Published in final edited form as:
Mucosal Immunol. 2013 July ; 6(4): 657–665. doi:10.1038/mi.2013.15.

Mucosal immunity in human and simian immunodeficiency lentivirus infections

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Abstract

Overwhelming evidence indicates that distinct pathological phenomenon occurs within the gastrointestinal (GI) tract of progressively simian immunodeficiency virus (SIV)-infected Asian macaques and HIV-infected humans compared with other anatomical sites. Massive loss of GI tract lamina propria CD4 T cells, alteration in the profile of lymphocytic cytokine production, changes in the landscape of GI tract antigen-presenting cells, and variations to the structural barrier of the GI tract are hallmarks of progressive HIV/SIV infections. The pathology within the GI tract results in translocation of microbial products from the lumen of the intestine into peripheral circulation. These translocated microbial products directly stimulate the immune system and exacerbate immune activation and, thus, disease progression. Initiation of combination antiretroviral therapy (cART) does not restore completely the immunological abnormalities within the GI tract. This incomplete restoration within the GI tract may contribute to the increased mortality observed within HIV-infected individuals treated for decades with cART. Novel therapeutic interventions aimed at enhancing GI tract anatomy and physiology may improve the prognosis of HIV-infected individuals.

INTRODUCTION

Given that HIV and simian immunodeficiency virus (HIV/SIV) are cytopathic viruses and that they have tropism for leukocytes that express the receptor CD4 and one of a set of chemokine receptors, it follows that progressive loss of CD4 T cells is a hallmark of the disease and is, ultimately, associated with the development of AIDS.[1–4] Although the virus has specific tropism for CD4 T cells and loss of these cells underpins susceptibility to opportunistic infections, cessation of viral replication does not appear to allow HIV/SIV-infected individuals to return to health completely. Indeed, the immunological pathology that occurs during the course of untreated HIV/SIV infections cannot be completely reversed by therapeutic administration of combination antiretroviral therapy (cART). The pathological occurrences during the course of untreated, progressive HIV/SIV infections are

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DISCLOSURE

The author declared no conflict of interest.

complex and multifaceted. In particular, there are specific pathological occurrences within the GI tract that are thought to be of critical importance in perpetuating disease progression.

The gastrointestinal (GI) tract is the predominant structural and immunological barrier against the microorganisms of the outside world. In addition, the enterocytes that form the single cell layer of the mucosal epithelium provide essential roles in nutrient absorption. Hence, pathology to the structural and immunological anatomy of the GI tract during progressive HIV/SIV infections results in multiple deleterious sequelae. Here I review the numerous alterations observed within the GI tracts of HIV-infected individuals and/or SIV-infected Asian macaques and how these are thought to influence the overall health of such individuals and therapeutic interventions that might restore the anatomy and physiology of the GI tract.

PATHOLOGY WITHIN THE GI TRACT

In 1983, HIV was defined as the infectious agent that causes AIDS.[5,6] Only one year later, in 1984, Kotler *et al.*[7] observed that HIV-infected individuals had histological abnormalities of the GI mucosa. These observed abnormalities included malabsorption and lymphocyte depletion and they concluded that: “The histologic findings suggest that a specific pathologic process occurs in the lamina propria of the small intestine and colon in some patients with the syndrome.” This finding was incredibly insightful in its anticipation of subsequent discoveries. Indeed, the term “HIV enteropathy” has been appreciated for as long as it has been known that HIV causes AIDS. The enteropathy that afflicts HIV-infected individuals can occur from the acute phase of the infection through to advanced disease. It involves diarrhea, increased GI inflammation, increased intestinal permeability, and malabsorption.[8–10] Subsequently, considerable effort has investigated immunological abnormalities within the GI tract of progressively HIV/SIV-infected individuals, and overwhelming evidence suggests distinct immunological pathologies within the GI tract compared with other anatomical sites.

