Microbial Translocation in the Pathogenesis of HIV Infection and AIDS

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SUMMARY
In pathogenic simian immunodeficiency virus (SIV) and human immunodeficiency virus (HIV) infections, the translocation of microbial products from the gastrointestinal (GI) tract to portal and systemic circulation has been proposed as a major driver of the chronic immune activation that is associated with disease progression. Consistently, microbial translocation is not present in nonpathogenic SIV infections of natural host species. In vivo studies demonstrated that HIV/SIV-associated microbial translocation results from a series of immunopathological events occurring at the GI mucosa: (i) early and severe mucosal CD4+ depletion, (ii) mucosal immune hyperactivation/persistent inflammation; (iii) damage to the integrity of the intestinal epithelium with enterocyte apoptosis and tight junction disruption; and (iv) subverted the gut microbiome, with a predominance of opportunistic bacteria. Direct in situ evidence of microbial translocation has been provided for SIV-infected rhesus macaques showing translocated microbial products in the intestinal lamina propria and distant sites. While the mechanisms by which microbial translocation causes immune activation remain controversial, a key pathogenic event appears to be innate immune activation via Toll-like receptors and other pathogen recognition receptors. Accumulating clinical observations suggest that microbial translocation might affect HIV disease progression, response to therapy, and non-AIDS comorbidities. Given its detrimental effect on overall immunity, several interventions to prevent/block microbial translocation are currently under investigation as novel therapeutic agents for HIV/AIDS.

INTRODUCTION
The distinctive feature of untreated human immunodeficiency virus (HIV) infection is the progressive depletion of CD4+ T lymphocytes from blood, lymphoid organs, and mucosal tissues. While HIV infects and kills CD4+ T lymphocytes, virus cytopathicity alone is unlikely to provide a satisfying explanation for the course of the disease, which seems to result from complex virus-host interactions ultimately leading to chronic immune system activation and a substantial disruption of T-cell homeostasis (1). Studies conducted using both simian immunodeficiency virus (SIV)-infected macaques and HIV-infected individuals have clearly demonstrated that this immune dysfunction arises within a few weeks from virus transmission (acute infection), when viral dissemination at mucosal tissues results in the massive depletion of mucosal CCR5+ CD4+ T cells from direct and indirect virus cytopathicity (2, 3) as well as CD8+ T-cell-mediated killing of infected CD4+ T cells (4). CD4+ T-cell depletion is particularly rapid within the gastrointestinal (GI) tract, where a significant fraction of T lymphocytes reside, and is relatively slower in peripheral blood.

During the transition from acute to chronic infection, HIV

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replication is reduced but not fully suppressed by the host adaptive immune response, and during the long chronic phase of the infection, the CD4+ T-cell loss continues to progress. Importantly, the tempo of HIV- and SIV-associated CD4+ T-cell depletion is related to both the set-point level of viremia and the magnitude of virus-associated chronic immune activation (1, 5). HIV/SIV-associated chronic immune activation is characterized by polyclonal B-cell activation (6), high T-cell turnover of both CD4+ and CD8+ T cells with an enhanced cell surface expression of molecules indicative of cell activation (7), and high levels of circulating proinflammatory cytokines and chemokines (8).

HIV/SIV-associated chronic immune activation is not just a consequence of continuous viral replication but rather represents a complex and multifactorial phenomenon that ultimately plays a major role in disrupting T-lymphocyte homeostasis, affecting normal immune function, and even compromising the response to antiviral therapy (reviewed in reference 9). In addition, newly generated activated T cells are themselves targets of HIV infection, in turn sustaining viral replication (10) and therefore creating a self-perpetuating vicious circle between HIV replication and immune activation. For these reasons, it is not surprising that the magnitude of this chronic immune activation has been long identified as a major determinant of disease progression independent of the level of virus replication (5, 11, 12). Of note, antiretroviral therapy (ART), which results in the complete suppression of HIV replication (5, 11, 12), of the level of virus replication (5, 11, 12). Of note, antiretroviral therapy (ART), which results in the complete suppression of HIV replication (5, 11, 12), and, indeed, HIV-infected individuals with poor CD4+ recovery on virologically suppressive ART often exhibit higher levels of immune activation (13, 14). In support of an independent pathogenic role of chronic immune activation is the classical observation that nonpathogenic SIV infection in natural nonhuman primates such as sooty mangabeys and African green monkeys is associated with high levels of viral replication and low levels of immune activation (15).

