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Current Opinion in Immunology

HIV and mucosal barrier interactions: consequences for transmission and pathogenesis

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The mucosal barrier plays an integral function in human health as it is the primary defense against pathogens, and provides a critical transition between the external environment and the human internal body. In the context of HIV infection, the most relevant mucosal surfaces include those of the gastrointestinal (GI) and genital tract compartments. Several components help maintain the effectiveness of this mucosal surface, including the physical anatomy of the barrier, cellular immunity, soluble factors, and interactions between the epithelial barrier and the local microenvironment, including mucus and host microbiota. Any defects in barrier integrity or function can rapidly lead to an increase in acquisition risk, or with established infection may result in increased pathogenesis, morbidities, or mortality. Indeed, a key feature to all aspects of HIV infection from transmission to pathogenesis is disruption and/or dysfunction of mucosal barriers. Herein, we will detail the host–pathogen relationship of HIV and mucosal barriers in both of these scenarios.

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Current Opinion in Immunology 2015, **36**:22–30

This review comes from a themed issue on **Host pathogens**

Edited by **Peter A Barry** and **Guido Silvestri**

For a complete overview see the Issue and the Editorial

Available online 4th July 2015

http://dx.doi.org/10.1016/j.coi.2015.06.004

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HIV transmission

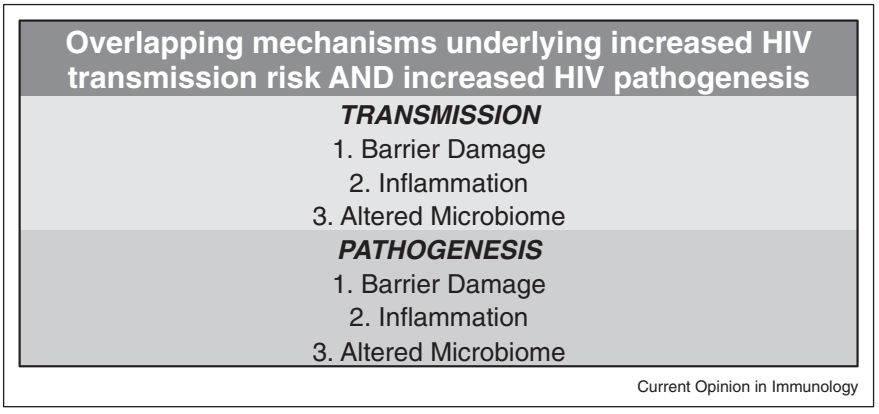
Extensive research has been dedicated to the development of HIV prevention intervention strategies. A significant challenge in developing these interventions is an incomplete understanding of correlates of sexual transmission including the role of mucosal inflammation. What is exceedingly clear is that increased mucosal inflammation enhances the rate of sexual transmission of HIV at mucosal surfaces [1–3]. Given the recent failure of the HVTN505 [4] and STEP [5] vaccine trials, where increased HIV transmission risk was observed in vaccine recipients, understanding the role of these mechanisms is a growing concern for the HIV prevention field. Recent studies have helped increase our understanding of how sexual activity, inflammation, and host microbiota can influence the mucosal barrier and will be the focus of the transmission portion of this review (Figures 1 and 2a).

Physical and biological barriers for HIV at mucosal surfaces

Routes for HIV transmission include the vagina (and other compartments in the female genital tract; FGT), anus, rectum, and penis/foreskin. These surfaces have both anatomical (epithelial barrier and secreted mucus) and biological barriers (immune cells and antimicrobial factors) to resist viral infection. Per coital frequency of HIV transmission is quite low; approximately 0.1% for unprotected receptive vaginal intercourse and 1.4% for unprotected receptive anal intercourse [6], which demonstrates the effectiveness of these barriers against HIV. The FGT is protected from virus penetration by a multilayered squamous and columnar epithelium in the ectocervix/vaginal vault and endocervix, respectively. On the other hand, the rectal compartment (and other GI sites) is only comprised of a single layer columnar epithelium likely contributing to higher transmission rates. Epithelial integrity at all sites is mediated by protein structures acting to adhere cells to one another (i.e. tight and adherens junctions) or to the extracellular matrix. While the FGT is not considered keratinized, it also contains a layer of flattened differentiated epithelial cells called the stratum corneum which provides further physical and biological barriers to microorganisms [7]. On top of all mucosal surfaces is a layer of secreted mucus, which contains hundreds to thousands of biologically relevant soluble proteins, including immune factors and antimicrobial agents [8], mucins [9] and antiproteases [10,11], which provide immune defense and anti-inflammatory protection against epithelial damage. In addition, mucus itself provides a substantial physical barrier against HIV migration and penetration [12,13].