Loss of CD4 T cells

There is overwhelming evidence to suggest that CD4 T cells are disproportionately depleted from the lamina propria of the GI tract compared with peripheral blood of acutely HIV-infected humans and SIV-infected Asian macaques. Indeed, after the original observations of enteropathy and histological abnormalities in the HIV-infected GI tract, immunohistochemical analysis showed a proinflammatory infiltration of lymphocytes, yet concomitant with a striking absence of CD4 T cells.[11,12] These findings lead to the suggestion that the intestinal mucosa could be a site of significant HIV replication and CD4 T-cell infection and consequent depletion.[12] Subsequently, other studies also using immunohistochemistry confirmed that the GI tract was, indeed, significantly depleted of CD4 T cells.[13–15] The mechanism underlying this preferential loss of GI tract CD4 T cells is likely multifaceted; however, it seemed unlikely that HIV coincidentally has tropism for CD4 T cells and these were the very cells depleted. Studies in SIV-infected Asian macaques has shown significant viral replication within the GI tracts of acutely SIV-infected animals, suggesting much of the CD4 T-cell depletion is attributed to direct infection with SIV.[16,17]

Use of flow cytometric analysis has also allowed for quantitative examination and phenotypic assessment of GI CD4 T cells in HIV/SIV-infected individuals.[18–25] Several of these studies examined CD4/CD8 ratios in intestinal biopsies and peripheral blood to compare CD4 T-cell depletion between these two anatomical sites, and these studies also suggested that GI tract was preferentially depleted of CD4 T cells. Additional studies that concentrated on examining depletion of the specific cells targeted by HIV—CD4 T cells that express the HIV coreceptor CCR5 (chemokine (C-C motif) receptor 5)—have shown that these cells are almost completely depleted from GI tract tissues.[22–26] Indeed, phenotypic analysis of CD4 T cells in GI mucosa of healthy individuals demonstrated that the majority of T cells in mucosal tissues expressed CCR5 and were very permissive to *in vitro* infections with HIV.[27,28]

Altered functionality of GI tract lymphocytes

Although CD4 T-cell depletion is nearly complete in progressively HIV-infected humans and SIV-infected Asian macaques, it is important to note that CD4 T-cell depletion is not the only abnormality observed within the GI tract. Indeed, there are many other pathological phenomena that likely contribute to disease progression. In healthy individuals, GI tract lymphocytes contribute to antibacterial defenses and can promote maintenance of the structural barrier of the GI tract. In particular, specialized lymphocytic subsets that reside in the GI tract are capable of producing effector cytokines such as interleukin-17 (IL-17) and interleukin-22 (IL-22). These cytokines can recruit neutrophils and myeloid cells to effector sites by inducing granulocyte colony-stimulating factor,[29] and are involved in epithelial regeneration in mucosal tissues.[30] This IL-22/IL-17-mediated maintenance of the mucosal layers is associated with induction expression of claudins (which are protein components of epithelial tight junctions), defensins (which have antibacterial activities), and mucin.[31] Moreover, IL-22 is, itself, a growth factor for epithelial cells as the majority of epithelial cells in the body express the IL-22 receptor and IL-22 can cause epithelial cells to divide *in vitro*.[32]

Several studies have shown that although CD4 T cells are massively depleted from the GI tracts of progressively HIV/SIV-infected individuals, CD4 T cells that produce the effector cytokine IL-17 are preferentially depleted.[33–35] Given the known roles for IL-17/IL-22 in maintenance of mucosal barriers, preferential loss of T helper type 17 (Th17) cells was hypothesized to provide mechanistic insights into damage to the structural barrier of the GI tract observed in chronically HIV/SIV-infected individuals.[36–39] However, although CD4 Th17 cells are classically considered to be the predominant source of IL-17 and IL-22, recent studies have clearly demonstrated that subsets of CD8 T cells and innate lymphocytes are also capable of producing these effector cytokines. Subsequent studies have demonstrated that the CD8 T cells and innate lymphocytes that produce IL-17 and IL-22 are, similar to Th17 cells, present at decreased frequencies within the GI tracts of HIV/SIV-infected individuals.[40–43] This immunological skewing away from GI tract lymphocytes capable of producing IL-17 is associated with systemic immune activation[36,40] and focal damage to the structural barrier of the GI tract (discussed below).[40] Taken together, these data suggest that immunological devastation within the GI tract is a critically important factor to consider for HIV/SIV disease progression. Understanding the mechanisms

underlying this immunological abnormality may lead to novel therapeutic interventions to decrease systemic immune activation that predicts disease progression better than either plasma viral loads of peripheral blood CD4 T-cell counts.[1]

