV hosts: showing AIDS the door. Science 335: 1188-1193.

75. Apetrei C, Gautam R, Sumpter B, Carter AC, Gaufin T, et al. (2007) Virus subtype-specific features of natural simian immunodeficiency virus SIVsmm infection in sooty mangabeys. J Virol 81: 7913-7923.

76. Gordon SN, Klatt NR, Bosinger SE, Brenchley JM, Milush JM, et al. (2007) Severe depletion of mucosal CD4+ T cells in AIDS-free simian immunodeficiency virus-infected sooty mangabeys. J Immunol 179: 3026-3034.

77. Pandrea IV, Gautam R, Ribeiro RM, Brenchley JM, Butler IF, et al. (2007) Acute loss of intestinal CD4+ T cells is not predictive of simian immunodeficiency virus virulence. J Immunol 179: 3035-3046.

78. Nigam P, Kwa S, Velu V, Amara RR (2011) Loss of IL-17-producing CD8 T cells during late chronic stage of pathogenic simian immunodeficiency virus infection. J Immunol 186: 745-753.

79. Ujhelyi E, Králl G, Zimonyl I, Bánhegyi D, Hollán SR, et al. (1995) Longitudinal immunological follow-up of HIV infected haemophiliacs in Hungary. Acta Microbiol Immunol Hung 42: 189-198.

80. Sankaran S, Guadalupe M, Reay E, George MD, Flamm J, et al. (2005) Gut mucosal T cell responses and gene expression correlate with protection against disease in long-term HIV-1-infected nonprogressors. Proc Natl Acad Sci U S A 102: 9860-9865.

81. Hatano H, Delwart EL, Norris PJ, Lee TH, Dunn-Williams J, et al. (2009) Evidence for persistent low-level viremia in individuals who control human immunodeficiency virus in the absence of antiretroviral therapy. J Virol 83: 329-335.

82. Hunt PW, Brenchley J, Sinclair E, McCune JM, Roland M, et al. (2008) Relationship between T cell activation and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. J Infect Dis 197: 126-133.

83. Kaul R, Ball TB, Hirbod T (2011) Defining the genital immune correlates of protection against HIV acquisition: co-infections and other potential confounders. Sex Transm Infect 87: 125-130.

84. Piacentini L, Biasin M, Fenizia C, Clerici M (2009) Genetic correlates of protection against HIV infection: the ally within. J Intern Med 265: 110-124.

85. Novembre J, Galvani AP, Slatkin M (2005) The geographic spread of the CCR5 Delta32 HIV-resistance allele. PLoS Biol 3: e339.

86. Kaul R, Plummer FA, Kimani J, Dong T, Kiama P, et al. (2000) HIV-1-specific mucosal CD8+ lymphocyte responses in the cervix of HIV-1-resistant prostitutes in Nairobi. J Immunol 164: 1602-1611.

87. Ji H, Ball TB, Kimani J, Plummer FA (2004) Novel interferon regulatory factor-1 polymorphisms in a Kenyan population revealed by complete gene sequencing. J Hum Genet 49: 528-535.

88. Ball TB, Ji H, Kimani J, McLaren P, Marlin C, et al. (2007) Polymorphisms in IRF-1 associated with resistance to HIV-1 infection in highly exposed uninfected Kenyan sex workers. AIDS 21: 1091-1101.

89. Ji H, Ball TB, Ao Z, Kimani J, Yao X, et al. (2010) Reduced HIV-1 long terminal repeat transcription in subjects with protective interferon regulatory factor-1 genotype: a potential mechanism mediating resistance to infection by HIV-1. Scand J Infect Dis 42: 389-394.

90. Boulet S, Kleyman M, Kim JY, Kamya P, Sharafi S, et al. (2008) A combined genotype of KIR3DL1 high expressing alleles and HLA-B*57 is associated with a reduced risk of HIV infection. AIDS 22: 1487-1491.