In order for HIV transmission to occur, infectious virions or infected cells from the donor must cross these barriers to find a susceptible cell in the host. HIV can penetrate as much as 10 μm into the squamous epithelium in the FGT

Figure 1



Similar mechanisms for transmission and pathogenesis in HIV infection.

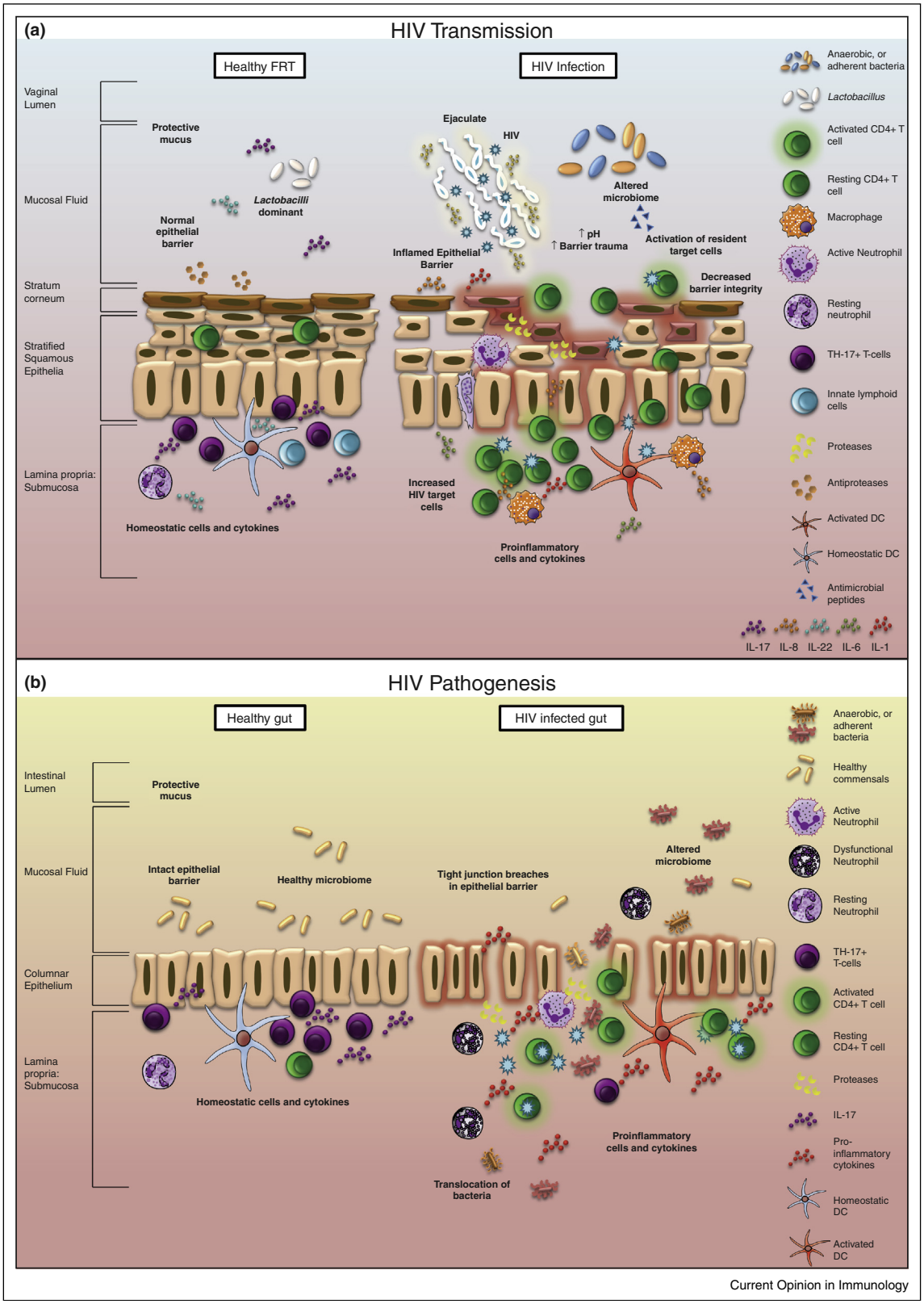
where target cells reside, which is significantly increased upon tight junction disruption, and migration varies between individuals [14•]. HIV preferentially infects CD4+ T-cells co-expressing CCR5 at mucosal sites [1] and particularly those that are HIV-specific [15] or activated [16•]. Not all CD4+ T-cells are equal, however, and T-helper type 17 (Th17) CD4+ T-cells [17•], as well as activated CD4+ T-cells expressing $\alpha_4\beta_7$ or $\alpha_4\beta_1$ [18], are highly susceptible cells in the FGT compartment. However, the exact mechanism by which HIV infects rectal tissue is uncertain. The rectal lamina propria contains abundant CD4+CCR5+ T-cells that express multiple markers of cell activation, thus key targets for HIV [19]. A network of dendritic cells (DCs) resides within the distal rectum that could facilitate transfer of HIV to target T-cells [20,21,22•]. Another intriguing possibility is that HIV triggers DCs to migrate between rectal epithelial cells, capture virus, and transfer it to activated T-cells in the lamina propria [23]. Therefore immunological factors that affect the number of available target cells, activation status, their accessibility, and/or dissemination, could affect the likelihood of infection.