That IL-17/IL-22-producing CD8 T cells, CD4 T cells, and innate lymphocytes are present at decreased frequencies within the GI tracts of SIV-infected animals provides some insight into the mechanisms underlying this observed decrease. Indeed, as neither CD8 T cells nor innate lymphocytes express the CD4 receptor for HIV/SIV, it seems unlikely that preferential infection by HIV/SIV in progressively infected individuals underlies depletion of IL-17/IL-22-producing cells. Moreover, we previously used quantitative real time-PCR for HIV DNA and flow cytometric sorting of mitogenically stimulated lymphocytes from chronically HIV-infected individuals to determine the relative infection frequencies of Th17 and Th1 cells and found equal infection of Th1 and Th17 cells.[33] Given the uniformity in loss of all IL-17/IL-22-producing lymphocytes and lack of preferential HIV/SIV infection of Th17 cells, several groups began examining the nature of the landscape of antigen-presenting cells (APCs) within the GI tract of SIV/HIV-infected individuals. These studies have uniformly demonstrated significant alterations to the APC landscape in progressively infected GI tracts. High numbers of APCs expressing the tryptophan-metabolizing enzyme indoleamine 2,3 dioxygenase (IDO) have been observed in chronically HIV-infected invididuals,[44,45] and IDO activity (measured by serum levels of tryptophan and tryptophan metabolites) has been associated with low frequencies of Th17 cells in progressively HIV-infected individuals.[46] Moreover, the metabolites produced by IDO directly inhibited IL-17-producing lymphocytes *in vitro*.[42] The high levels of IDO^+ APCs observed in HIV/SIV-infected individuals was associated with decreased frequencies of $CD103^+$ APCs, which are thought to provide factors required for differentiation and maintenance of IL-17/IL-22-producing lymphocytes.[40] $CD103^+$ APCs were also excellent in inducing Th17 cells from naive CD4 T cells *in vitro*.[40] Hence, alterations to the landscape of GI tract APCs may limit the ability of progressively HIV/SIV-infected individuals to maintain IL-17/IL-22-producing lymphocytes.

Structural alterations of the GI tract

Given that the majority of epithelial cells in the body express the IL-22 receptor,[32] loss of lymphocytes producing IL-17 and IL-22 might be expected to have a detrimental effect on maintenance of the GI tract epithelial barrier. Indeed, the tight epithelial barrier is a single layer of cells that serves to separate the host from the enormous luminal bacterial microbiome. The epithelium also absorbs luminal nutrients. Several studies have indicated impaired integrity of the epithelial barrier of the GI tract in chronically HIV-infected individuals. Indeed, as previously stated, Kotler *et al.*[7] originally observed malabsorption, and lymphocyte depletion in HIV-infected individuals. This enteropathy afflicting HIV-infected individuals could occur from the acute phase of the infection through to advanced disease and involved diarrhea, increased GI inflammation, increased intestinal permeability, and malabsorption.[8–10] Demonstration of damage to the structural barrier of the GI tract has been provided by focal breaches in the barrier, massive apoptosis of enterocytes, decreased expression of epithelial repair tight junction genes, and increased GI tract permeability.[10,47–50] Indeed, focal breaches of the epithelial barrier were in spatial

juxtaposition to infiltrating microbial products *in vivo*, and the extent of damage to the GI tract barrier during HIV/SIV was directly associated with the degree of microbial translocation, both locally within the GI tract and systemically (discussed below).[47–49]

