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Mucosal Immunity to HIV: A Review of Recent Literature

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Abstract

Purpose of review—This review summarizes recent literature in the field of mucosal immunology as it applies to human immunodeficiency virus (HIV) transmission and pathogenesis.

Recent findings—Pertinent recent findings include elucidation of the role of mucosal antigen-presenting cells and retinoic acid in imprinting a gut-homing phenotype on antigen-specific T and B cells, and the identification of Th17 and Treg cells as key modulators of the balance between tolerance and inflammation in mucosal tissues.

Summary—Mucosal surfaces of the body serve as the major portal of entry for human immunodeficiency virus (HIV). These tissues also house a majority of the body's lymphocytes, including the CD4+ T-cells that are the major cellular target for HIV infection. Elucidating mucosal immune responses is critical to our understanding of the host-pathogen relationship for two reasons: first, mucosal barriers are defended by a range of innate and adaptive defenses that might be exploited to develop effective vaccines and/or microbicides; second, adaptive immune responses in mucosal lymphoid tissues may serve to limit viral replication, decreasing the host's viral burden as well as reducing the likelihood of sexual transmission to a naïve host.

Keywords

CTL; gut; IgA; Treg; Th17

Introduction

Mucosal tissues are generally accepted as the largest collection of lymphoid tissues in the body, and the majority of the body's T- and B-lymphocytes are housed in the gastrointestinal mucosa, an assessment based upon enumeration of Peyer's patches and lymphoid follicles in human intestine [1*,2]. The distribution of mucosal tissues throughout the body has recently been reviewed in detail, along with the standardized nomenclature adopted by the Society for Mucosal Immunology [1*].

The genetic and immunologic correlates of protection from human immunodeficiency virus (HIV) infection have still not been fully elucidated. Intensive studies are underway to characterize potentially protective immune responses in relevant cohorts, including HIV Controllers, nonhuman primates with non-progressive simian immunodeficiency virus (SIV) infection, and highly exposed, persistently seronegative (HEPS) individuals [3-5]. Given that the majority of HIV transmission occurs via mucosal surfaces, there is great interest in understanding how the complex interplay of innate and adaptive mucosal defenses may shape

the outcome of HIV infection. This review will highlight recent developments in the field of mucosal immunology within the context of HIV/SIV infection.

Recent advances in the field of mucosal immunology

Much remains to be learned about the induction and trafficking patterns of mucosal lymphocytes in humans. Recent advances in murine models have led to a new appreciation for the role of mucosal antigen-presenting cells in imprinting a gut-homing phenotype on antigen-specific lymphocytes. A series of studies demonstrated that rodents deficient in vitamin A have reduced IgA-producing antibody secreting cells (ASC) and effector/memory T cells in intestinal mucosa [6,7]. Retinoic acid (RA), a metabolite of vitamin A, induces expression of integrin $\alpha 4\beta 7$ and chemokine receptor CCR9, which are implicated in lymphocyte homing to the intestinal mucosa [7]. Intestinal dendritic cells (DC) express enzymes critical for RA biosynthesis [7], and deliver the instructive signal triggering expression of $\alpha 4\beta 7$ and CCR9 [8-11] (reviewed in [12]). Intriguingly, the activated $\alpha 4\beta 7$ heterodimer, expressed on gut-homing lymphocytes as they transit through blood, can bind HIV gp120 on infected CD4⁺ T-cells. The gp120- $\alpha 4\beta 7$ interaction, in turn, triggers activation of $\alpha L\beta 2$ integrin (LFA-1) on the CD4⁺ T-cell surface. LFA-1 is critical for cell-cell interactions and 'immunological synapse' formation; accordingly, its upregulation on infected CD4 cells may facilitate cell-to-cell spread of HIV [13**].

Second, renewed attention has been devoted to oral tolerance, and to the balance between tolerogenic and pro-inflammatory T-cell responses in the intestinal mucosa. In the mouse intestine, RA and TGF- $\beta 1$ induce differentiation of regulatory CD4⁺ T cells expressing the forkhead box transcription factor FoxP3 [14,15**]. However, in a proinflammatory cytokine environment in which both TGF- $\beta 1$ and IL-6 are present, the "balance" is tipped in favor of Th17-type responses [15**,16]. Th17 cells are CD4⁺ T cells whose induction depends on the orphan nuclear receptor ROR γ t [17*]. These cells express IL-17 and IL-22, and are critical in clearing fungal and extracellular bacterial infections [18**], but can also mediate inflammation and autoimmunity [19]. These findings relate to rodent models for tolerance and inflammation, so their applicability to HIV/SIV infection remains to be determined. Nevertheless, mucosal Treg and Th17 cells have been identified in HIV-infected humans and SIV-infected macaques, as discussed below.