Mucosal inflammation

Inflammatory responses are largely initiated by epithelial cells, through activation of pattern recognition receptors, to secrete soluble defense factors (antimicrobial peptides, AMPs) and cytokines to stimulate a response from immune cells. HIV can turn this process to its advantage. Indeed, an inflammatory cascade at the mucosal level following virus exposure is required for the establishment of productive viral infection [24]. Although it would seem logical that increase secretion of AMPs that can inhibit HIV infectivity *in vitro* would have the same effect *in vivo*, paradoxically this is not the case. In fact, elevated levels of α-defensins at mucosal surfaces are associated with increased risk of HIV infection [25,26], presumably exacerbating risk by recruiting target cells for infection and/or increasing the susceptibility of cells [27,28]. In addition, increased levels of pro-inflammatory cytokines/chemokines are associated with increased rates of HIV acquisition [29,30••]. Recently, Masson *et al.* demonstrated that in the CAPRISA-004 trial, a 3-fold increased risk of HIV infection was observed in women who had elevated levels of at least five mucosal pro-inflammatory cytokines, including MIP-1α, IL-8, MIP-1β, IL-1β, IL-1α, and TNF-α [30••]. Proteomic analysis by Arnold/Burgener *et al.* demonstrated this pro-inflammatory cytokine profile is linked with increased neutrophil protease levels, barrier disruption, and increased frequency of cervicovaginal CD4+ T-cells [31••]. Thus, a model is proposed that mucosal barrier disruption via neutrophil proteases may drive immune cell migration and frequency, and increase virion access to susceptible cells, and thus the risk of HIV acquisition.

Conversely, *reduced* inflammation and immune activation at mucosal surfaces are associated with HIV protection. Much of this insight has been gained from studying HIV exposed seronegative (HESN) individuals. Decreased immune activation [32], characterized by reduced systemic CD4+ T-cell gene expression [33] coupled with lowered mucosal cytokine/chemokine expression (MIG, IP10, and IL1α) [34] may collectively limit target cell availability and activation and hence reduced risk of infection. Alterations in the mucosal proteome, such as elevated levels of protective serpins, elafin and other antiproteases [35–37], are also associated with protection. Importantly, these factors are essential for *reducing* and *controlling* inflammatory responses [38,39,10,40], particularly due to the direct inhibition of proteases, which are important for immune cell migration, activation, and

Figure 2



Proposed mechanisms of barrier breakdown and inflammation in **(a)** HIV transmission and **(b)** HIV pathogenesis.

tissue barrier breakdown [41]. Indeed, the absence of specific serpins can lead to increased levels of activated Th17+ cells at mucosal surfaces [42], which can be targets of initial infection for HIV [43••]. Furthermore, these antiproteases and their cleavage products have antiviral activity [10,44–46] but are not inherently pro-inflammatory. Therefore it is clear that soluble factors within mucosa are important players in affecting inflammation status of mucosal surfaces and the likelihood of HIV transmission.

