Microbial translocation, immune activation and HIV disease

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Abstract
The advent of combination antiretroviral therapy (cART) has significantly improved the prognosis of HIV-infected individuals. However, individuals treated long term with cART still manifest increased mortality compared to HIV-uninfected individuals. This increased mortality is closely associated with inflammation, which persists in cART-treated HIV-infected individuals despite levels of plasma viremia being below detection limits. Chronic, pathological immune activation is a key factor in progression to AIDS in untreated HIV-infected individuals. One contributor to immune activation is microbial translocation, which occurs when microbial products traverse the tight epithelial barrier of the gastrointestinal tract. Here we review the mechanisms underlying microbial translocation and its role in contributing to immune activation and disease progression in HIV infection.

Keywords
HIV; Microbial translocation; Immune Activation; Mucosal Immunity

Persistent immune activation and microbial translocation during HIV infection

The pathology of disease caused by human immunodeficiency virus (HIV) infection is complex and multifaceted. In addition to high levels of systemic viral replication, HIV infection results in chronic immune activation and overall immunological dysfunction, which is closely associated with progression to acquired immunodeficiency syndrome (AIDS)¹-³. Furthermore, in HIV-infected individuals who are successfully treated with combined antiretroviral therapy (cART), persistent immune activation is associated with increased morbidity and mortality⁴-⁶. Several lines of evidence indicate that a key contributor to immune activation and disease progression during HIV infection is microbial translocation⁶-⁵¹. During HIV infection and pathogenic simian immunodeficiency virus (SIV) infection of non-human primates, several factors underlie microbial translocation, including breakdown of the tight epithelial barrier of the gastrointestinal (GI) tract and mucosal immune dysfunction⁶, ¹⁶, ¹⁷, ²⁴, ⁴². A more complete understanding of the

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mechanisms underlying microbial translocation may provide insight into the development of novel therapeutic interventions aimed at decreasing microbial translocation and subsequent immune activation, thus decreasing morbidity and mortality in HIV infection.

**Immunological abnormalities in progressive infection**

One of the earliest insults to the immune system after infection with HIV is direct infection and depletion of CD4 T cells. This depletion first occurs rapidly in mucosal tissues, where high numbers of activated, memory CD4 T cells (the preferred target of HIV) reside. A slower, and progressive, depletion of CD4 T cells in peripheral tissues and blood ensues. Given that the majority of CD4 T cells reside within mucosal tissues, early loss of mucosal CD4 T cells represents loss of the majority of these cells within the body. While this depletion likely induces homeostatic pressure to restore mucosal CD4 T cells, low levels of mucosal CD4 T cells are insufficient to cause progression to AIDS. Indeed, during non-progressive SIV infection of natural hosts, who do not progress to AIDS, moderate depletion of mucosal CD4 T cells is observed, yet does not result in disease progression.

While CD4 T cells represent the most drastically affected lymphocyte cell type after HIV infection, other leukocyte subsets are also altered. Indeed, increased turnover, cell cycle perturbations, apoptosis, immune senescence and altered functionality among CD8 T cells, B cells, and innate immune cells are also observed. Among both CD4 and CD8 T cells, loss of essential naïve and central memory cell populations occurs, resulting in an increased frequency of short-lived effector cells. Among natural killer (NK) cells, altered functionality, including decreased cytokine production, increased cytotoxicity, and dysfunctional homing has been observed. Defective B cell responses have also been reported in HIV infection, including hyperactivation of B cells and dysregulation of B cell subsets, leading to altered antibody production. Dendritic cell (DC) subsets are also significantly altered, including abnormal levels of plasmacytoid and myeloid DCs, and loss of mucosal CD103+ DCs. Macrophages are also dysfunctional during HIV infection, with a reduced capability for phagocytosing bacterial products in mucosal tissues, and in addition, may be targets for HIV infection. High plasma levels of proinflammatory cytokines such as interferon-α (IFN-α), interleukin-1 (IL-1), IL-6, IL-18 and tumor necrosis factor-α (TNF-α) are also thought to result from innate cell activation. Finally, lymphoid tissue fibrosis, manifested by collagen deposition in lymphoid tissues and associated with expression of transforming growth factor-β (TGF-β), limits immunological responses and CD4 T cell restoration, even after initiation of cART.