The mechanisms underlying damage to the structural barrier of the GI tract are likely numerous and complex. Given the data published, it is reasonable to suggest that the focal damage observed to the structural barrier of the GI tract is attributed, at least in part, to the alterations to the immunological environment within the GI tract. Indeed, the acute inflammatory response associated with acute HIV/SIV infection is a very likely culprit in initiating a cyclical process. Part of the acute inflammatory process observed in HIV infection involves production of tumor necrosis factor (TNF).[51] Although production of TNF is a critical immunological response, overproduction of TNF can have deleterious effects on the epithelial barrier of the GI tract. Indeed, exposure to TNF could induce apoptosis in epithelial cells *in vitro*,[52] and *in vivo* studies also suggested that excess production of TNF perturbs the tight epithelial barrier, in part by induction of enterocyte apoptosis (reviewed in ref. 53). Consistent with this premise, therapeutic interventions aimed solely at decreasing TNF levels *in vivo* resulted in improved integrity of the structural barrier of the GI tract in individuals with Crohn's disease.[54] There are also recent data demonstrating that *in vivo* treatment with anti-TNF therapies ameliorated some of the pathogenic consequences of acute SIV infection in Asian macaques.[55] However, the efficacy of anti-TNF therapy in decreasing enterocyte apoptosis and focal damage to the structural barrier of the GI tract during progressive SIV infection is yet to be examined. After GI tract damage has been initiated, alterations in the functionality of local lymphocyte populations (discussed above) likely contributes to an inability to repair the damage considering that the amount of damage to the structural barrier of the GI tract is negatively associated with the degree to which IL-17/IL-22-producing lymphocytes are decreased.[40]

MUCOSAL HIV/SIV-SPECIFIC CELLULAR RESPONSES

The vast majority of HIV transmission occurs via introduction of the virus to a mucosal site.[56] Moreover, after acquisition of the virus, the first cells that become productively infected, leading to viral spread, appear to be mucosal CD4 T cells.[17] As such, there is significant effort in producing vaccines that stimulate HIV/SIV-specific immune responses within mucosal tissues.[57] The immune response that is elicited during natural infection is, in general, insufficient to control viral replication. However, a small fraction (<0.5%) of HIV-infected individuals, termed elite controllers, spontaneously control viral replication to undetectable levels and many of these individuals express the HLA types HLA B57 or HLA B27.[58] It is widely believed that cellular immune responses mounted against the virus by such elite controllers is responsible for control of viral replication.[58] Thus, there has been significant attention given to understanding the types of HIV-specific and SIV-specific immune responses elicited in elite controllers. Similar frequencies of HIV-specific CD8 T cells are present within the mucosa of the GI tract in cohorts of HIV-infected individuals irrespective of their ability to control viral replication.[22,59] Although the magnitude of the HIV-specific CD8 T-cell response does not appear to differ between elite controllers and individuals with progressive HIV disease, the functional quality of the HIV-specific CD8 T-cell response appears to be improved in elite controllers.[60] In particular, HIV-specific CD8

T cells in elite controllers are able to better simultaneously produce several effector cytokines compared with HIV-specific CD8 T cells in individuals with detectable plasma viremia. Similar multifunctionality is also observed among HIV-specific CD4 T cells in the GI tract of elite controllers compared with individuals with detectable plasma viremia.[61] This association between increased functionality of HIV-specific T cells and decreased pathology is also across different anatomical sites within individuals. Indeed, increased functionality of HIV-specific T cells in broncheoalveolar lavage is associated with increased frequencies of CD4 T cells in broncheoalveolar lavage compared with the GI tract.[59]

Although the functionality of virus-specific T cells has been associated with controlled viral replication *in vivo*, it is unclear whether the polyfunctional T cells control viral replication or the polyfunctional T cells result from low levels of viral replication. To help clarify a potential role for T cells in controlling viral replication, Hansen *et al.*[62,63] developed a replication-competent cytomegalovirus (CMV) viral vector expressing SIV proteins. Infection with the CMV vector induced high frequencies of SIV-specific CD8 T cells with an effector memory phenotype and these effector memory CD8 T cells could protect rhesus macaques from mucosal SIV infection. The degree of protection against mucosal SIV infection was associated with the degree of SIV-specific effector memory CD8 T cells elicited by the CMV vectors.[63] Hence, specific functional and phenotypic subsets of T-cell immunity against HIV/SIV appear to provide protection against viral replication *in vivo*. The superior protection against infection seen in animals vaccinated with CMV vectors compared with animals vaccinated with adenovirus vectors may be attributed to the increased differentiation status of the virus-specific CD8 T cells induced by CMV vectors compared with virus-specific CD8 T cells induced by adenovirus vectors that tend to be less differentiated and express surface markers such as CD27 and CD28.[64]