Immune regulation and mucosal tissues: The Th17/Treg balance

Recent reports indicate that Th17 cells are important targets for acute HIV/SIV infection in the GI tract. These cells may be preferentially lost in HIV/SIV-infected hosts with progressive disease [20*-22*], but not in non-progressing hosts such as sooty mangabeys [21*] or African green monkeys [20*]. In addition, SIV-infected rhesus macaques coinfecting with *S. typhimurium* showed increased bacterial dissemination relative to SIV-uninfected controls, coincident with a loss of mucosal Th17 cells [23**]. This finding mirrored the observation that IL-17 receptor deficient mice showed increased systemic dissemination of *S. typhimurium* from the gut, suggesting that IL-17 deficiency causes defects in mucosal barrier function [23**]. Taken together, these observations suggest a model to explain the mucosal translocation of bacterial products in individuals with HIV/SIV infection [24,25]. Systemic immune activation has been identified as a key feature differentiating pathogenic versus non-pathogenic outcomes of HIV/SIV infection [3].

The role of Treg in HIV/SIV pathogenesis remains less clear; these cells may reduce immune activation, but may also limit HIV-specific adaptive responses [26*]. Treg have been phenotypically identified in blood and mucosal tissues of HIV-infected humans [27,28] and SIV-infected macaques [29,30]. In acute SIV infection, Treg are reportedly expanded in lymph node paracortical regions [29], but depleted from ileal mucosa [31]. In chronic SIV infection,

FoxP3 and CTLA-4 mRNA were increased in mucosal tissues of macaques with high viremia, and FoxP3 mRNA correlated with SIV RNA levels in tissues [32*]. Indirect markers for Treg function, including indoleamine 2,3 dioxygenase, were also associated with high viral load. It remains unclear whether the presence of Treg in mucosal tissues simply reflects a consequence of inflammation or whether these cells promote virus replication and dissemination by limiting HIV/SIV-specific adaptive responses.

Acute HIV/SIV infection and the gastrointestinal tract

There is now an extensive literature documenting the rapid depletion of gut lamina propria CD4⁺ T-cells during acute HIV/SIV infection [33,34]. This depletion may be mediated by direct infection of target cells [35], bystander apoptosis [36], or a combination of mechanisms. Studies of rhesus macaques vaginally exposed to SIVmac revealed that the SIV-specific CD8⁺ T-cell response in gut occurred “too little and too late” to prevent depletion of lamina propria CD4⁺ T-cells and systemic viral dissemination [37,38].

Innate immune responses at mucosal surfaces could theoretically provide an important means of limiting HIV replication and dissemination. However, there are few reports describing the role of innate mucosal effector cells in HIV disease. Mucosal NK and NK-T cells have not been extensively studied; one report showed depletion of colonic NK cells in HIV-infected subjects [39]. Similarly, few studies have focused on intestinal $\gamma\delta$ T cells in HIV infection [40]. However, one paper reported an expansion of $\gamma\delta$ T cells in immunized macaques protected from rectal challenge with SIVmac251 [41]. It was recently demonstrated that sooty mangabeys have a higher frequency of $\gamma\delta$ T cells in blood than humans or rhesus macaques [42*]. Although their role in immunosurveillance remains poorly understood, mucosal $\gamma\delta$ T cells may limit opportunistic infections and/or maintain intestinal epithelial integrity [40, 42*].

Mucosal cell-mediated immunity during chronic infection

In HIV/SIV infection, intestinal lamina propria CD4⁺ T-cells are rapidly depleted and there is an expansion and/or influx of CD8⁺ T-cells [43]. The persistence of virus in mucosal tissues throughout chronic infection argues that these tissues remain sites of ongoing interaction between the virus and the immune system. MHC Class I-restricted cytotoxic T cells (CTL) have been identified throughout the gastrointestinal mucosa of individuals with chronic HIV disease [44,45] and SIV-infected rhesus macaques [46,47]. Studies characterizing the TCR hypervariable regions of CTL clones from rectal mucosa, semen and cervix revealed that many such clones were shared between mucosal sites and PBMC [48]. Due to the difficulty of obtaining large numbers of lymphocytes from biopsy tissue, comprehensive mapping of response breadth has been challenging. However, studies to date suggest that CD8⁺ T-cells from rectal mucosa and blood recognize similar HIV peptide pools [49] and epitopes [50].

