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Mucosal Immune Dysfunction in AIDS Pathogenesis

Mirko Paiardini[1], Ian Frank[2], Ivona Pandrea[3], Cristian Apetrei[3] and Guido Silvestri[1,4]

[1]Departments of Pathology and Laboratory Medicine and [2]Medicine, University of Pennsylvania, Philadelphia, USA; [3]Tulane National Primate Research Center; [4]Yerkes National Primate Research Center, Emory University, Atlanta, USA

Abstract

The mucosal immune system plays a central role in both the transmission of HIV infection and the pathogenesis of AIDS. Most HIV infections are acquired through mucosal transmission, and quantitative and qualitative defects of mucosal immunity are consistently present in all stages of pathogenic HIV and SIV infections. A series of recent studies has emphasized the role of a rapid, dramatic, and largely irreversible depletion of mucosa-associated lymphoid tissue-based memory $CD4^+CCR5^+$ T-cells as a key determinant of disease progression in HIV-infected individuals and SIV-infected macaques. It has also been proposed that, in order to be effective, an AIDS vaccine should prevent the early depletion of these mucosal $CD4^+$ T-cells. However, the observation of depletion of mucosal $CD4^+$ T-cells during the primary phase of nonpathogenic SIV infection of natural SIV hosts, such as sooty mangabeys and African green monkeys, suggests that additional pathogenic factors are involved in the AIDS-associated mucosal immune dysfunction. These factors may include: (i) selective depletion of specific $CD4^+$ T-cell subsets; (ii) dysfunction of other (non-$CD4^+$) immune cells; and (iii) generalized immune activation. Importantly, the mucosal immune dysfunction observed during pathogenic HIV and SIV infection is associated with translocation of microbial products (i.e. lipopolysaccharide) from the intestinal lumen to the systemic circulation where they may be responsible, at least in part, for the chronic immune activation that follows pathogenic HIV and SIV infections. The role of mucosal immunity in AIDS pathogenesis emphasizes the importance of understanding whether and to what extent the HIV-associated depletion of mucosal $CD4^+$ T-cells is reversible after prolonged suppression of virus replication with antiretroviral therapy. Further studies of mucosal immunity during primate lentiviral infections will be needed to better understand, and ultimately prevent and treat, the mechanisms underlying the AIDS-associated mucosal immune dysfunction.

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Corresponding author: Guido Silvestri, gsilvest@mail.med.upenn.edu

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Introduction

The mucosa-associated lymphoid tissue (MALT) represents a critical element in the interaction between primate lentiviruses and the host immune system. Numerous studies have shown that (i) most transmissions of HIV occur via a mucosal route, either vaginal or rectal; (ii) a significant fraction of virus replication occurs at the level of MALT in all stages of diseases; and (iii) progressive dysfunction of the mucosal immunity is a key feature of the HIV/SIV-associated immune deficiency (as reviewed[1]). However, the exact cellular and molecular mechanisms underlying the complex interaction between HIV/SIV and the primate mucosal immune system are still poorly understood. This lack of basic knowledge is, in all likelihood, the main reason why we still do not have an

Correspondence to:
Guido Silvestri
University of Pennsylvania School of Medicine
705 Stellar Chance Laboratories
422 Curie Boulevard
19104 Philadelphia, PA, USA
E-mail: gsilvest@mail.med.upenn.edu

effective AIDS vaccine or antiviral treatments that can eradicate the infection.

A series of recent studies have proposed that the depletion of CD4+ T-cells, which is known to be the immunologic hallmark of progression to AIDS, is more rapid and severe at the level of MALT than in peripheral blood and secondary lymphoid organs (i.e. lymph nodes and spleen) (as reviewed[1]). As such, monitoring the effect of HIV infection at the level of MALT, both in the natural history as well as after treatment with antiretroviral therapy (ART), may be considered as part of the clinical management of HIV-infected individuals. In this article, we will summarize our current understanding of the interaction between primate lentiviruses and the host mucosal immune system, and how this interaction leads to the severe mucosal immune dysfunction associated with progression to AIDS.

The mucosal immune system: structure and function

The mucosal immune system (here also referred to as MALT) plays a major role in protecting the host from environmental antigens and microbial infections. Its largest component is the gastrointestinal immune system (here also referred to as gut-associated lymphoid tissue) that is present throughout the digestive gastrointestinal (GI) tract from the oral cavity to the rectum[2,3]. The digestive and absorbing function of the GI tract is fundamental for the uptake of nutrients and fluids, but also inevitably exposes the intestinal mucosa to an enormous amount of microbes (both commensals and pathogens) that may act as antigens and/or allergens[4,5]. The GI tract is, in fact, by far the largest surface of the body in contact with the external environment, and not surprisingly, many human pathogens enter the body through the GI mucosal surface. On the other hand, the intestinal microbial flora plays an essential role in the digestive function of the GI tract, so that the integrity and functionality of this apparatus requires the maintenance of a delicate equilibrium between the need to preserve the commensal microbes and protection of the mucosal surface from enteroinvasive pathogenic microorganisms[6-11]. Several mechanisms contribute to this equilibrium. First, epithelial cells form a continuous layer that separates the gut flora in the lumen from the intestinal lamina propria. Being firmly joined together by tight junctions, the epithelial cells form a physical barrier against the penetration of pathogens. Second, this physical barrier is reinforced by the presence of a continuous layer of mucus and glycocalyx lining the apical side of intestinal epithelial cells. Third, a broad spectrum of antimicrobial molecules, that represent a very important biological barrier against bacterial pathogens, is produced in the mucosal environment[12-16].