The mechanisms causing HIV/SIV-associated immune activation remain incompletely understood, and it is possible that specific factors contribute differently to this phenotype in different subsets of patients. The mechanisms that have been proposed to cause HIV-associated immune activation include (i) the direct effect of specific virus gene products (Env, Nef, and Tat, etc.), (ii) the innate and adaptive immune responses to the virus, (iii) an ineffective regulation of normally generated antiviral immune responses, (iv) bystander activation of T and B lymphocytes caused by an increased level of production of proinflammatory cytokines (e.g., tumor necrosis factor alpha [TNF-α], interleukin-1 [IL-1], IL-6, and several others), (v) the presence of clinical or subclinical coinfections, and, more recently, (vi) the preferential infection of central memory CD4+ T cells (as opposed to effector memory CD4+ T cells) as a factor responsible for concentrating the bulk of the antigenic load in central lymphoid tissues (16, 17). In this rather complex immunopathogenic context, the translocation of microbial products from the intestinal lumen to the systemic circulation appears to be a central factor that determines the severity of HIV/SIV-associated chronic immune activation.

The specific purpose of this article is to comprehensively review the literature on the pathogenic role of microbial translocation in the setting of SIV and HIV disease, its possible causes, and the consequences in terms of the loss of immune function, HIV disease progression, and response to therapy. An understanding of the mechanisms responsible for HIV-associated immune activation and microbial translocation will set the basis for the development of therapeutic strategies to prevent or reduce this phenomenon and thus improve the prognosis of HIV infection.

DEFINITION OF MICROBIAL TRANSLOCATION

Gut “translocation” of bacteria or other microbes is defined as the nonphysiological passage of the gastrointestinal microflora through the intestinal epithelial barrier and the lamina propria and eventually to local mesenteric lymph nodes and, from there, to extranodal sites (18–20). Under normal circumstances, translocating microbes and microbial products are phagocytosed within the lamina propria and the mesenteric lymph nodes (21). However, if the host mucosal immune system is compromised, these defense mechanisms may fail, thus permitting the egress and survival of bacteria at distant, extraintestinal sites (21–23).

Lipopolysaccharide (LPS), a component of Gram-negative bacterial cell walls and a known agonist of Toll-like receptor 4 (TLR-4) (24), is considered a major marker of microbial translocation (25–27). In addition to local defense against microbial translocation at the level of the GI mucosa and within the liver, several lines of protection are active in the systemic circulation to neutralize translocating LPS and its downstream effects as a potent immune-activating molecule, thus limiting the detrimental effects of microbial translocation. These protective factors include IgM, IgG, and IgA specific for the LPS core antigen and endotoxin core antibodies (EndoCAb), and indeed, clinical conditions featuring an acute excess of circulating endotoxin result in the consumption of EndoCAb (e.g., sepsis) as they bind and neutralize LPS (28). In other clinical settings (e.g., inflammatory bowel disease [IBD]), ongoing chronic microbial translocation results in elevated LPS levels that are associated with high EndoCAb titers. Importantly, LPS induces several responses in the innate immune system, with the interaction of LPS with LPS binding protein (LBP), which catalytically transfers LPS onto membrane or soluble CD14 (sCD14), leading to NF-κB activation and cytokine production (29), thus determining a state of aberrant immune activation.

Microbial translocation has been described for various diseases, with immunocytochemical evidence for a role of bacteria such as Listeria, Escherichia coli, and Streptococcus in the pathogenesis of Crohn’s disease (30). Similarly, in the mouse model of graft-versus-host disease following stem cell transplantation, microbial translocation negatively affects the expression of intestinal α-defensins, leading to the outgrowth of E. coli, which causes sepsis (31). High levels of endotoxemia have also been described for subjects who have undergone laparotomic cholecystectomy, suggesting that intestinal manipulation may impair gut mucosal barrier function (27). The idea that microbial translocation is a key mechanism of mucosal immune dysfunction, chronic systemic immune activation, and, ultimately, disease progression during HIV infection was originally proposed by Brenchley et al. in a landmark hypothesis article (32). Several months later, those authors reported significantly increased levels of circulating LPS in both chronically HIV-infected individuals and SIV-infected rhesus macaques (RM) that positively correlated with measures of innate and adaptive immune activation (33). After that initial report, numerous studies have confirmed that HIV infection is associated with a substantial loss of CD4+ T cells residing in the gut, in turn leading to microbial translocation (34, 35).