Intestinal CD8⁺ T-cells exhibit a partially activated, effector memory phenotype [51-53]. Despite abundant expression of granzymes, rectal CD8⁺ T-cells express low levels of perforin as compared to their counterparts in blood [38,54]. Nevertheless, when stimulated *in vitro* with HIV peptides, rectal CD8⁺ T-cells produce cytokines and release CD107-containing granules [55*,56*]. One study reported that HIV-specific CD8⁺ T-cell responses in terminal ileum were weak, in contrast to bronchoalveolar lavage, which contained a high frequency of polyfunctional CD8⁺ T cells; it remains unclear whether this finding reflects the role of terminal ileum as an inductive site [57*]. Nevertheless, additional studies comparing responses at multiple mucosal sites are warranted. Many “elite controllers”, defined as individuals with chronic HIV infection and plasma viremia <50 copies/mL in the absence of antiretroviral therapy [4], have unusually robust and polyfunctional HIV-specific T-cell responses in rectal mucosa [58*]. Taken together, these findings suggest that MHC-restricted T cells in mucosal

tissues serve as a critical component of the host's adaptive immune armamentarium throughout chronic infection.

Mucosal antibodies and HIV transmission

Can mucosal antibodies protect against HIV/SIV transmission? In two landmark studies, systemically administered combinations of neutralizing antibodies protected rhesus macaques from mucosal challenge with pathogenic simian/human immunodeficiency virus (SHIV) [59, 60]. In one case, the challenge was administered orally to neonates [59]; in the other, the challenge was administered vaginally to adult female macaques [60]. Thus, high levels of systemic IgG antibodies can protect some mucosal surfaces from pathogenic challenge. This finding implies that IgG from the peripheral circulation can access mucosal secretions [61].

While the protection in these studies was attributed mainly to neutralization, HIV-specific IgG antibodies may also mediate antibody-directed cell-mediated cytotoxicity (ADCC) through interaction with NK cell Fc receptors. In one study, women with ADCC in cervicovaginal fluids had lower genital HIV viral loads than women with ADCC in serum only [62]. HIV-specific ADCC has also been identified in saliva [63]. Polyclonal or polymeric antibodies specific for HIV envelope may also mediate surface agglutination of viral particles, blocking virus entry via a mechanism distinct from classic neutralization [64*].

The issue of “protective” mucosal antibodies in high-risk cohorts has been controversial [5]. Several groups have reported HIV-specific IgA in plasma or secretions from HEPS individuals [65-71]; these antibodies have been described as neutralizing [72,73] and inhibiting transcytosis across an epithelial monolayer [70,74], although they were unable to block transfer of virus from DC to susceptible target cells [75]. Other studies have failed to detect such antibodies in HEPS cohorts [61,76,77], and a recent study of rhesus macaques repeatedly exposed to low-dose rectal SIV challenge found no evidence for SIV-specific adaptive responses, either humoral or cell-mediated [78*].

Mucosal antibodies in chronic infection

In chronically infected individuals, HIV-specific IgG is readily detected in secretions, yet HIV-specific IgA is detected infrequently and at lower levels than IgG [61,79,80]. Analysis of antibody-secreting cells (ASC) from blood revealed a similar trend: gp160-specific IgG-producing ASC were more abundant than IgA-producing ASC in the same individuals [81]. One detailed study compared the Ig heavy chain gene repertoire of intestinal Ig-secreting cells from HIV-infected and control subjects [82]. Subtle decreases in IgA-producing plasma cells were noted in colon and duodenum of HIV-positive subjects as compared to controls; however, the overall V(H) repertoire of mucosal plasma cells was relatively unperturbed [82]. In another study, the relative proportions of total IgA, G and M-secreting plasma cells were similar in duodenal samples from HIV-positive and healthy individuals [83]. However, the total number of duodenal ASC was greater in HIV-infected subjects; this was also true for IgA and IgG1 secreting cells. The density of lamina propria ASC was highest in patients off therapy or taking nucleoside analogs alone, and was normalized in patients on HAART [83]. These findings are consistent with a recent report describing normalization of B cell populations in blood following HAART [84].