In addition to maintaining efficient physical and biological barrier functions, the intestinal mucosa is actively involved in the generation of innate and adaptive immune responses that take place within the MALT, which represents the most abundant lymphoid structure in the human body, with the gut-associated lymphoid tissue alone containing > 40% of all body lymphocytes[17,18]. Mucosal immune responses are directed against pathogens that invade the gut epithelium and are typically generated in the backdrop of a general immunologic tolerance to the numerous nonpathogenic antigens present in the intestinal lumen. From a functional point of view, the mucosal immune system can be functionally divided into inductive and effector sites. In the inductive sites (i.e. mesenteric lymph nodes, Peyer's patches and isolated lymphoid follicles), the antigens of the mucosal lumen are collected and immune responses are induced. The effector sites include the epithelium and the epithelial lamina propria, where the adaptive immune cells differentiate and exert their immune effector function, either cellular response mediated by T-cells or humoral response mediated by B-cells and plasma cells. Anatomically, the lymphocyte populations can be divided into those present in epithelium (intraepithelial lymphocytes containing predominantly CD8+ T-cells as well as T$\gamma\delta$ cells) and those in the underlying lamina propria (lamina propria lymphocytes containing predominantly CD4+ T helper cells as well as CD8+ T-cells, natural killer cells, and B-cells)[17-19].

The HIV might be considered a mucosal pathogen, since its natural transmission occurs mainly through a mucosal surface. Experimental mucosal transmission of SIV in nonhuman primates has been shown to occur in the oral, rectal, and genital mucosae[20,21]. While epithelial cells are not productively infected by HIV, they can selectively capture virions and then transfer them to dendritic cells, macrophages and CD4+ T-cells that are located in the subepithelial layers[21-23]. Dendritic cells expressing dendritic cell-specific intracellular adhesion molecule-grabbing nonintegrin and other C-type lectin receptors then facilitate infection of mucosal CD4+ T-cells by HIV and SIV in the form of an "infectious synapse", a dendritic cell/T-cell conjugate that also promotes the spread of infection to adjacent cells[24-32].

Consistent with these observations is the finding that during experimental SIV infection of macaques, the GI

mucosa is the initial and predominant site of SIV infection, as indicated by the early local accumulation of SIV-infected lymphocytes and the high viral load in this tissue when compared to blood and lymph nodes[33-35]. As the focus of this review is on the mucosal immune dysfunction associated with or induced by HIV and SIV infection, we will not here discuss in any further detail the current understanding of the virologic, immunologic, and anatomical determinants of mucosal transmission of primate lentiviruses.

Mucosal immune dysfunction during acute HIV and SIV infections

A series of influential studies have elucidated the early immunologic consequences of HIV and SIV infection at the level of mucosal tissues[33,36-42]. These studies were mainly conducted in SIV-infected macaques, where sequential longitudinal sampling of mucosal tissues (i.e. respiratory mucosa via bronchoalveolar lavage, and intestinal mucosa via biopsy) can be performed. The main conclusion of these studies is that early HIV/SIV infection is consistently associated with a rapid, dramatic, and largely irreversible depletion of mucosal $CD4^+$ memory T-cells, particularly those expressing the HIV/SIV coreceptor CCR5[33,36-42]. This observation is related to the well-known facts that the majority of newly transmitted HIV strains, as well as the commonly used SIV of macaques (SIVmac), are CCR5-tropic[43], and that primate lentiviruses preferentially infect and kill activated $CD4^+$ T-cells[44-46]. As such, the large population of memory/activated $CD4^+CCR5^+$ T-cells residing in the effector mucosal sites (particularly the lamina propria) represents a highly susceptible target for virus replication, especially at a time when no antiviral adaptive immune response has yet been generated[33,36-42]. This is in contrast with the relative preservation of the $CD4^+$ T-cell pool residing at the level of peripheral blood and lymph node, i.e. sites where a significant fraction of $CD4^+$ T-cells are resting, naive or central memory, and for the vast majority $CCR5^-$. As expected, experimental infection of macaques with CXCR4-tropic SIV or SHIV results in a more dramatic depletion of $CD4^+$ T-cells in lymph nodes, with relative preservation of mucosal tissues[38,47].