Translocating bacteria and microbial components may stimulate innate immune cells through the TLR pathways as well as
other innate immune receptors, thus contributing to the proinflammatory cytokine milieu and systemic immune activation associated with chronic HIV infection (33). Consistent with the above-mentioned findings on several biological and pathological models, acute HIV infection is associated with relatively normal LPS levels, with increased LBP, sCD14, and EndoCAb levels compared to levels in uninfected individuals, thus suggesting that the ongoing translocation of LPS is rapidly counteracted by the host Ig response. However, EndoCAb titers decrease progressively as HIV infection enters its chronic phase, which becomes characterized by higher plasma levels of LPS.

**MEASUREMENT OF MICROBIAL TRANSLOCATION**

The extent of microbial translocation can be assessed either directly through the measurement of bacterial by-products in plasma, such as LPS and bacterial DNA or RNA fragments, or indirectly by sCD14, LBP, EndoCAb, and antiflagellin antibodies. Recently, plasma levels of intestinal fatty acid binding protein (I-FABP), a marker of enterocyte damage (36), have also been used to correlate intestinal impairment and microbial translocation (37, 38). Table 1 summarizes the characteristics of the principal microbial translocation markers measured in plasma/serum of HIV-infected patients. In the majority of published studies, sCD14, LBP, and EndoCAb are measured in the plasma or serum by a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA), which generally produces reliable and reproducible results (47). In particular, sCD14 has been widely used as a marker of microbial translocation, given the relatively easy standardization of its measurement between different laboratories. It must be noted, however, that sCD14 serves as a biomarker of monocyte activation (29), and although it correlates with LPS, it is not a direct and specific marker of microbial translocation per se. Conversely, the commercial *Limulus* amebocyte lysate (LAL) assay allows for the quantitative determination of LPS in reference to known endotoxin concentrations and is therefore a direct measure of endotoxemia. However, the assay presents technical difficulties due to possible reagent contamination and the inhibitory nature of some plasma components which have rendered the assay difficult to reproduce. The pretreatment of samples by heating has been shown to provide more reliable results and is thus mandatory for plasma LPS measurements using the LAL technique (48). Finally, it should be remembered that as LPS is a specific product of the cell wall of Gram-negative bacteria, the LPS assay fails to detect translocating Gram-positive bacteria.

An alternative method to assess microbial translocation is the detection and quantification of the universally conserved microbial 16S rRNA gene in plasma (49). The majority of 16S rRNA gene PCR assays are designed for the qualitative detection of amplicons, which allows for the identification of different bacterial species (39, 40, 50). Novel and highly sensitive real-time PCRs have recently been developed, focusing on the accurate quantification of 16S rRNA gene sequences (45, 46, 51, 52). However, exogenous DNA contamination during workflow or the presence of DNA traces in PCR reagents, including *Taq* DNA polymerase (46), the removal of human background DNA, and bacterial DNA enrichment (55). Despite the attempts to implement sensitive techniques to detect traces of microbial translocation in peripheral blood of HIV-infected patients, concerns remain regarding the risk of contamination. Bacterial flagellin is known as a microbial compound with strong immune-modulatory properties that has an essential role under conditions of intestinal damage, such as inflammatory bowel disease. Recently, elevated levels of flagellin-specific IgG were described for three cohorts of HIV-1-infected patients with a significantly elevated ratio of flagellin-specific IgG to total IgG (56) and were subse-
sequently demonstrated to be reduced following highly active ART (HAART) despite not reaching the levels in HIV-negative controls (57). Combined, these data suggest that flagellin should be considered an important microbial product that plays an important role in immune activation due to translocation from the gut.