91. Boulet S, Sharafi S, Simic N, Bruneau J, Routy JP, et al. (2008) Increased proportion of KIR3DS1 homozygotes in HIV-exposed uninfected individuals. AIDS 22: 595-599.

92. Burgener A, Boutilier J, Wachihi C, Kimani J, Carpenter M, et al. (2008) Identification of differentially expressed proteins in the cervical mucosa of HIV-1-resistant sex workers. J Proteome Res 7: 4446-4454.

93. Mestecky J (2006) Humoral immune responses to the human immunodeficiency virus type-1 (HIV-1) in the genital tract compared to other mucosal sites. J Reprod Immunol 72: 1-17.

94. Devito C, Broliden K, Kaul R, Svensson L, Johansen K, et al. (2000) Mucosal and plasma IgA from HIV-1-exposed uninfected individuals inhibit HIV-1 transcytosis across human epithelial cells. J Immunol 165: 5170-5176.

95. Choi RY, Levinson P, Guthrie BL, Lohman-Payne B, Bosire R, et al. (2012) Cervicovaginal HIV-1-neutralizing immunoglobulin A detected among HIV-1-exposed seronegative female partners in HIV-1-discordant couples. AIDS 26: 2155-2163.

96. Kaul R, Trabattoni D, Bwayo JJ, Arienti D, Zagliani A, et al. (1999) HIV-1-specific mucosal IgA in a cohort of HIV-1-resistant Kenyan sex workers. AIDS 13: 23-29.

97. Mestecky J, Wright PF, Lopalco L, Staats HF, Kozlowski PA, et al. (2011) Scarcity or absence of humoral immune responses in the plasma and cervicovaginal lavage fluids of heavily HIV-1-exposed but persistently seronegative women. AIDS Res Hum Retroviruses 27: 469-486.

98. Fiore JR, Laddago V, Lepera A, La Grasta L, Di Stefano M, et al. (2000) Limited secretory-IgA response in cervicovaginal secretions from HIV-1 infected, but not high risk seronegative women: lack of correlation to genital viral shedding. New Microbiol 23: 85-92.

99. Dorrell L, Hessell AJ, Wang M, Whittle H, Sabally S, et al. (2000) Absence of specific mucosal antibody responses in HIV-exposed uninfected sex workers from the Gambia. AIDS 14: 1117-1122.

100. Horton RE, Ball TB, Wachichi C, Jaoko W, Rutherford WJ, et al. (2009) Cervical HIV-specific IgA in a population of commercial sex workers correlates with repeated exposure but not resistance to HIV. AIDS Res Hum Retroviruses 25: 83-92.

101. Ghadially H, Keynan Y, Kimani J, Kimani M, Ball TB, et al. (2012) Altered dendritic cell-natural killer interaction in Kenyan sex workers resistant to HIV-1 infection. AIDS 26: 429-436.

102. Tomescu C, Duh FM, Lanier MA, Kapalko A, Mounzer KC, et al. (2010)

Increased plasmacytoid dendritic cell maturation and natural killer cell activation in HIV-1 exposed, uninfected intravenous drug users. AIDS 24: 2151-2160.

103. Scott-Algara D, Truong LX, Versmisse P, David A, Luong TT, et al. (2003) Cutting edge: increased NK cell activity in HIV-1-exposed but uninfected Vietnamese intravascular drug users. J Immunol 171: 5663-5667.

104. Montoya CJ, Velilla PA, Chougnet C, Landay AL, Rugeles MT (2006) Increased IFN-gamma production by NK and CD3+/CD56+ cells in sexually HIV-1-exposed but uninfected individuals. Clin Immunol 120: 138-146.

105. McLaren PJ, Ball TB, Wachihi C, Jaoko W, Kelvin DJ, et al. (2010) HIVâ€ exposed seronegative commercial sex workers show a quiescent phenotype in the CD4+ T cell compartment and reduced expression of HIVâ€ dependent host factors. J Infect Dis 202 Suppl 3: S339-344.