In addition to studies of virus-specific T-cell responses, much effort has gone into understanding humoral responses that neutralize broad stains of HIV (reviewed in ref. 65). Indeed, preventing initial infection events would likely require a mucosal humoral immune response. Consistent with this premise, application of SIV-specific neutralizing antibodies to the vaginal surface can prevent vaginal infection of rhesus macaques using a pathogenic strain of SIV.[66] Moreover, evidence of protective humoral immunity in HIV comes from observations that individuals repetitively exposed to the virus and yet who remain uninfected produce HIV-specific immunoglobulin A antibodies at mucosal sites.[67,68] However, the majority of chronically HIV-infected individuals do not mount vigorous HIV-specific immunoglobulin A antibody responses in mucosal sites or systemically.[69] Thus, these findings suggest that induction of a functional HIV-specific immune response would aid in control of viral replication and may inhibit viral replication. However, vaccines that induce immunological responses that prevent acquisition of HIV do not currently exist and significant pathology within the GI tract occurs during progressive infection and the majority of HIV-infected individuals will succumb to AIDS if not treated with cART.

MICROBIAL TRANSLOCATION

That the human GI tract is colonized with approximately 10^{14} normal flora bacteria, the structural and immunological perturbations to the GI tracts in progressively HIV/SIV-

infected individuals was hypothesized to result in microbial translocation.[70] Microbial translocation during HIV infection was first described in 2006, where it was demonstrated that bioactive microbial products were significantly elevated in plasma from HIV-infected individuals and from progressively SIV-infected Asian macaques.[71] Furthermore, the levels of lipopolysaccharide (LPS) in these individuals directly correlated with activation of both the adaptive and innate arms of the immune system.[71] Subsequently, at least 50 groups corroborated the existence of microbial translocation during progressive HIV/SIV infections (reviewed in refs. 72 and 73). Hence, there is overwhelming evidence that the immunological and epithelial damage that occur in progressively HIV/SIV infected individuals lead to an inability of the host to restrict the microbiome and all of its immunostimulatory components to the lumen of the GI tract. Moreover, it is quite clear that the microbial products that translocate during progressive HIV/SIV infections are bioactive *in vivo* and exacerbate chronic immune activation. However, the degree to which microbial translocation contributes to the chronic immune activation is less clear. Indeed, microbial translocation is but one cause of immune activation in chronically HIV/SIV-infected individuals and other factors likely contribute. There has been only one study that did not observe microbial translocation in chronically HIV-infected individuals.[74] Here the authors used repeatedly thawed plasma samples from longitudinally sampled Ugandans before infection and then into the chronic phase of HIV infection and failed to find elevated plasma LPS or soluble CD14 (sCD14). However, in a follow-up study using exactly the same plasma samples, the authors found elevated levels of plasma C-reactive protein that correlated with sCD14.[75] It is unclear why these two reports from the same authors appear to contradict one another and why the findings of these authors were inconsistent with the numerous other reports of microbial translocation in chronic HIV infection, but factors such as nonfasting blood draws and repeated freeze thaws of plasma samples might have played a role.