These cumulative immunological abnormalities of both the innate and adaptive arms of the immune system have been referred to as immune activation, given that they seem to result from the immune system being overtly activated. The degree to which the immune system is activated is of extreme importance to HIV-infected individuals considering that it represents the strongest correlate of disease progression, morbidity, and mortality. Thus, chronic, generalized immune activation is far reaching, and it is really no surprise that untreated individuals eventually succumb to opportunistic infections, given the vast dysfunction of the immune system during progressive HIV disease.

The mechanisms which underlie immune activation during HIV infection are multifaceted and complex, and are likely both direct and indirect. One factor which can directly contribute to immune activation is viral replication. Direct stimulation of the immune system by HIV can occur via at least two mechanisms; stimulation of HIV-specific T and B cells and stimulation of innate cells via viral RNA binding to Toll-like receptor 7 (TLR7) and TLR8. However, the extent to which the immune system is activated during chronic HIV infection cannot solely be attributed to the virus itself. Indeed, the immune
compromised state associated with chronic HIV infection can lead to reactivation of viruses normally controlled by the immune system including Epstein Barr virus (EBV) and cytomegalovirus (CMV). Replication of these viruses can also contribute to immune activation via recognition of virally infected cells by antigen-specific T and B cells and viral products binding to pattern recognition receptors. One of the direct consequences of activation of the immune system is secretion of proinflammatory cytokines, such as IFN-α, TNF-α, IL-1, IL-6 and IL-18, which can contribute to additional immune activation and apoptosis of immune cells. High levels of systemic proinflammatory cytokines can also decrease the immune system’s ability to maintain healthy levels of lymphocytes that normally produce homeostatic effector cytokines such as IL-17 and IL-22. This skewing away from IL-17/IL-22+ lymphocytes likely contributes to immune activation, particularly in mucosal tissues where cells producing effector cytokines such as IL-17 and IL-22 are critical to maintain the integrity of the epithelial barrier. Indeed, an inability to maintain immunological and epithelial integrity of the GI tract results in translocation of luminal antigens, including microbial products, into peripheral circulation. Microbial products, which can directly stimulate the immune system, in peripheral circulation and other anatomical sites have been shown to contribute to immune activation in HIV-infected individuals and progressively SIV-infected Asian macaques.

Evidence for microbial translocation

The human GI tract is colonized with approximately 10^{14} normal flora bacteria, which live in symbiosis with the host and can enhance immune function. These organisms are essential for efficient metabolic function and digestion, and the human body has coevolved with microbiota leading to a symbiotic relationship that promotes a healthy GI tract. Given the critical importance for these bacteria to avoid direct contact with the systemic immune system, several structural and immunological host factors exist to prevent microbial products from translocating from the lumen of the intestine. These include a physical barrier of epithelial cells, intestinal IgA, and a network of immune cells such as macrophages, dendritic cells (DCs), T cells, and innate lymphoid cells. However, during HIV infection, there is an assault to several of these protective mechanisms, and microbial products translocate into the lamina propria of the GI tract, and, eventually, into systemic circulation.

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. Furthermore, the levels of lipopolysachharide (LPS) in these individuals directly correlated with activation of both the adaptive and innate arms of the immune system. These data provided insights into an underlying cause of immune activation observed during HIV infection. In the six years since these data were published, microbial translocation during pathogenic HIV or SIV infections was corroborated by several groups, with at least 44 reports of microbial translocation occurring during progressive HIV or SIV infection. Microbial translocation is not a phenomena isolated to HIV infection. Indeed, microbial translocation has been reported in several diseases, including inflammatory bowel diseases (IBD), infection by hepatitis C virus (HCV), alcoholism, and cardiovascular disease.