The first description of the early depletion of mucosal $CD4^+$ T-cells was published in 1998 by Veazey and Lackner, who observed a 70-95% loss of $CD4^+$ T-cells in the jejunum, ileum, and colon by day 21 post SIVmac239 infection[33]. This observation was confirmed by other studies[41,42], as well as studies where the genital and respiratory mucosae were examined[38,48]. More recently, the mechanisms underlying the early SIV-associated mucosal $CD4^+$ T-cell loss were elucidated in a study of SIVmac251-infected macaques, where up to 60% of $CD4^+$ T-cells were found to harbor SIV DNA by day 10 postinfection, thus suggesting a direct, virus-mediated killing of infected $CD4^+$ T-cells as the main mechanism responsible for their depletion[41]. Interestingly, this study also shows that SIV DNA was present in *bona fide* $CCR5^-$ $CD4^+$ T-cells as measured by flow cytometry[41]. However, another study reported that only up to 7% of mucosal $CD4^+$ T-cells were infected with SIV (as measured by SIV RNA by *in situ* hybridization, a most stringent measure of productive infection) at any given time, thus suggesting that, for the vast majority, the early SIV-associated depletion of $CD4^+$ T-cells is related to indirect death of uninfected, bystander $CD4^+$ T-cells, likely due to CD95-mediated apoptosis[42]. Interestingly, a peculiar feature of acute SIV infection is that "resting" (i.e. $Ki67^-$, $CD69^-$, $CD25^-$) $CD4^+$ T-cells are more likely to be productively infected than during the chronic phase[42]. A possible explanation for these somewhat discrepant observations is that the depletion of $CD4^+$ T-cells from the GI tract may in fact be multifactorial, with direct viral infection accounting for the earliest (within days of infection) loss of $CD4^+$ T-cells and activation-induced cell death (and perhaps host cytotoxic cellular response as well) responsible for the subsequent depletion (within weeks). The pathogenic role of the early loss of mucosal $CD4^+$ T-cells has been defined in an elegant study where sequential sampling of bronchoalveolar lavage in SIVmac239-infected macaques after *in vivo* labeling with bromodeoxyuridine (BrdU) indicated that rapid disease progression is closely associated with insufficient production and tissue delivery of $CD4^+$ effector memory cells[38].

Studies where the very early (within days or weeks) events occurring in the MALT following HIV infection of humans are extremely difficult to perform for obvious ethical reasons. In work conducted independently by the groups of Dandekar, Markowitz and Douek, mucosal samples were obtained through either jejunal, colon or terminal ileum biopsy in HIV-infected individuals with acute/early infection[37,39,40]. Collectively, these studies clearly indicated that HIV infection is also characterized by an early, severe, and largely irreversible depletion of mucosal $CD4^+CCR5^+$ memory T-cells, although the level of this depletion appears to be less prominent than what was observed in SIVmac239-infected macaques[38,41,42,48]. Based on these findings, a model of AIDS pathogenesis has been formulated whereby the selective depletion of memory $CD4^+$ T-cells from mucosal tissues during acute HIV or SIV infection is a key

determinant of disease progression[1,49-52]. According to this model, the early loss of MALT CD4+ T-cells induces a significant impairment of mucosal immunity that may result in a series of pathogenic sequelae that are mostly apparent during chronic infection (see next paragraph for more detail). It should also be noted, however, that the exact pathogenic role of the early loss of mucosal CD4+ T-cells is still unclear, particularly if one considers that the early, post-acute stages of HIV infection are virtually always asymptomatic, while clear clinical evidence of mucosal immune dysfunction (i.e. opportunistic infections at the level of the GI and respiratory tracts) occurs usually only after years of untreated infection and at a time when severe systemic CD4+ T-cell depletion is present.

Mucosal immune dysfunction during chronic HIV and SIV infections

The pathogenesis of AIDS may be schematically simplified as being comprised of two distinct phases: an acute phase, lasting a few weeks, dominated by the direct cytopathic effects of an unchecked virus replication, resulting in a significant loss of memory/activated CD4+CCR5+ T-cells (which is particularly prominent in the MALT), is followed by a chronic phase lasting several years, where the immune system exerts some control over a steady-state virus replication, but host-specific factors, such as a state of chronic, generalized immune activation, play a central role in further damaging a progressively dysfunctional immune system[49,53]. As previously reported, early acute HIV/SIV infection is associated with a rapid and significant depletion of mucosal CD4+ memory T-cells that appears to be more dramatic in the highly pathogenic SIVmac infection model[38,41,42,48]. During chronic HIV infection, the extension of MALT CD4+ T-cell depletion remains more severe than that of circulating CD4+ T-cells, as first shown in 1995 by Thomas Schneider, et al.[36]. In this important study, peripheral blood lymphocytes, as well as lymphocytes isolated from duodenal biopsy specimens, were analyzed in 34 HIV-infected individuals, eight asymptomatic and 26 with AIDS[36]. The main finding was that both groups of patients exhibited a preferential (i.e. more severe compared to blood) but similarly severe loss of duodenal CD4+ T-cells (average frequencies CD4+ T-cells in the duodenum was 3% in asymptomatic and 1% in AIDS individuals) compared to healthy controls. These earlier results were largely confirmed and then expanded upon by studies showing that chronic HIV infection is associated with severe MALT CD4+ T-cell depletion, mainly involving CD4+CCR5+ T-cells resident in the lamina propria, as observed in tissue specimens collected from the terminal ileum[39] and the rectosigmoid colon[40]. Interestingly, the level of CD4+ T-cell depletion in the MALT was remarkably high in HIV-infected individuals defined as long-term nonprogressors, that is to say, those who remain asymptomatic and with high CD4+ T-cell counts for many years[54]. Collectively, these data clearly indicate that MALT CD4+ T-cell depletion is consistently present during all stages of HIV infection. As the overwhelming majority of HIV-infected individuals receive medical attention when they are already past the acute phase of infection, studies aimed at defining the clinical relevance of the chronic depletion of mucosal CD4+ T-cells are important. Some of the open questions include: Is the mucosal depletion of CD4+ T-cells more predictive of disease progression and risk of AIDS than the peripheral depletion? Should the level of MALT CD4+ T-cells and, more generally, the state of the immune function, be monitored in HIV-infected individuals and the results used to decide when to start antiretroviral therapy? How reversible is the mucosal CD4+ T-cell depletion when virus replication is suppressed?