Consequences of microbial translocation

To understand more completely the degree to which microbial translocation, as opposed to HIV/SIV replication, contribute to immune activation and disease progression, several groups have turned attention to chronically HIV/SIV-infected individuals in which the contribution of viral replication is diminished. Indeed, there are at least two cohorts of HIV-infected individuals in which viral replication is reduced to a minimum: elite controllers and cART-treated individuals. Although elite controllers have a significantly improved prognosis compared with viremic HIV-infected individuals, they tend to have higher levels of immune activation compared with HIV-uninfected individuals, and some of these individuals nevertheless lose peripheral blood CD4 T cells and some even progress to AIDS.[4] Elevated levels of plasma LPS have been detected in elite controllers that positively correlated with the frequency of activated phenotype $CD38^{+}HLA\text{-}DR^{+}$ CD8 T cells and negatively with peripheral blood CD4 T-cell counts.[4]

Similar studies have been performed using samples from cART-treated individuals. Current era cART is sufficient to control HIV replication to undetectable levels for decades.[76] However, recent studies have shown clearly that although cART can suppress plasma viral loads to undetectable levels, cART-treated individuals nevertheless have increased mortality

and morbidity. This increased mortality was closely associated with residual inflammation, which was in turn associated with cardiovascular disease,[77,78] osteopenia,[79] and cognitive decline.[80] Given that viral replication is decreased to very low levels in these individuals, it seems highly unlikely that the residual inflammation is directly attributable to ongoing viral replication. This increased mortality seemed to be linked to microbial translocation given that elevated plasma levels of sCD14 (a bacterial LPS receptor that is produced after LPS stimulation *in vivo*) independently predict increased mortality in cART-treated HIV-infected individuals.[78] Moreover, plasma levels of sCD163 (a scavenger receptor) were associated with unstable noncalcified coronary plaques in cART-treated HIV-infected individuals.[78,81] Consistent with the premise that microbial translocation contributes to immune activation and immunological pathology, microbial translocation has been suggested to play an important role in limiting immunological reconstitution of peripheral blood CD4 T cells after administration of cART.[71,82–87]

Given the strength of the data suggesting microbial translocation plays a major role in disease pathogenesis of HIV-infected individuals treated with long-term cART, it is important to consider mechanisms underlying the increased microbial translocation and consequent immune activation in these individuals. Significant efforts have been taken to understand more completely the state of the GI tract in HIV-infected individuals treated for long periods of time with cART. From these studies, it is quite clear that significant pathology remains despite cART adequately controlling systemic viral replication to very low levels. Several studies of the small intestine of cART-treated, HIV-infected individuals have concurred that CD4 T-cell reconstitution is poor and occurs at a much slower rate than the CD4 T-cell reconstitution observed in peripheral blood.[23,88–90] Moreover, individuals treated with cART during the early stages of HIV infection have improved reconstituted CD4 T cells in the GI tract compared with individuals treated with cART during chronic HIV infection (who rarely reconstituted significant numbers of GI tract CD4 T cells).[89,90] Continued depletion of GI tract CD4 T cells after administration of cART likely contributes to an inability to preserve the integrity of the GI tract that has been suggested from gene chip analysis of epithelial cells where decreased expression of GI tract repair genes was observed,[90] which is also consistent with the premise that the local immunological environment influences the ability of the host to maintain epithelial integrity (discussed above). The observed inability of cART-treated, HIV-infected individuals to reconstitute healthy frequencies of GI tract CD4 T cells was related to inflammation-associated fibrosis within Peyer's patches and lamina propria of the GI tract,[91,92] suggesting that fibrosis to the GI tract prohibits healthy immunological responses. Fibrosis appears to limit bioavailability of IL-7 within the lymphoid tissue that might provide mechanistic insight into how fibrosis limits reconstitution of CD4 T cells.[93]

GI TRACT BACTERIOME/VIROME

Although it is clear that microbial translocation occurs during progressive HIV/SIV infections and contributes to immune activation, microbial translocation also occurs in other diseases associated with inflammation of the GI tract. In other diseases where microbial translocation has been implicated in pathogenesis, an altered balance in the composition of the commensal microflora (dysbiosis) has been suggested to play an important role in the

disease.[94,95] From this, several hypothesized that dysbiosis may facilitate microbial translocation and consequent immune activation in progressive HIV/SIV infections. Cross-sectional analysis of the fecal microbiome of different cohorts of individuals seemed to suggest that dysbiosis in HIV-infected individuals may contribute to disease progression. These studies tended to agree that HIV-infected individuals had increased levels of potentially pathogenic fecal bacteria including clostridia and proteobacter species and lower levels of probiotic organisms such as *Lactobacilli* and *Bifidobacteria*.[96–98] Moreover, higher levels of fecal proteobacteria were associated with local immune activation within the GI tract.[99]