Microbial translocation and immune activation

While the degree to which microbial translocation, viral replication (e.g. by HIV, CMV, and EBV), and proinflammatory cytokines cause immune activation in progressively HIV or SIV-infected individuals is unclear, several specific effects of microbial product-mediated
immune activation can be considered. Indeed, chronic TLR activation in HIV-disease, through recognition of translocated bacterial products and/or viral products, can cause dysregulation of immune responses\textsuperscript{30, 75} and has been potentially linked to a number of co-morbidities, including dementia in AIDS patients\textsuperscript{8}.

Monocytes and macrophages express a variety of pattern recognition antigen receptors, and stimulation of these cells via these receptors can contribute to inflammation and cardiovascular disease\textsuperscript{76}. Specifically, increased levels of activated monocyte subsets are observed in progressively HIV or SIV-infected individuals and are likely being activated by a variety of microbial products\textsuperscript{8, 77}. Moreover, elevated plasma levels of soluble markers of monocyte and macrophage activation (sCD14 and sCD163) have been reported in HIV infection\textsuperscript{38, 78}. Furthermore, plasma levels of sCD14 (a bacterial LPS receptor) independently predict mortality in cART-treated HIV-infected individuals and plasma levels of sCD163 (a scavenger receptor) are associated with unstable non-calcified coronary plaques in cART-treated HIV-infected individuals\textsuperscript{38, 78}. Thus, chronic innate immune activation may directly contribute to the increased thrombosis risk observed in HIV-infected individuals. Indeed, monocytes and platelets in peripheral blood of HIV-infected individuals express high levels of tissue factor\textsuperscript{18, 79}. Tissue factor can initiate the extrinsic clotting pathway, potentially leading to increased plasma levels of D-dimers (products of fibrinolysis), which have been linked to mortality (all causes) in HIV infected patients\textsuperscript{4}. Moreover, in SIV-infected non-human primates, levels of several coagulation biomarkers, including D-dimers, have been linked to plasma LPS levels; potentially linking thrombosis and cardiovascular disease risk to microbial translocation\textsuperscript{21, 40}.

While recognition of bacterial products by TLR-expressing myeloid cells likely contributes to immune activation and the related co-morbidities observed in HIV-infected individuals, LPS exposure can also contribute to increased T cell turnover in HIV infection. Indeed, increased central memory CD4 T cell turnover is an important aspect of immune activation\textsuperscript{58} and turnover of CD4 central memory T cell pools may contribute to the depletion of other CD4 T cell populations in progressive HIV and SIV infections\textsuperscript{59}. Interestingly, exposure of peripheral blood mononuclear cells to bacterial TLR ligands \textit{in vitro} results in increased cell cycling and death of memory CD4 T cells, but not CD8 T cells\textsuperscript{51}, presenting a potential model whereby microbial products may be contributing to increased CD4 T cell turnover in HIV and SIV infection independent of antigen recognition via T cell receptors.

Increased plasma levels of bacterial products are associated with increased turnover of GI tract CD4 T cells and alterations in relative frequencies of individual subsets of GI tract T cells\textsuperscript{15}. Indeed, microbial translocation has been linked to an increased proportion of T regulatory cells compared to T helper 17 (Th17) cells, as well as dysregulation of GI tract γδ T cells in SIV-infected macaques\textsuperscript{44, 80}. Furthermore, low frequencies of GI tract Th17 cells persist in cART-treated, HIV-infected individuals, which is associated with ongoing microbial translocation\textsuperscript{31}. These T cell dysfunctions in the GI tract may be due to decreased homing of T cells to the gut and/or skewing of local T cell populations\textsuperscript{39}, however the precise mechanism(s) that connect T cell subset alterations, T cell turnover and dysfunctionality, damage to the structural barrier of the GI tract and microbial translocation requires further exploration.