Some interesting clues as to the pathogenic role of mucosal CD4+ T-cell depletion during chronic infection were provided by a recent study by Picker, et al.[55]. In this study, lung-derived CD4+ T-cells were examined by sampling the bronchoalveolar lavage of a group of eight SIVmac239-infected rhesus macaques, followed longitudinally from the infection until the onset of an AIDS-related event and subsequent sacrifice[55]. The main observation of this study is that after the acute depletion, a consistent and progressive decline of the fraction of CD4+ T-cells in the bronchoalveolar lavage (particularly the effector memory T-cells) is observed in the chronic infection, coincident with a similarly progressive exhaustion of CD4+ central memory T-cells at the level of blood and lymph nodes[55]. In addition, short-term BrdU labeling experiments showed that the homeostasis of mucosal CD4+ effector memory T-cells was largely dependent on the production and migration of new cells, and that a progressive, systemic decline of CD4+ central memory T-cells resulted in the insufficiency of mucosal CD4+ effector memory T-cells that is associated with progression to AIDS[55]. Importantly, the systemic loss of CD4+ central memory T-cells during chronic SIVmac239 infection of macaques was more closely associated with the level of immune activation than viral load[55], thus consistent with recent observations underlining the pathogenic role of chronic immune activation in HIV infection of humans[56]. In all, these findings led to the hypothesis

that, at least in the SIVmac239 model of SIV infection of macaques, progression to AIDS is closely associated to the depletion of mucosal CD4+ effector memory T-cells below a critical "threshold level". This hypothesis is consistent with the observation that in HIV-infected humans, low levels of mucosal CD4+ T-cells and increased MALT immune activation are associated with increased collagen deposition in the lymph nodes, thus suggesting a parallel loss of effector memory and central memory T-cell function emerging in mucosal and systemic lymphoid tissues, respectively, during chronic progressive HIV disease[39]. An interesting link between mucosal immune dysfunction and overall function of the intestinal mucosa was identified in studies by Dandekar, et al., reporting the development of malabsorption and nutritional complications in CD4+ T-cell depleted, SIV-infected rhesus macaques[57]. In more recent work by the same group, high throughput gene expression indicated that mucosal CD4+ T-cell depletion is associated with downregulation of host genes involved in mucosal growth and enterocyte function[58].

These important observations notwithstanding, a clear correlation between loss of mucosal CD4+ effector memory T-cells and progression to AIDS in HIV-infected humans has not yet been established. In fact, the exact mechanisms by which low levels of mucosal CD4+ T-cells relate to the generalized immunodeficiency typical of human AIDS, – in which, it should be remembered, many "non-mucosal" infectious complications occur, from cytomegalovirus retinitis to central nervous system infections and lymphomas, just to name a few – remain poorly understood. Over the past year, a more comprehensive theory of the relationship between mucosal CD4+ T-cell depletion, mucosal immune dysfunction, and progression to AIDS has been proposed in a series of elegant and somewhat provocative studies that are described in detail in the next paragraph.

The microbial translocation theory: a mechanistic link between breakdown of the mucosal barrier and high levels of systemic immune activation

A tremendous amount of experimental evidence derived from studies conducted in both HIV-infected individuals and SIV-infected macaques indicates that the establishment of a state of chronic, generalized immune activation is a characteristic feature of pathogenic HIV/SIV infection that is consistently associated with disease progression[56]. Salient features of the HIV/SIV-associated chronic immune activation include: (i) increased frequencies of T-cells and B-cells with an activated phenotype, (ii) accelerated lymphocyte turnover with abnormalities in cell cycle regulation, and (iii) high serum levels of proinflammatory cytokines and chemokines[56]. Importantly, the extension of chronic immune activation is a strong correlate of disease progression in HIV-infected individuals[59,60]. Unfortunately, the pathogenesis of the HIV-associated immune activation remains obscure, especially when one considers that the number of activated T-cells appears to exceed the number of virus-specific ones, and that chronic immune activation is typically not present in natural hosts for SIV infection in which virus replication is as high as or even higher than in HIV-infected individuals[61].

A possible mechanism linking the early and persistent loss of MALT CD4+ T-cells with the generalized immune activation that is typical of the chronic phase of pathogenic HIV/SIV infections has been recently proposed by Daniel Douek and Jason Brenchley[52,62]. These authors showed that the plasma levels of lipopolysaccharide, a component of the bacterial cell wall that can be used as an indicator of microbial translocation from the intestinal lumen to the systemic circulation, are significantly increased in chronically HIV-infected individuals and SIV-infected rhesus macaques[62]. Importantly, plasma levels of lipopolysaccharide correlated with the level of systemic immune activation in HIV-infected individuals[62]. Based on these studies, a mechanistic link was proposed between the defects in mucosal immunity that are related to the loss of MALT CD4+ T-cells, and the establishment of the typically high levels of immune activation seen during pathogenic HIV/SIV infections[52,62]. The idea is that in the setting of a significant depletion of CD4+ T-cells, the loss of mucosal immune function favors a breakdown of the physical and/or biological mucosal barrier that results in the translocation of microbial products (e.g. lipopolysaccharide and others) from the gut to the systemic circulation (Fig. 1). These microbial products would, in turn, cause a broad activation of the immune system via their binding to certain toll-like receptors and consequent "bystander" activation of lymphocytes that are not specific to HIV antigens[62]. Several additional pieces of evidence support this mechanistic link: (i) enteropathy with increased apoptosis of enterocytes has been documented in patients with HIV infection and AIDS[63,64] (Haase A, Abstract #11, 24th Annual Symposium on Non-Human Primate Models for AIDS, Atlanta, October, 2006); (ii) high levels of viral replication and CD4+ T-cell depletion in gut-associated lymphoid tissue correlate with decreased expression of