### MICROBIAL TRANSLLOCATION IN THE PATHOGENESIS OF HIV AND SIV INFECTIONS

A seminal study by Brenchley et al. demonstrated for the first time that both chronically HIV-infected individuals and SIV-infected rhesus macaques exhibit significantly increased levels of circulating LPS. Particularly, HIV-infected patients in an advanced stage of disease (<200 CD4⁺ T cells/ml) had significantly higher plasma LPS levels than uninfected individuals, thus suggesting the presence of increased microbial translocation in this study population. Interestingly, a positive correlation was found between LPS levels in plasma and measures of innate and adaptive immune activation (33, 39), thus supporting the hypothesis that microbial translocation may result in T-cell activation, which is known to influence HIV disease progression (5, 11, 12, 58). Table 2 shows an overview of studies suggesting that microbial translocation through the gastrointestinal tract is a cause of immune activation and immune dysfunction in HIV/SIV infections.

The possibility that microbial TLR ligands might affect T-lymphocyte activation in HIV-uninfected individuals was elegantly investigated by Funderburg et al. (67). In that study, in vitro exposure to microbial TLR ligands promoted the cell surface expression of lymphocyte-homing and activation/apoptosis molecules on CD4⁺ and CD8⁺ T cells (67). Consistent with those observations, in vitro stimulation by microbial and viral TLR ligands was shown to induce T-cell activation in both antiretroviral-naïve and ART-treated, HIV-infected patients (68, 69). By extension, those in vitro findings suggested that systemic exposure to TLR ligands in HIV-infected patients results in heightened immune activation, effecting T-cell sequestration in lymphoid tissues, and T-cell turnover, thus reinforcing the hypothesis that microbial translocation is a crucial determinant of immune activation.

### Pathogenesis of HIV/SIV-Associated Microbial Translocation

From the early phase of acute HIV or SIV infection, and throughout the course of chronic disease, the gastrointestinal tract suffers from a substantial immunological and structural disruption, which includes the depletion of CD4⁺ T cells, with a preferential loss of the IL-17-producing subpopulation (Th17 cells), the establishment of local mucosal hyperactivation/inflammation, the exhaustion of intestinal macrophage phagocytic function, and structural epithelial damage (apoptosis of enterocytes and disruption of tight junctions, etc.). Collectively, these alterations may result in the increased passage of gut microflora and microbial products into the systemic circulation. Interestingly, several aspects of HIV-associated mucosal immune dysfunction were described prior to the original formulation of the microbial translocation hypothesis, including the presence of a rapid and severe loss of CD4⁺ T cells in the gastrointestinal tract of SIV-infected macaques and HIV-infected humans (34, 35, 70–72).

During acute HIV/SIV infection, CD4⁺ T-cell depletion is more prominent in the gastrointestinal tract than in peripheral blood and lymph nodes. Such severe CD4⁺ T-cell impairment has been shown to preferentially involve gut-residing memory-activated CD4⁺ CCR5⁺ T cells, reflecting the well-known prevalence of CCR5-tropic viruses in the early phases of infection. Almost concomitantly, Veazey et al. and Keweni et al. showed that more than 70% of intestinal CD4⁺ cells were depleted as early as 21 days post-SIVmac239 infection (72, 73), and several subsequent studies then confirmed these findings and shed further light onto the mechanistic pathways underlying CD4⁺ T-cell depletion (34, 74–76). Among those studies, Mattapalil et al. were able to demonstrate the presence of SIV DNA in up to 60% of intestinal CD4⁺ T cells as early as 10 days after experimental SIVmac251 infection, therefore emphasizing the role of direct virus-mediated killing of CD4⁺ T cells as a mechanism causing the observed depletion (70). However, Li et al., using in situ hybridization to detect SIV RNA, concluded that only ~7% of mucosal CD4⁺ cells were productively infected by SIV during acute infection, thus supporting the hypothesis that apoptosis-mediated death is a pivotal mechanism sustaining mucosal T-lymphocyte depletion (77). Similar data have also been obtained by serial gastrointestinal tract sampling in HIV-infected individuals during early stages of infection, although the depletion of intestinal CD4⁺ CCR5⁺ T cells appeared to be somewhat less dramatic than in the SIVmac infection model (34, 35, 71). Following the acute phase of infection, CD4⁺ T-cell depletion in the intestinal mucosa has been shown to continue throughout chronic disease to a much greater extent than in the peripheral blood and lymph nodes. Taken together, these data indicate that the depletion of gut CD4⁺ T cells is a multifactorial phenomenon, which involves both direct virus-mediated killing and bystander death of uninfected cells, with a subsequent impairment of the structure and function of the intestinal immune system and mucosal barrier, which ultimately leads to sustained microbial translocation.