SIV infected natural hosts do not progress to AIDS despite high levels of virus replication, and do not have evidence of microbial translocation. This may help explain the lack of immune activation and disease progression in chronically SIV-infected natural hosts\textsuperscript{7, 55}. Furthermore, experimental administration of bacterial products in natural hosts induces immune activation, which is in turn associated with increased viral loads and CD4 T cell

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depletion81. These data support a role of microbial translocation in immune activation and disease progression during progressive HIV and SIV infections. Indeed, microbial translocation can induce immune activation in the absence of HIV or SIV infections. For example, in pigtail macaques, which have increased levels of damage to their GI tract even in the absence of SIV infection, microbial translocation into the lamina propria of the GI tract correlates with immune activation, both locally in mucosal tissues and systemically21. Furthermore, in idiopathic CD4 lymphocytopenia (ICL), a disorder characterized by low CD4 counts without HIV infection, high CD4 T cell activation directly correlates with increased microbial translocation82. Given these studies, microbial translocation can drive activation of the immune system, even in the absence of chronic virus infection.

**Causes of microbial translocation**

Several key mechanisms underlying microbial translocation have recently been identified (Figure 1). One defense mechanism which protects against microbial translocation is the structural barrier of the GI tract. The tight epithelial barrier is a single layer of cells which serves to protect the body from translocating bacteria and to absorb luminal nutrients72. During HIV or SIV infection, the integrity of the tight epithelial barrier of the GI tract is compromised, which has been demonstrated by focal breaches in the barrier, decreased expression of epithelial repair tight junction genes, and increased GI tract permeability16, 24, 50, 83. Indeed, focal breaches of the epithelial barrier are in spatial juxtaposition to microbial translocation in vivo, and the extent of damage to the GI tract barrier during HIV and SIV infection is directly associated with the degree of microbial translocation, both locally within the GI tract and systemically16, 24, 50. Similarly, even in the absence of lentiviral infection, damage to the tight epithelial barrier correlates with local levels of LPS, demonstrating the importance of maintaining barrier integrity to avoid microbial translocation21.

Maintenance of GI tract structural integrity is dependent, in part, on a functional mucosal immune system. Indeed, Th17 cells, which respond to bacterial products and tend to localize to the GI tract, are significantly depleted during HIV and SIV infection, which is associated with GI tract structural damage, microbial translocation and immune activation19, 42, 71. Furthermore, transcriptional profiling of IL-17 producing lymphocytes demonstrates that these cells express several genes important for homeostasis of epithelial cells, including IL-2242. Furthermore, innate lymphoid cells and specialized NK cells that produce IL-17 and IL-22 are associated with a healthy GI tract barrier, and are lost after SIV and HIV infection42, 84. Other mechanisms may contribute to damage to the barrier in addition to loss of IL-17 and IL-22 producing cells in the GI tract. Indeed, HIV replication itself can drive epithelial damage, likely due to the production of proinflammatory cytokines which can directly induce apoptosis of epithelial cells24.

In a healthy individual, if microbes traverse the epithelial barrier and enter the lamina propria, several immune mechanisms exist to kill or transport the bacteria back to the lumen. However, the massive dysfunction of the mucosal immune system during HIV and SIV infection fails to prohibit systemic microbial translocation once microbial products enter the lamina propria (Figure 1). As discussed above, specialized GI tract macrophages are essential in the clearance of microbial products from the lamina propria, however, these cells are dysfunctional during HIV infection18. Another immunological irregularity observed during HIV and SIV infection is B cell dysfunction, including significantly lower levels of IgA62, 85. IgA antibodies are a key component of the mucosal immune system, and in particular are essential in protection against microbial translocation as IgA can directly bind bacteria and prohibit microbes from binding to the epithelium, can neutralize the bioactivity...
of bacterial products, and IgA can transport bacteria from the lamina propria back into the lumen.\textsuperscript{73}

Taken together, it appears that dysfunction of the GI tract immune system is multifaceted, includes alterations of lymphocyte and myeloid subsets, as well as depletion of CD4 T cells, and this overall dysfunction contributes to microbial translocation. However, whether the immunological abnormalities cause microbial translocation or whether microbial translocation, immune activation, and immunological abnormalities are all dependent upon one another is unclear. Most likely, damage to the epithelial barrier of the GI tract, mucosal immune dysfunction, CD4 T cell depletion, microbial translocation, immune activation, and chronic virus replication all are part of a chronic and perpetual cycle which drive HIV disease progression.