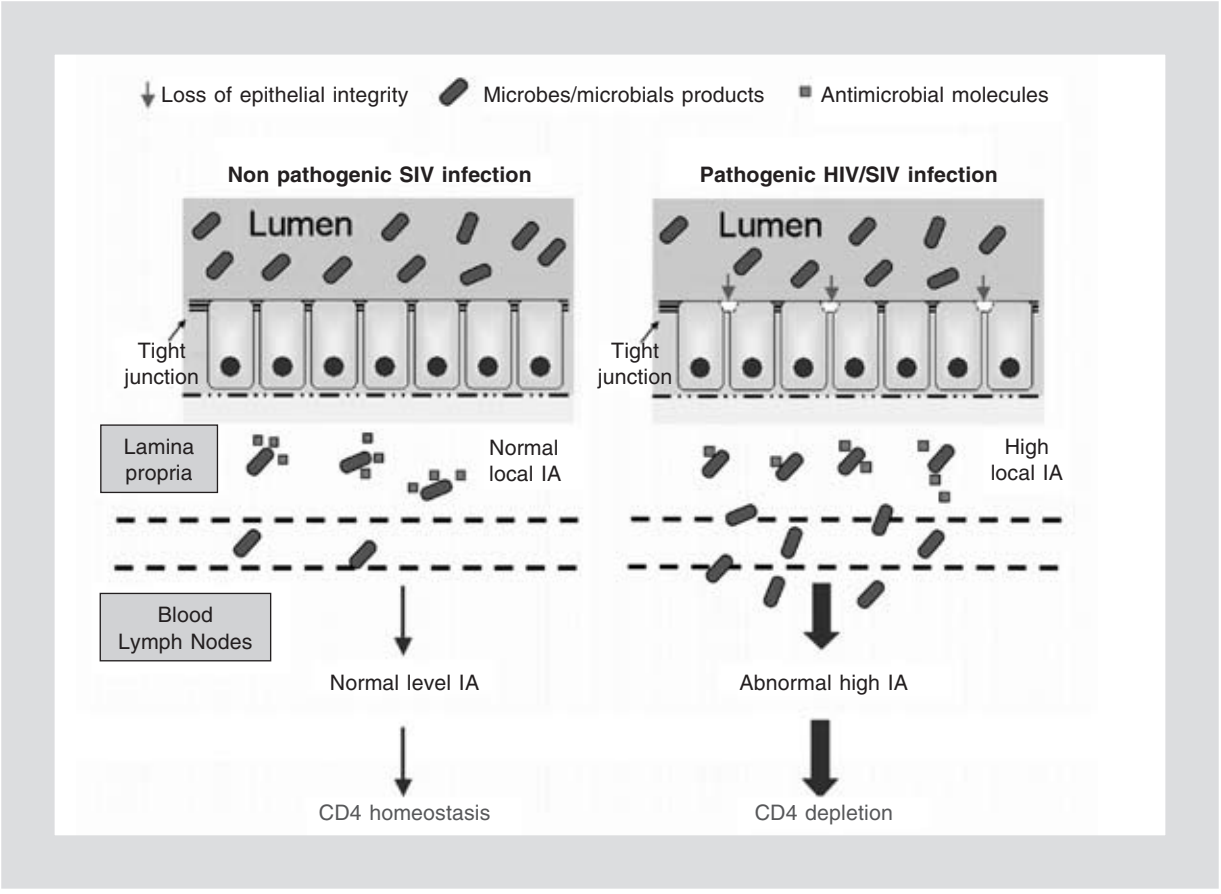


Figure 1. *Mechanistic link between mucosal functionality and systemic levels of immune activation in HIV/SIV infection.* Right panel: *During pathogenic HIV/SIV infections, significant depletion of CD4+ T-cells and chronic high levels of immune activation (IA) result in a loss of mucosal immune function and breakdown of the mucosal barrier that allows the translocation of microbial products to the systemic circulation. These microbial products may, in turn, cause a toll-like receptor-mediated broad activation of the immune system with consequent activation-induced cell death of bystander lymphocytes.* Left panel: *Despite a similar level of mucosa-associated lymphoid tissue (MALT) CD4+ T-cell depletion, in the context of normal level of IA, natural SIV hosts preserve mucosal immune function and show no evidence of microbial translocation. This may account for the absence of a high level of systemic IA in these animals.*

genes regulating epithelial barrier maintenance and increased transcription of immune activation and inflammation-associated genes[65]; (iii) microbial translocation has been noted in individuals with inflammatory bowel disease[66] and after damage to the GI tract during conditioning for hematopoietic transplantation[67,68].

While the breakdown of the gut mucosal barrier with stimulation of immune cells by microbial products appears to be at least one of the causes of immune activation during chronic HIV infection, some key issues in the proposed mechanism need to be clarified. First, so far only indirect correlative evidence indicates that microbial translocation causes immune activation in HIV-infected individuals; a direct cause-effect has not yet been shown and will likely be provided by studies wherein the level of microbial translocation is experimentally manipulated in SIV-infected nonhuman primates. Second, it is still unclear whether and to what extent microbial translocation is caused by loss of mucosal CD4+ T-cells, other forms of mucosal immune dysfunction, or direct damage to epithelial cells. Third, it is unclear how exactly the translocation of lipopolysaccharide and similar molecules leads to the specific type of chronic immune activation found in HIV-infected individuals. Answering these fundamental questions will provide important clues in our understanding of HIV pathogenesis and may hopefully define new molecular targets for innovative therapeutic strategies to be used, in addition to standard ART, for the clinical management of HIV-infected individuals.