106. Juno J, Burgener A, Rahman S, Mogk K, Wachihi C, et al. (2012) A distinct cytokine and chemokine profile at the genital mucosa is associated with HIV-1 protection among HIV-exposed seronegative commercial sex workers. Mucosal Immunol 5: 277-287.

107. Chege D, Chai Y, Huibner S, Kain T, Wachihi C, et al. (2012) Blunted IL17/IL22 and pro-inflammatory cytokine responses in the genital tract and blood of HIV-exposed, seronegative female sex workers in Kenya. PLoS One 7: e43670.

108. Berlier W, Cremel M, Hamzeh H, Lévy R, Lucht F, et al. (2006) Seminal plasma promotes the attraction of Langerhans cells via the secretion of CCL20 by vaginal epithelial cells: involvement in the sexual transmission of HIV. Hum Reprod 21: 1135-1142.

109. Sharkey DJ, Macpherson AM, Tremellen KP, Robertson SA (2007) Seminal plasma differentially regulates inflammatory cytokine gene expression in human cervical and vaginal epithelial cells. Mol Hum Reprod 13: 491-501.

110. Sharkey DJ, Macpherson AM, Tremellen KP, Mottershead DG, Gilchrist RB, et al. (2012) TGF-Î² mediates proinflammatory seminal fluid signaling in human cervical epithelial cells. J Immunol 189: 1024-1035.

111. Denison FC, Grant VE, Calder AA, Kelly RW (1999) Seminal plasma components stimulate interleukin-8 and interleukin-10 release. Mol Hum Reprod 5: 220-226.

112. Doncel GF, Joseph T, Thurman AR (2011) Role of semen in HIV-1 transmission: inhibitor or facilitator? Am J Reprod Immunol 65: 292-301.

113. Robertson SA, Sharkey DJ (2001) The role of semen in induction of maternal immune tolerance to pregnancy. Semin Immunol 13: 243-254.

114. Robertson SA, Ingman WV, O'Leary S, Sharkey DJ, Tremellen KP (2002) Transforming growth factor beta--a mediator of immune deviation in seminal plasma. J Reprod Immunol 57: 109-128.

115. Kafka JK, Sheth PM, Nazli A, Osborne BJ, Kovacs C, et al. (2012) Endometrial epithelial cell response to semen from HIV-infected men during different stages of infection is distinct and can drive HIV-1-long terminal repeat. AIDS 26: 27-36.

116. Gutsche S, von Wolff M, Strowitzki T, Thaler CJ (2003) Seminal plasma induces mRNA expression of IL-1beta, IL-6 and LIF in endometrial epithelial cells in vitro. Mol Hum Reprod 9: 785-791.

117. Robertson SA, Guerin LR, Moldenhauer LM, Hayball JD (2009) Activating T regulatory cells for tolerance in early pregnancy - the contribution of seminal fluid. J Reprod Immunol 83: 109-116.

118. Peters B, Whittall T, Babaahmady K, Gray K, Vaughan R, et al. (2004) Effect of heterosexual intercourse on mucosal alloimmunisation and resistance to HIV-1 infection. Lancet 363: 518-524.

119. Kingsley C, Peters B, Babaahmady K, Pomeroy L, Rahman D, et al. (2009) Heterosexual and homosexual partners practising unprotected sex may develop allogeneic immunity and to a lesser extent tolerance. PLoS One 4: e7938.

120. Jennes W, Evertse D, Borget MY, Vuylsteke B, Maurice C, et al. (2006) Suppressed cellular alloimmune responses in HIV-exposed seronegative female sex workers. Clin Exp Immunol 143: 435-444.

121. Jennes W, Sawadogo S, Koblavi-Dème S, Vuylsteke B, Maurice C, et al. (2003) Cellular human immunodeficiency virus (HIV)-protective factors: a comparison of HIV-exposed seronegative female sex workers and female blood donors in Abidjan, Côte d'Ivoire. J Infect Dis 187: 206-214.