The existence of the SIV, nonhuman primate model of HIV infection allowed for the longitudinal sampling of feces at different amounts of time after infection. This allowed for a more controlled examination of the microbiota while minimizing differences that might be attributed to lifestyle and diet. Three studies have used next-generation sequencing approaches to study the composition of the fecal micro-biota in SIV-infected and SIV-uninfected nonhuman primates.[100–102] Two of these studies examined the microbiome of cross-sectional cohorts of SIV-infected and SIV-uninfected animals,[100,101] and one study examined the microbiome of several animals longitudinally, before and after SIV infection.[102] There was agreement among all three studies regarding the major communities of fecal microbes within nonhuman primates and there was agreement among all three studies that SIV infection did not result in significant alteration in the fecal bacteriome of progressively SIV-infected Asian macaques.

The bacteriome is clearly the largest microbial community within the GI tract; however, other potentially pathogenic microorganisms can also contribute to the microbiome. To examine the composition of the microbiome of progressively HIV/SIV-infected individuals in a more unbiased approach, Handley *et al.*[101] used 454 “shot gun” sequencing technology to analyze all DNA and RNA species present in fecal samples. The authors found a significant increase in the size of the fecal virome in SIV-infected Asian macaques and the virome contained potentially pathogenic viruses, and adenoviruses, in particular, were found to colocalize with areas of damage to the structural barrier of the GI tract. Importantly, these same fecal viruses were absent in SIV-infected natural hosts of SIV who manifest a nonprogressive infection with SIV. Hence, subclinical GI tract viral infections might contribute to microbial translocation and immune activation in progressive HIV/SIV infections.

Given the discrepancy between studies of HIV-infected humans and SIV-infected Asian macaques, it is unclear if the GI tract bacteriome is significantly altered during progressive HIV/SIV infections. One possible explanation for the discrepancy is inherent differences between the cohorts of individuals examined in the human studies and not the viral infection *per se*. With the discrepancies between data retrieved from nonhuman primates and humans, and the lack of comparable virome analysis in humans, and considering that is not entirely clear whether dysbioses cause or result from immune activation and immunodeficiency (or both), further studies are certainly warranted.

TREATMENTS

With the several studies indicating that microbial translocation plays an important role in exacerbating immune activation and, potentially, in comorbidities in cART-treated individuals, therapeutic interventions aimed at decreasing microbial translocation have been considered. The therapeutic intervention aimed at decreasing microbial translocation that has been investigated most thoroughly is probiotic bacteria. Probiotic bacteria are thought to impart benefits to the host via a variety of mechanisms including production of short-chain fatty acids (important for proliferation of epithelial cells), competition with potentially pathogenic bacteria for nutrients, and decrease in GI tract inflammation. Several studies have investigated potential benefits imparted to HIV-infected individuals by probiotic dietary supplementation. The majority of these studies have focused analyses on systemic effects of probiotic supplementation. Three studies have found modest improvement in reconstitution of peripheral blood CD4 T cells and decreased GI disorders after treatment with cART and probiotics.[103–106] A fourth study using two probiotic organisms found moderately decreased microbial translocation and immune activation after probiotic supplementation of cART.[107] A fifth study aimed to alter the composition of the GI tract microbiota by supplementing cART with a unique oligosaccharide mixture of “prebiotics” that are thought to promote the growth of probiotic organisms.[98] The prebiotic treatment was, indeed, associated with increased probiotic organisms and treated individuals had decreased markers of microbial translocation and systemic immune activation compared with placebo-treated individuals.[98] Finally, a sixth study using a combination of four probiotic organisms and a combination of prebiotics found marginal decreases in immune activation in individuals treated with these “synbiotics” and cART.[108] Importantly, probiotics were associated with some sort of immunological improvements in all five human studies. However, the mechanisms by which probiotics exerted these effects was unclear. Moreover, with the multitude of species of bacteria that have probiotic characteristics, it is unclear which probiotics might be most beneficial to cART-treated, HIV-infected individuals. Of the numerous species of bacteria that have been used as probiotics, the two formulations of probiotics that have been most exhaustively studied in other diseases characterized by microbial translocation are *Lactobacillus rhamnosis* GG and VSL-3.[109–112] VSL-3 is a combination of four strains of *Lactobacilli*, three strains of *Bifidobacteria*, and one strain of *Streptococcus.* A combination of *Lactobacillus* GG and VSL-3 was recently shown to impart an immunological benefit to cART-treated, SIV-infected Asian macaques.[102] This probiotic treatment led to decreased fibrosis within GI tract lymphoid follicles that was associated with increased reconstitution of GI tract CD4 T cells. Indeed, in these animals, just 5 months of therapy was sufficient for reconstitution of GI tract CD4 T cells to near healthy levels.[102] Hence, the immunological benefits that seem to be imparted by probiotic use likely involves restoration of GI tract physiology. It is important to note, however, that these published studies have, in general, involved fairly small sample sizes and the largest effects of the probiotic treatments tend to be within the GI tract itself. Moreover, there are numerous preparations of probiotic therapeutics and it is unclear if all probiotics will have similar effects. Hence, large placebo interventional trials are certainly warranted.