Sexual activity and sexually transmitted infections (STIs)

Sexual activity results in increased inflammation and thus may allow an opportunity for HIV transmission [47]. The act of coitus itself can result in microabrasions in the mucosal surface, resulting in wound healing processes and increased vascularity, infiltration and recruitment of immune cells, and increased inflammatory cytokines and proteins [48–50]. In particular, wound healing is associated with neutrophil recruitment, which may increase susceptibility to HIV infection via protease production (described above) [51,52]. Semen itself is highly basic and increases vaginal pH, which may in turn alter the protective mucus layer in the FGT [47,53]. Semen exposure increases pro-inflammatory cytokines in the FGT (such as IL-6, IL-8 and IL-1β) compared to protected coitus or abstinence [54], and is associated with increased CD4+ T-cells and macrophages shortly after coitus [54,55]. Interestingly, progressive sexual exposure, as observed in sex workers from Kenya, results in decreased mucosal immune activation over time [56], suggesting that frequent sexual activity may induce tolerance and precede a reduced immune activation phenotype. Endogenous sex hormones during luteal phase of menses and injectable contraceptives have also been associated with increased HIV infection risk [4,57–63], where progesterone or progestin-based contraceptives may affect HIV target cell levels or host innate immune factors. Recent studies also implicate epithelial tissue remodeling, immune cell movement, and protease levels as potential underlying drivers for this observation [64].

Concurrent STIs also have a strong epidemiological link with increased risk of HIV acquisition. Two of the best-described STIs that increase HIV risk are Herpes simplex virus 2 (HSV-2) and human papilloma virus (HPV); each has been associated with a 2–3 fold increased rate of HIV acquisition in large meta-analyses [65,66]. While the mechanism by which this occurs remains unclear, one hypothesis is that innate host pro/anti-inflammatory mediators modulate HIV susceptibility [67]. Indeed, a higher pro-inflammatory profile characterized by increased numbers of HIV-1 target cells (both CCR5+CD4+ T-cells and DC-SIGN+ DCs) upon HSV-2 infection can persist in mucosal tissues [68,69]. HSV-2 also associates with the amplification of target cells expressing homing markers (α4β7) [70] in the absence of viral shedding and increased activated CD4+CCR5+ T cells (and subsequently higher HIV infectivity) in FGT mucosal explant tissue [71]. Less is known about HPV, although Th1 responses have been linked with HPV clearance [72]. In the rectal compartment, co-infections can be a cause of proctitis in men who have sex with men (MSM) [73,74], and associated with epithelial disruption, inflammation and mucosal ulceration; thus not surprisingly increased risk of HIV acquisition [65,75•]. HPV infection is also prevalent in MSM and associated with an increased risk of HIV acquisition although the mechanism underlying this process is unclear [76]. Thus, STI's may increase HIV target cells and homing to mucosa, and potentially epithelial disruption, but further work is needed to understand these mechanisms given high the prevalence of these STIs and increased risk for HIV.