**Microbial translocation despite suppressed viremia**

Current cART treatments can suppress viremia to undetectable levels for the life of an individual and their use vastly improves the life expectancy of HIV-infected individuals. Further, suppressed viremia diminishes the majority of immune activation contributed to direct HIV replication. However, despite this virus suppression, cART-treated individuals still have increased morbidity and mortality compared to HIV-uninfected individuals, which, is associated with ongoing immune activation\textsuperscript{4-6}. One factor contributing to immune activation despite virus suppression may be ongoing low levels of virus replication from reservoirs that are maintained despite cART. Indeed, complete eradication of HIV is likely impossible even with robust treatments\textsuperscript{86}. Thus, persistence of latently HIV-infected cells may contribute to the ongoing immune activation observed during cART treatment. Another factor which may contribute to immune activation and microbial translocation despite cART is co-infection with other viruses, such as herpes and hepatitis viruses. Indeed, coinfection with hepatitis viruses during HIV infection is associated with higher levels of microbial translocation\textsuperscript{87}.

Microbial translocation likely underlies a substantial amount of the ongoing immune activation in cART-treated individuals. Indeed, while microbial translocation is lower in HIV-infected, cART-treated individuals than untreated individuals, it is significantly higher than in uninfected subjects.\textsuperscript{7} High levels of microbial translocation are associated with immune activation and limited immune reconstitution in HIV-infected pediatric patients on cART\textsuperscript{88}. During cART treatment, individuals can be broadly classified in two groups: immunological responders, which exhibit CD4 T cell reconstitution and slightly decreased immune activation, and immunological non-responders (INR), who maintain high immune activation and low levels of CD4 T cells\textsuperscript{89-92}. Several groups have demonstrated that lack of immune restoration in these INRs is associated with increased levels of microbial translocation\textsuperscript{10, 12, 26, 36, 45, 93, 94}. In addition, the bacterial products which translocate in INRs are polymicrobial and these individuals tend to lose beneficial flora such as the probiotic bacteria Lactobacillaceae\textsuperscript{36}. Furthermore, in cART-treated individuals, there is an inverse correlation between levels of microbial translocation and CD4 T cell reconstitution\textsuperscript{11, 12}, as well as decreased homing of CD4 T cells to the GI tract\textsuperscript{39}. Also, persistence of HIV-infected cells within the GI tract of cART-treated individuals is associated with microbial translocation\textsuperscript{32}. Perhaps the data which most convincingly demonstrate negative effects of microbial translocation are based on findings that levels of microbial translocation independently predict mortality in cART-treated, HIV-infected individuals\textsuperscript{38}.

Ongoing mucosal immune dysfunction during cART treatment likely underlies the microbial translocation observed. Indeed, while cART induces peripheral CD4 T cell reconstitution,
this reconstitution is limited in the GI tract\textsuperscript{52}. Collagen deposition in lymphoid tissues of the GI tract is not resolved during cART treatment, which may prohibit GI tract CD4 T cell reconstitution\textsuperscript{66}. However, it may not just be bulk CD4 T cell reconstitution that underlies dysfunctional mucosal immunity, as Th17 cells, which are essential for maintaining homeostasis of epithelial cells, are not reconstituted despite cART, which may exacerbate the ongoing GI tract dysfunctions\textsuperscript{71}. Moreover, during cART, damage to the tight epithelial barrier of the GI tract still occurs, including decreased epithelial repair genes and increased enterocyte apoptosis\textsuperscript{95}. Thus, despite suppression of viral replication by cART, mucosal immune dysfunctions persist and likely underlie microbial translocation.