Can the HIV-associated mucosal CD4 depletion be reverted or prevented?

A central question in contemporary AIDS research is whether and to what extent the level of mucosal CD4+

T-cells, and the overall mucosal immune system function, of HIV-infected individuals can be restored when virus replication is fully suppressed by ART. While the kinetics of viral suppression and CD4+ T-cell reconstitution in the peripheral blood of HIV-infected individuals on ART have been extensively investigated, the potential for ART to restore mucosal CD4+ T-cells has been more difficult to assess, chiefly because mucosal tissues are rather inconvenient to sample. As a consequence, our understanding of the impact of ART on the restoration of the GI mucosal immune system and function is limited, with somewhat controversial results being generated.

The observation that residual virus replication occurs in the GI tract of HIV-infected individuals treated with ART, despite undetectable levels of viral replication in the blood[69,70], is consistent with the finding that mucosal effector CD4+ T-cells are not restored in patients receiving ART during chronic infection[69,70]. In addition, a large study (40 patients) describing the effects of ART initiated during acute/early HIV infection in the reconstitution of mucosal CD4+ T-cells showed that most patients (70% of the cohort) do not experience complete restoration of CD4+ T-cells in the intestinal lamina propria despite several years of therapy[71]. Interestingly, this study also demonstrated that in ART-treated patients with persistent CD4+ T-cell depletion, the levels of immune activation in memory cells returned to levels seen in the uninfected controls in the blood, but remain elevated in the GI tract[71]. Collectively, these studies suggest that HIV replication in mucosal tissues is likely to be the major reason behind the persistently low levels of mucosal CD4+ T-cells in HIV-infected individuals on ART with undetectable virus in blood. This possibility, however, is quite puzzling when one considers the dramatic clinical effect, in terms of both morbidity and mortality, associated with the suppression of virus replication, and leaves one wondering how it is possible that mucosal CD4 depletion is an important marker of disease progression if its persistence under ART is not associated with major morbidity? Similarly, if mucosal CD4+ T-cells are the main sources of HIV replication at all stages of infection, how come this residual virus replication does not translate in at least some levels of circulating virus?

While more work is required to explain these apparent paradoxes, it should also be noted that more recent data in SIV-infected macaques suggest that mucosal CD4+ T-cell reconstitution can in fact be achieved if therapy is started early enough. George, et al. showed that in ART-treated SIV-infected macaques, viral suppression, near complete restoration of CD4+ T-cells (four out of five animals showed > 80% restoration of mucosal CD4+ T-cells) and enhanced expression of genes regulating mucosal repair/regeneration could be achieved in the GI tract if therapy is initiated during primary SIV infection[72]. In another study from the same group, the effects of ART on CD4+ T-cell reconstitution were longitudinally assessed in three HIV-infected individuals identified during primary HIV infection (within 4-6 weeks following HIV exposure) and immediately treated. The results were discordant; two patients did not show any significant changes in the CD4+ T-cell levels, but one patient experienced a complete recovery of the mucosal CD4+ T-cell compartment[73]. These data suggest that ART-treated SIV-infected rhesus macaques and HIV-infected individuals might experience a near complete recovery of mucosal CD4+ T-cells if therapy is initiated early after infection, thus indicating that CD4+ T-cells have the capacity to repopulate the MALT as long as their depletion is stopped before the restoration capacity of the immune system is irreversibly compromised. While these latter findings may be very important for the clinical management of HIV infection, the limited number of patients makes it difficult to reach definitive conclusions. Plainly, a better understanding of whether and to what extent prolonged virus replication is associated with restoration of CD4+ T-cells in the GI tract of HIV-infected individuals is urgently needed. At present, the need to reduce the risk of developing drug-dependent side effects as well as drug-resistant HIV strains has changed treatment guidelines to recommend initiation of therapy only when blood CD4+ T-cell counts drop below 350/mm^3, without considering the level of CD4+ T-cell depletion in the MALT and assuming that the ART-induced immune reconstitution involves the entire immune system.

An equally important question is whether and to what extent and HIV/SIV vaccination may inhibit or at least reduce mucosal CD4+ T-cell depletion. This issue was in part addressed in a recent study conducted by Mattapallil, et al., where the effect of systemic vaccination with a DNA-prime recombinant adenovirus boost immunization regimen expressing SIVmac239 genes was examined in macaques challenged with high-dose, intravenous SIVmac251[74]. In this study, the authors found a statistically significant, although not striking, increase in the level of mucosal memory CD4+ T-cells (as well as memory CD4+ T-cells in peripheral blood mononuclear cells and lymph nodes) in vaccinated animals compared to unvaccinated controls[74]. These results are consistent with unpublished data suggesting that mucosal CD4+ T-cell depletion during acute SIV infection is more prominent if the animals are experimentally