Microbiota

Changes in vaginal microbial communities are consistently associated with increased HIV risk. This is exemplified by a striking increase in HIV susceptibility with bacterial vaginosis (BV) by as much as 60% [77–79]. BV occurs when protective microbiota in the vagina, dominated by lactic acid-producing *Lactobacillus* species which are associated with protection from HIV transmission [80], are replaced by diverse strains of bacteria such as *Gardnerella*, *Atopobium*, *Prevotella*, *Fusobacterium* spp., and other BV-associated bacteria (BVAB) [79,81]. BV is associated with increased pro-inflammatory cytokine levels, particularly IL-1β [82,83]. Some BVAB are associated with cervicitis in humans [84] which may induce recruitment of T-cells, and treatment of BV with metronidazole led to decreases in mucosal CCR5+CD4+ T-cells [85]. Studies of the mucosal proteome during BV [86] demonstrated lower levels of factors important for an effective physical barrier (small proline-rich proteins and involucrin) [7,87,88]. A recent study by Anahtar *et al.* demonstrated that high ecological diversity of FGT microflora drives enhanced stimulation of TLR4 and NFkB, leading to increased pro-inflammatory cytokines and a subsequent increase in activated CCR5+CD4+ T-cells [89••]. Thus, it is possible that microflora diversity may be an underlying component and/or driver of host inflammation responses and HIV acquisition.

Less is known about the role of the rectal microbiome and HIV transmission. It has been hypothesized that the diversity of sexual repertoire and the number of sexual partners found in many MSM may create a novel ecosystem that facilitates the generation and transmission of antibiotic resistant *Neisseria gonorrhea* infection. It remains to be seen whether this ecosystem might also facilitate HIV transmission. Although dysbiosis of gut microbiota increases with progressive HIV infection (discussed below) [90,91•], it is not clear whether the gut microbiota of HIV-negative MSM differs from that seen in HIV-negative heterosexual men. Given that the interactions of the

microbiota with the host may lead to significant changes in the mucosal barrier altered HIV susceptibility, understanding these mechanisms may uncover important microbial targets for biomedical intervention.

Taken together, further studies of the mechanism of how these inflammatory/anti-inflammatory mediators lead to HIV infection risk at mucosal surfaces may shed light on susceptibility mechanisms and targeted strategies for prevention technology development.

Pathogenesis

Once HIV infection occurs, the mucosal landscape is dramatically and rapidly altered. While the underlying mechanisms of this are unclear, what is evident is that destruction to the mucosal tissues is substantial and not completely reversible with ART. Here we will review recent evidence that demonstrate how multiple factors impact barrier integrity during HIV infection and contribute to host pathogenesis to HIV (Figure 2b).

Barrier

While early studies reported damage to the GI tract and alterations in mucosal immunity after HIV infection, the first indication that barrier function was involved in pathogenesis came from influential work from Brenchley *et al.* demonstrating that microbial products translocate during HIV infection [92,93]. The mechanism for microbial translocation was subsequently demonstrated to be focal breaches that occur in the GI tract during HIV infection, allowing microbial products to translocate from the lumen [94,95], thus further driving inflammation. More recently, the consequences of barrier damage in HIV infection in mortality has been further highlighted. Namely, Hunt *et al.* demonstrated that soluble factors in periphery associated with barrier damage, including the tight junction protein zonulin, and the epithelial death biomarker intestinal fatty acid binding protein (I-FABP) predict mortality in HIV infected individuals [96••]. Furthermore, studies in the SIV model have demonstrated that *preexisting* barrier damage before SIV infection predicts disease progression to AIDS after infection [97•], demonstrating the importance of barrier health even before infection occurs.

Homeostatic immunity

Studies of early infection during HIV have been limited due to difficulty in both early diagnoses of HIV as well as obtaining samples during these early time points. A major contribution to our understanding of acute HIV infection has been by Schuetz *et al.*, where mucosal samples were collected in acute HIV infection, and patients were initiated on ART extremely early [98••]. These studies demonstrated that several key immunological components are altered early in HIV infection including rapid loss of Th17 cells in Feibig III, which is not reversible unless ART is initiated early in Fiebig I/II [98••]. Additionally, Th17 cells are rapidly lost in the FGT after HIV infection, demonstrating that multiple mucosal sites are targets [43••]. Th17 cells are of particular interest in the context of mucosal immunology given that these cells are critical in mucosal homeostasis, and loss of Th17 cells in chronic HIV infection has been associated with damage to the tight epithelial barrier and ensuing microbial translocation [99–101]. While it is promising that early ART treatment can prevent Th17 loss, it should be noted that the reality of being able to treat as early as Fiebig I/II, or essentially two weeks after infection, would be nearly impossible. Thus, further understanding of the early kinetics of Th17 loss, why they are an early target, and their relationship to overall CD4+ T-cell depletion and pathogenesis, will be important for treatment strategies.