**Therapeutic interventions to decrease microbial translocation**

Given the GI tract dysfunctions that lead to microbial translocation and immune activation despite cART, in order to decrease morbidity and mortality in cART-treated individuals, therapeutic interventions aimed at improving GI tract function should be investigated (Box 1). Indeed, several studies have demonstrated promising results in enhancing mucosal immunity during HIV and SIV infection. For example, PD-1 blockade reduces immune activation and microbial translocation by decreasing type-I IFN signaling in the GI tract\textsuperscript{96}. A particularly exciting area of research is modulation of the composition of the GI tract microbiota, and effects of probiotic administration during HIV and SIV infection. Several studies in mice and humans have demonstrated that probiotics enhance mucosal immunity\textsuperscript{97}. Indeed, probiotic administration in HIV-infected individuals has shown promising results with modest improvement in CD4 T cell levels in the periphery, as well as decreased GI disorders\textsuperscript{98, 99}. Given that loss of IL-17 and IL-22 producing lymphocytes is associated with damage to the GI tract\textsuperscript{42}, future studies aimed at increasing these cell populations in mucosal tissues will be essential. Other potential interventions to enhance these cells may be treatment with retinoic acid or probiotic treatments that include increased segmented filamentous bacteria species, both which have been shown to induce IL-17 production by T cells\textsuperscript{100, 101}. Furthermore, enhancing the epithelial barrier itself will be essential, with candidates such as probiotics and glucagon-like peptides, which have shown to enhance epithelial barrier integrity in mice\textsuperscript{102}.

Use of mucosal adjuvants in vaccination strategies may also provide improved GI tract immunity. Indeed, use of *Escherichia coli* enterotoxin with HIV DNA therapeutic vaccines reduces viral burden in both the GI tract and periphery\textsuperscript{103}. Furthermore, administration of HIV virus like particles with CCL28, which recruits IgA-secreting plasma cells in the GI tract lamina propria, induces enhanced IgA responses in the gut\textsuperscript{104}, which may be essential for improving mucosal immunity. In addition, vaccination using *Clostridium perfringens* expressing HIV-1 Gag induced robust HIV-specific responses in the GI tract\textsuperscript{105}. Improving mucosal immunity and decreasing damage to the tight epithelial barrier of the GI tract through either treatment strategies or therapeutic vaccination may decrease morbidity and mortality in HIV-infected individuals, thus future studies into potential treatments will be essential.

**Concluding remarks**

During HIV infection, the strongest predictor of disease progression is immune activation, which persists despite cART and is associated with morbidity and mortality. One cause of immune activation is microbial translocation, which occurs during HIV infection and is associated with driving immune activation and mortality even during cART treatment. Altered mucosal immunity and a damaged tight epithelial barrier of the GI tract likely underlie microbial translocation during HIV infection, and developing therapeutic
interventions aimed at restoring GI tract immunity and integrity may decrease HIV disease progression.

References


### Box 1. Outstanding questions

- How much immune activation is directly caused by microbial translocation during HIV infection?
- When does microbial translocation begin during HIV infection?
- What underlies dissemination of microbial products locally versus systemically?
- Which host factors are involved in control of microbial translocation both locally and systemically?
- Does dysbiosis play a role in microbial translocation?
- What therapeutic interventions may decrease microbial translocation?
Figure 1.
Mechanisms underlying damage to the tight epithelial barrier of the GI tract and microbial translocation during HIV infection. (a) In the absence of HIV infection, retinoic acid produced by CD103+ DCs helps to drive T cells and innate lymphoid cells (ILC) to produce IL-17 and IL-22, which contributes to a healthy epithelial barrier. B cells produce IgA in the lamina propria, which regulate microbial products in the lumen and prohibit microbes (purple rods) from interacting with and translocating across the epithelial barrier. Macrophages (Mφ) are capable of phagocytosing any bacterial products that translocate without a proinflammatory milieu. (b) However, during HIV infection, IL-17 and IL-22 producing lymphocytes are lost, both by direct infection of CD4+ T cells by HIV, and potentially by skewing of homeostatic responses to proinflammatory responses such as IFN-α production by plasmacytoid dendritic cells (pDCs). Further, hyperactivated macrophages no longer phagocytose bacterial products but respond with inflammatory cytokines such as TNF-α and IFN-α. In addition, decreased IgA may lead to decreased control of microbial products. Inflammation and loss of homeostatic cytokines such as IL-17 and IL-22 lead to damage to the tight epithelial barrier of the GI tract, allowing microbial products to translocate and furthering inflammation.