Does HIV infection perturb class switching recombination (CSR)? Such effects could be mediated indirectly by depleting CD4+ “helper” T-cells, or directly through the action of HIV gene products on B cell signaling. One report suggested that HIV Nef penetrates B cells, perturbing CD154 and cytokine signaling and blocking CSR [85]. However, it has also been reported that HIV gp120 can bind B cell mannose C-type lectin receptors, stimulating T-cell

independent class switching [86]. Additional work will be required to demonstrate the relevance of these findings to *in vivo* class switching and mucosal antibody production.

The Female Reproductive Tract

HIV-specific T-cells have been identified in cervical mucosa of women with chronic HIV infection [45,48,87-90], cervicovaginal mucosa of SIV-infected macaques [37,91,92], and cervical cytobrush from HEPS women [93]. In chronically infected individuals, HIV-specific cervical T-cells included MHC class I- and class II-restricted CTL [45]. As demonstrated by redirected lysis and HIV-specific ⁵¹Cr release assays, CTL exist throughout the female reproductive tract (FRT), including endo- and ectocervix, vaginal mucosa, uterine endometrium and fallopian tubes [87,94,95]. However, the immunological properties of the FRT change dramatically in response to hormonal fluctuations through the menstrual cycle [96-101]. Notably, CTL activity is specifically suppressed in uterine endometrium during the post-ovulatory phase, presumably to prevent immune rejection of the conceptus [94]. It has been speculated that this temporary suppression of CTL function might provide a window of opportunity for HIV infection and dissemination [102].

The FRT is also equipped with a variety of physical barriers and innate defenses (for review, see [103,104]). Physical defenses include low vaginal pH, multi-layered vaginal epithelium, local flora and a mucous layer. Secretory leukocyte protease inhibitor (SLPI) [105,106], and lactoferrin, a milk protein, are found in a variety of secretions and exhibit anti-HIV activity *in vitro* [107,108]. The chemokine RANTES [109,110] and several defensin family members [111,112] are also found in the FRT.

Immune defenses in the male reproductive tract, and their potential role in HIV infection and transmission, have been much less studied than those of the FRT. However, HIV/SIV-specific CTL have been identified in semen [113-115]. Renewed attention has focused on the male reproductive tract with the identification of a factor, termed Semen-derived Enhancer of Virus Infection (SEVI), that enhances *in vitro* infectivity of HIV by several orders of magnitude [116**,117].

Microbicides: the balance between mucosal protection and inflammation

A variety of agents have been tested for the ability to block HIV entry at mucosal surfaces [118*,119*]. Microbicides may act by several mechanisms: by directly inactivating virus particles; by serving as a physical barrier; by enhancing or restoring local bacterial flora; by interfering with virus adsorption, fusion or entry; or by blocking virus replication inside the host cell. One of the most daunting challenges in microbicide development is the identification of compounds that perform these functions without altering the structural integrity of the mucosal epithelium, facilitating HIV entry into target cells, or promoting local inflammation in a way that increases, rather than decreases, the likelihood of HIV transmission.

Several promising microbicide candidates have failed to demonstrate efficacy in clinical trials. Nonoxynol-9 (N-9), a nonionic surfactant, increased HIV incidence in one trial and was found to have a variety of cytotoxic and pro-inflammatory effects [120-122]. Clinical trials of cellulose sulphate (UsherCel) were prematurely halted when results suggested a trend towards increased transmission frequency in women receiving the compound [123]. Given these failures, there is now an urgent need to adopt a broader range of preclinical testing methods to facilitate early identification of compounds with cytotoxic or pro-inflammatory effects [124-126].

Preparations with specific anti-HIV properties, including entry inhibitors [127] and neutralizing antibodies [128], have shown promising microbicide activity in nonhuman primate

studies. A variety of antiretroviral compounds and monoclonal antibodies specific for viral or cellular proteins already exist, some of which may hold promise for development as topical microbicides [118*]. In addition, combination preparations incorporating both HIV-specific as well as nonspecific agents, perhaps alongside mucosally delivered vaccines, may be tested in the future.

Conclusions

Given the recent failures of highly publicized vaccine and microbicide trials, there is renewed interest in exploring novel vaccine strategies and further elucidating the role of mucosal immunity in HIV transmission [129**]. Future studies should be directed towards the following questions: (a) What innate and/or adaptive responses constitute “protective” immunity against HIV infection and/or disease progression? (b) How can mucosal responses be harnessed to develop better HIV vaccines and microbicides? (c) How can advances in fundamental mucosal immunology inform the design of HIV vaccines, microbicides and therapeutics? Addressing these questions will require a heightened awareness within the HIV research community of developments in the field of basic mucosal immunology, and increased exchanges of information between the two disciplines.

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