Immune activation

HIV-associated inflammation is one of the most unequivocally clear consequences of HIV infection and is highly associated with disease progression, morbidities and mortality, independent of antiretroviral therapy (ART) [102,103]. HIV itself can directly induce inflammation via stimulation of immune cells and induction of both innate and adaptive arms of the immune system [103]. However, it is likely that the majority of the inflammation in HIV infection is via indirect or 'bystander' mechanisms, due to factors such as microbial translocation, CMV reactivation, and other mechanisms, which can both cause and be an effect of CD4+ T-cell depletion [102]. Recently, a novel mechanism for inflammation and CD4+ T-cell depletion was described, whereby Doitsch *et al.* demonstrated that caspase-1 driven pyroptosis results in the spilling of cytosolic contents, containing highly inflammatory cytokines, namely IL-1β [104••]. The major consequence of this chronic inflammation is increased morbidities and mortality, highlighting the need to more precisely delineate factors underlying inflammation in HIV-infected individuals.

Microbiome

Several recent studies have highlighted that dysbiosis of the microbiome during HIV infection is associated with mucosal dysfunction. Vujkovic-Cvijin *et al.* recently demonstrated that during HIV infection, adherent bacteria such as Proteobacteria are enriched in the intestinal mucosa, together with depletion of Bacteroidia bacteria, which is associated with disease progression [91•]. The proposed mechanism by which this dysbiosis drives disease is by the ability for these bacteria to stimulate kynurenine pathways of tryptophan catabolism [91•], which is known to depress Th17 cells and associated with disease progression in HIV [96••,105,106,107••]. Dysbiosis can also directly alter immune cells in the GI tract; Dillon *et al.* demonstrated that increased Prevotella and Proteobacteria, together with decreased Firmicutes and Bacteroidetes in HIV infection is associated with increased T-cell and myeloid DC (mDC)

activation [108••]. In addition, mDCs can be directly stimulated by HIV-altered mucosal bacteria (HAMBs), which are associated with T-cell activation [109•]. Finally, Klase *et al.* demonstrated that in SIV-infected macaques, Proteobacteria were not only increased, but more metabolically active, and were identified as the major bacteria group that translocate during lentiviral infection [110••]. Taken together, it is clear that dysbiosis of the microbiome occurs during HIV infection, and is associated with microbial translocation and inflammation. However, given that this dysbiosis is associated with increased adherent Proteobacteria species, it can be hypothesized that these HAMBs could directly induce damage to the mucosal barrier. However, the precise mechanism of this damage is unknown and should be further studied.

Barrier function and soluble factors

While transmission studies clearly demonstrate that soluble factors including proteases and extracellular matrix proteins are associated with inflammation and increased HIV acquisition [31••], factors such as these have been largely understudied in terms of pathogenesis and will be essential to investigate. Transcriptional analysis of gut tissues in the SIV model has demonstrated that SIV infection is associated with decreased genes encoding for cell adhesion [111•] as well as a decrease in genes regulating focal adhesions, gap junctions and Wnt signaling in intestinal epithelium [112]. In addition, neutrophils have been shown to accumulate at high levels in the GI tissue of HIV infected individuals [107••]. Neutrophils secrete several inflammatory soluble factors that could contribute to inflammation and barrier disruption [31••], and could be a major contributor to GI dysfunction in the context of HIV. These data indicate that alterations in protein expression would therefore probably be altered and may contribute to a damaged mucosal barrier.

Conclusions

While the phenomena here are well described in both contributing to HIV transmission and pathogenesis, the specific mechanisms underlying these dysfunctions of the barrier are still unclear. To date, while there are many studies which have provided crucial information in regards to characterizing mucosal defects and HIV transmission or pathogenesis, we still lack specific mechanisms and thus targets to decrease HIV-associated disease. Overall, many issues are important for host barrier interactions in the context of HIV but remain unresolved. What is clear is that barrier function is a key parameter in both acquisition and pathogenesis of HIV infection, and determining specifically what induces inflammation, barrier breakdown, and altered microbiome will be critical in developing more effective prevention and treatment strategies for HIV infection.

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