122. Fowke KR, Nagelkerke NJ, Kimani J, Simonsen JN, Anzala AO, et al. (1996) Resistance to HIV-1 infection among persistently seronegative prostitutes in Nairobi, Kenya. Lancet 348: 1347-1351.

123. Kaul R, Rowland-Jones SL, Kimani J, Dong T, Yang HB, et al. (2001) Late seroconversion in HIV-resistant Nairobi prostitutes despite pre-existing HIV-specific CD8+ responses. J Clin Invest 107: 341-349.

124. Koning FA, Otto SA, Hazenberg MD, Dekker L, Prins M, et al. (2005) Low-level CD4+ T cell activation is associated with low susceptibility to HIV-1 infection. J Immunol 175: 6117-6122.

125. Card CM, Rutherford WJ, Ramdahin S, Yao X, Kimani M, et al. (2012) Reduced Cellular Susceptibility to In Vitro HIV Infection Is Associated with CD4+ T Cell Quiescence. PLoS One 7: e45911.

126. Songok EM, Luo M, Liang B, Mclaren P, Kaefer N, et al. (2012) Microarray analysis of HIV resistant female sex workers reveal a gene expression signature pattern reminiscent of a lowered immune activation state. PLoS One 7: e30048.

127. Shevach EM, DiPaolo RA, Andersson J, Zhao DM, Stephens GL, et al. (2006) The lifestyle of naturally occurring CD4+ CD25+ Foxp3+ regulatory T cells. Immunol Rev 212: 60-73.

128. Moreno-Fernandez ME, Rueda CM, Rusie LK, Chougnet CA (2011) Regulatory T cells control HIV replication in activated T cells through a cAMP-dependent mechanism. Blood 117: 5372-5380.

129. Card CM, McLaren PJ, Wachihi C, Kimani J, Plummer FA, et al. (2009) Decreased immune activation in resistance to HIV-1 infection is associated with an elevated frequency of CD4(+)CD25(+)FOXP3(+) regulatory T cells. J Infect Dis 199: 1318-1322.

130. Guerin LR, Moldenhauer LM, Prins JR, Bromfield JJ, Hayball JD, et al. (2011) Seminal fluid regulates accumulation of FOXP3+ regulatory T cells in the preimplantation mouse uterus through expanding the FOXP3+ cell pool and CCL19-mediated recruitment. Biol Reprod 85: 397-408.

131. Politch JA, Tucker L, Bowman FP, Anderson DJ (2007) Concentrations and significance of cytokines and other immunologic factors in semen of healthy fertile men. Hum Reprod 22: 2928-2935.

132. Balandya E, Wieland-Alter W, Sanders K, Lahey T (2012) Human seminal plasma fosters CD4(+) regulatory T-cell phenotype and transforming growth factor-Î²1 expression. Am J Reprod Immunol 68: 322-330.

133. Black CA, Rohan LC, Cost M, Watkins SC, Draviam R, et al. (2000) Vaginal mucosa serves as an inductive site for tolerance. J Immunol 165: 5077-5083.

134. Belkaid Y (2008) Role of Foxp3-positive regulatory T cells during infection. Eur J Immunol 38: 918-921.

135. Long SA, Rieck M, Tatum M, Bollyky PL, Wu RP, et al. (2011) Low-dose antigen promotes induction of FOXP3 in human CD4+ T cells. J Immunol 187: 3511-3520.

136. Weiner HL, da Cunha AP, Quintana F, Wu H (2011) Oral tolerance. Immunol Rev 241: 241-259.

137. Curti A, Trabanelli S, Salvestrini V, Baccarani M, Lemoli RM (2009) The role of indoleamine 2,3-dioxygenase in the induction of immune tolerance: focus on hematology. Blood 113: 2394-2401.

138. Genescà M, Ma ZM, Wang Y, Assaf B, Qureshi H, et al. (2012) Live-attenuated lentivirus immunization modulates innate immunity and inflammation while protecting rhesus macaques from vaginal simian immunodeficiency virus challenge. J Virol 86: 9188-9200.

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