Probiotics are one of many therapeutic interventions that have been envisioned to decrease microbial translocation. Other therapeutic interventions aimed at decreasing microbial translocation attempt to remove microbial products from circulation. One study supplemented cART with oral hyper-immune bovine colostrum. The bovine colostrum comprised high levels of proteins thought to be capable of mediating clearance of microbial products from circulation. However, the colostrum-treated individuals did not have lower levels of immune activation or microbial translocation compared with placebo-treated individuals.[113] These data suggested that the hyperimmune bovine colostrum was insufficient to clear microbial products from circulation and alternative modalities could be considered. A second study involved treating SIV-infected Asian macaques with sevelamer, a phosphate-binding molecule with anti-inflammatory properties thought to be attributed to LPS binding. Sevelamer-treated, SIV-infected animals had significantly reduced microbial translocation, immune activation, and cardiovascular comorbidities compared with control animals.[114] A large, placebo-controlled study of sevelamer in HIV-infected humans is currently underway in the AIDS clinical trials group (A5296). Taken together, the data suggest that supplementation of cART with therapeutics aimed at decreasing microbial translocation imparts immunological benefits and may improve the prognosis of cART-treated, HIV-infected individuals.

CONCLUDING REMARKS

Dysfunction of the immunological and structural barriers of the GI tract are becoming pathognomonic with progressive HIV/SIV disease. There have been significant strides in understanding the mechanisms underlying damage to the GI tract, its consequences, and in developing therapeutic interventions aimed in restoring the function of the GI tract. Although the precipitating events that occur during the acute phase of HIV/SIV infections leading to gastrointestinal damage remain elusive, it is clear that local immune activation, loss of total CD4 T cells, altered functionality of GI tract lymphocytes and myeloid cells, fibrosis of lymphoid and lamina propria tissues, and increased turnover of GI tract epithelial cells all play an important role in GI tract dysfunction, allowing microbial products to translocate systemically. Preliminary data using therapeutic interventions aimed at decreasing microbial translocation and improving GI tract dysfunction in HIV/SIV-infected individuals are looking very promising, but effects of blocked microbial translocation on systemic immune activation need to be better parsed out. However, long-term studies will be required to determine whether or not the immunological benefits imparted to individuals treated with cART and microbial translocation-reducing therapeutics have decreased comorbidities associated with long-term cART treatment.

Acknowledgments

I thank the Cleveland Immunopathogenesis Consortium (BBC) for helpful discussions. This work was supported by the Intramural NIAID, US NIH program. The content of this publication does not necessarily reflect the views or policies of DHHS, nor does the mention of trade names, commercial products, or organizations imply endorsement by the US Government.

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