$CD8^+$ cell depleted (Schmitz, et al., personal communication). Somewhat in contrast, an ongoing study from our group where 10 macaques were vaccinated with modified vaccinia Ankara virus expressing SIV genes where IV challenged with SIVmac239 showed that high levels of SIV-specific cytotoxic T lymphocyte (CTL) responses in mucosal tissues correlate with lower viral loads, but did not protect from the early depletion of mucosal $CD4^+$ T-cells (Engram, et al., unpublished observations). Along these lines, the recently released results of the Merck STEP clinical trial of an adenovirus-based candidate AIDS vaccine indicate fairly clearly that in fact the presence of robust, vaccine-elicited, virus-specific CTL responses does not correlate with protection from either HIV transmission or disease progression[75-77]. In all, these results underline how more studies are needed to determine whether or not a CTL-based AIDS vaccine directed at protecting from HIV-disease progression will have a clinical effect mediated by the preservation of higher levels of mucosal $CD4^+$ T-cells during primary infection.

Natural SIV infection is associated with mucosal $CD4^+$ T-cell depletion in absence of systemic immune activation and microbial translocation

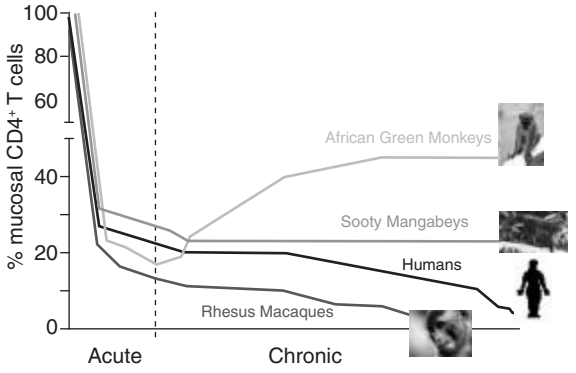
Important insights into the pathogenesis of HIV infection have been provided by studies of nonpathogenic SIV infection of African nonhuman primates that are natural hosts of SIV, such as sooty mangabeys (SM), African green monkeys (AGM), mandrills, and others[78]. The most intriguing feature of natural SIV infection is that in sharp contrast to pathogenic HIV infection in humans and SIV infection in macaques, African nonhuman primate hosts preserve a healthy level of peripheral $CD4^+$ T-cells and do not progress to AIDS despite comparable or even higher levels of plasma viremia[61,79]. In a long series of studies, we and others have been able to define some specific characteristics of nonpathogenic SIV infection in natural hosts. In particular, we found that limited immune activation is a consistent feature of natural SIV infection when compared with all known models of pathogenic HIV/SIV infection[80-85].

Two recent studies investigated the kinetics of mucosal $CD4^+$ T-cells during SIV infection of SM and AGM[86,87]. Gordon, et al. showed that in SIV-infected SM, memory $CD4^+$ T-cells are rapidly and severely depleted from the mucosal sites, but not from peripheral blood or lymph nodes, with kinetics remarkably similar to those observed during pathogenic SIVmac infection of macaques[86]. Intriguingly, the mucosal $CD4^+$ T-cell depletion observed in naturally SIV-infected SM occurred in the context of limited local and systemic immune activation and was partially reverted when virus replication was suppressed by ART[86]. In a similar study conducted in SIV-infected AGM, Pandrea, et al. observed a similar level of mucosal $CD4^+$ T-cell depletion during acute infection in these animals as compared to SIVmac-infected macaques[87]. In this study, a similarly profound depletion of mucosal $CD4^+$ T-cells was found during acute SIV from AGM infection of macaques, a model of infection where virus replication is rapidly controlled and the animals do not develop AIDS[87]. It should be noted that both experiments indicate that in contrast to pathogenic SIVmac infection of macaques (in which mucosal $CD4^+$ T-cell depletion becomes increasingly more severe as disease progresses to AIDS), the early mucosal $CD4^+$ T-cell depletion of natural hosts either does not progress further after reaching a stable plateau (in SM) or is followed by a significant recovery of these cells (in AGM) (Fig. 2). In another study, Milush, et al. described two experimentally infected SM, in which a multitropic (i.e. CCR5/CXCR4-tropic) SIV emerged coincident with the development of an extreme (< 0.5%) depletion of $CD4^+$ T-cells from blood, lymph nodes, and MALT[88]. This persistent (lasting > 6 years) $CD4^+$ T-cell depletion was surprisingly well tolerated by these two animals that have not showed any sign of AIDS-like disease[88].

The fact that natural SIV hosts maintain a normal mucosal immune function despite a significant level of $CD4^+$ T-cell depletion in these tissues is manifested by the complete absence of any increased susceptibility to infections. As expected in the context of preserved mucosal immune function and no systemic hyperimmune activation, there is no evidence of microbial translocation during SIV infection of natural hosts[62] (Fig. 2). These findings notwithstanding, it is still quite intriguing that natural SIV hosts maintain normal mucosal immune function with levels of $CD4^+$ in the MALT that are comparable to those described in HIV-infected humans who progress to AIDS. In the next paragraph we will discuss in detail the implications of and possible explanations for this interesting observation.

Pathophysiologic meaning of the HIV/SIV-associated mucosal $CD4^+$ T-cell depletion

In a way, the studies of mucosal immunity in natural SIV hosts suggest that during pathogenic HIV and SIV



MALT CD depletion	AIDS	Immune activation	LPS translocation
Yes	No	No	No
Yes	No	No	No
Yes	Yes	Yes	Yes
Yes	Yes	Yes	Yes

Figure 2. *Severe and acute MALT CD4+ T-cell depletion is necessary but not sufficient for progression to AIDS. Similarities and differences in mucosa-associated lymphoid tissue (MALT) CD4+ T-cell depletion, immune activation, microbial translocation and AIDS outcomes in natural (sooty mangabey, African green monkey) and non-natural (human, rhesus macaque) hosts. LPS: lipopolysaccharide.*

infection of humans and macaques, a severe and acute mucosal CD4+ T-cell depletion is necessary, but neither sufficient nor predictive of progression to AIDS. If this is indeed the case, what is the true pathophysiologic meaning of HIV/SIV-associated mucosal CD4+ T-cell depletion? And what factors allow natural SIV hosts to remain healthy despite low levels of mucosal CD4+ T-cells? Unfortunately, there is no simple answer to these compelling questions, other than the reiteration of how studies of natural SIV infection indicate that HIV/SIV-associated immunodeficiency is a complex phenomenon, initially triggered by the virus, but ultimately related to the nature of the host response to infection. In this context, AIDS pathogenesis is the cumulative result of multiple immunologic abnormalities, including CD4+ T-cell depletion and generalized immune activation, that exacerbate proliferation and apoptosis[78,89-93]. Having said that, the fact remains that we do not know yet what the key differences are in the mucosal immune system between natural and non-natural hosts for primate lentivirus infections.

One relatively simple possibility is that natural SIV hosts have progressively adapted, over time, to become less dependent on CD4+ T-cells to maintain the overall function of the mucosal immune system, thus becoming able to tolerate levels of mucosal CD4+ T-cell depletion that would be associated with disease progression in humans or macaques. This possibility is supported by the fact that even uninfected AGM show very low levels of CD4+ T-cells in mucosal tissues[81]. Along similar lines is the possibility that the CD4+ T-cell depletion in natural hosts is qualitatively different than that of non-natural hosts, with better preservation and/or balance of specific CD4+ T-cell subsets (T helper cells, Th1, Th2, Th17, etc.). Alternatively, natural SIV hosts may preserve mucosal immune function in the context of a marked reduction of CD4+ T-cells due to the lack of local and systemic immune activation. This hypothesis is consistent with the observation that in both SM and AGM, the level of mucosal immune activation, measured as the fraction of T-cells expressing markers of activation and proliferation, is clearly lower than in SIV-infected macaques[86,87]. An intriguing concept is that chronic immune activation may affect mucosal immunity by reducing the function of other cell types (i.e., non CD4+, such as NK cells, macrophages, γ-δ T-cells, etc.) in a scenario wherein the loss of CD4+ T-cells is more a marker than a determinant of a more generalized mucosal immune dysfunction. A final possibility is that the early mucosal CD4+ T-cell depletion observed in both pathogenic and nonpathogenic lentiviral infections is not *per se* sufficient to initiate the events leading to AIDS, but rather serves as a key mechanism to promote virus dissemination and establish chronic infection by generating a large and diffuse pool of infected cells during acute infection. In this view, the main marker of AIDS pathogenesis would be the progressive depletion of mucosal CD4+ T-cells occurring during chronic infection, coincident with the well-known loss of circulating CD4+ T-cells.

Future directions and conclusions

Over the past few years significant progress has been made in our understanding of the role played by the

mucosal immune system in the pathogenesis of AIDS. In particular, it is now very well established that a rapid, dramatic, and persistent depletion of MALT-based memory $CD4^+CCR5^+$ T-cells is a characteristic feature of the immunodeficiency observed in HIV-infected individuals and SIV-infected macaques[1,49-51]. However, the observation of depletion of mucosal $CD4^+$ T-cells during the primary phase of nonpathogenic SIV infection of natural SIV hosts, such as SM and AGM, suggests that additional pathogenic factors, most likely acting during the chronic phase of infection, must be involved in the AIDS-associated mucosal immune dysfunction. While it is clear that studies of mucosal immunity during SIV infection of natural hosts have provided an illuminating frame of reference to interpret data generated in studies of pathogenic HIV/SIV infections, much work needs to be done to understand how natural SIV hosts avoid disease progression and maintain low levels of local immune activation and microbial translocation, even in the presence of severe CD4 depletion in mucosal tissues.

A second major outstanding issue is to define the implications of these findings in terms of clinical management of HIV infection. The fact that the loss of $CD4^+$ T-cells, the clinical hallmark of progression to AIDS, is more rapid and severe in the MALT than in peripheral blood suggests that monitoring the effects of HIV infection at the level of MALT by performing GI biopsies may be clinically useful. Of note, it will be important to determine whether or not a better reconstitution of mucosal $CD4^+$ T-cells in ART-treated HIV-infected individuals is associated with a lower risk of disease progression. Another important point for the clinical treatment of HIV infection is to define whether the new data on mucosal immune dysfunction represent a sufficient rationale to initiate therapy earlier than according to the existing guidelines. It will also be useful to define if the severity of mucosal $CD4^+$ T-cell depletion and local immune activation is comparable in the different anatomic sites (i.e. jejunum vs. ileum, vs. colon/rectum).

In conclusion, further studies are needed to solve the complex riddle of how the interaction between primate lentiviruses and the host mucosal immune system leads to the severe mucosal immune dysfunction associated with progression to AIDS. The elucidation of these mechanisms will allow us to better understand, prevent, and treat HIV pathogenesis and will represent a major step forward in the fight against AIDS.

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