Interestingly, studies of nonpathogenic SIV infection in the natural hosts of SIV, e.g., sooty mangabeys (SM) and African green monkeys (AGM), have also shown severe CD4⁺ T-cell depletion in mucosal tissues during acute infection (78, 79). However, this depletion is not accompanied by sustained microbial translocation and chronic mucosal immune activation, and in fact, in contrast to pathogenic SIV and HIV infections, the early mucosal CD4⁺ T-cell loss does not seem to progress further in SM and is even followed by a partial recovery in AGM (80). The latter findings notwithstanding, it remains intriguing that natural SIV hosts show no evidence of microbial translocation despite significant intestinal CD4⁺ T-cell loss, with this finding suggesting that mucosal CD4⁺ T-cell depletion is necessary but not sufficient for microbial translocation to ensue. In addition to the evolutionary hypothesis that natural SIV hosts have progressively adapted to maintain mucosal immunity despite reduced local CD4⁺ T-cell levels, several non-mutually exclusive mechanisms might be hypothesized to explain differences in microbial translocation between pathogenic SIV/HIV infections and infections of natural SIV hosts. First and foremost, it is possible that a different “quality” of CD4⁺ T-cell depletion in natural versus nonnatural SIV hosts at the intestinal mucosal level plays a major role. Indeed, both SIV-infected RM and HIV-infected persons experience a significant and preferential reduction of the IL-17-producing CD4⁺ (Th17) and CD8⁺ (Tc17) T-cell subsets throughout the gastrointestinal tract (81–84). Similarly, the depletion of IL-17-producing innate lymphocytes in the GI tracts of SIV-infected Asian macaques has been demonstrated (85, 86).

In marked contrast, natural SIV hosts have been shown to maintain frequencies of functional gut-based Th17 and Th1
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<td>33</td>
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<td>HIV-infected patients SIV-infected rhesus macaques</td>
<td>Higher levels of LPS in HIV-infected patients LPS level increases following SIV infection in RM Positive correlation between plasma LPS levels and circulating CD8⁺ CD38⁺ HLA-DR⁺ T cells Inverse correlation between plasma LPS levels and TNF⁺ IL-1⁺ monocytes upon LPS stimulation Inverse correlation between plasma LPS levels and plasma EndoCAB titers</td>
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<td>39</td>
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<td>INRs on HAART Full-responder patients on HAART Advanced naïve patients</td>
<td>Higher LPS levels in INRs and advanced naïve patients Positive correlation between LPS and proliferating Ki67⁺ CD8⁺ T cells in INRs Higher plasma enterobacterial DNA levels in INRs</td>
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<td>39</td>
<td>Cross-sectional</td>
<td>Case Western Reserve University Cohort, with (i) HIV-negative patients, (ii) HIV-positive naïve patients, (iii) HIV-positive treated patients with undetectable viremia, and (iv) HIV-positive treated patients with detectable viremia UCSF SCOPE Cohort ACTG study 5014</td>
<td>Increased bacterial 16S rRNA levels in HIV-infected patients Correlation of bacterial 16S rRNA with LPS in HAART-treated patients Correlation of 16S rRNA levels (i) directly with the frequency of CD38⁺ and HLA-DR⁺ CD8⁺ T cells and (ii) inversely with the degree of CD4⁺ restoration on HAART Decrease in 16S rRNA levels following HAART</td>
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<td>Correlation between Ki67-expressing T cells in the gut and plasma LPS levels</td>
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<td>Higher LPS levels in patients with AIDS than in controls Correlation between LPS levels, CD4⁺ T-cell lymphopenia, and HIV RNA load Inverse correlation between plasma LPS levels and expression of IL-12 and IFN-α following TLR stimulation No difference between HIV-1- and HIV-2-infected individuals within the same disease stage, CD4⁺ T-cell count, or viral load intervals</td>
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<td>39</td>
<td>Cross-sectional</td>
<td>SIV-uninfected pigtail macaques SIV-uninfected rhesus macaques HIV-negative controls</td>
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<td>39</td>
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<td>HIV-infected patients, HAART naïve HIV-infected patients on HAART HIV-negative controls</td>
<td>Decrease in activated CD14⁺ CD16⁺ monocytes, correlating with HIV RNA, in course of HAART Elevated sCD14 and TNF-α levels persist in course of HAART</td>
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<td>39</td>
<td>Cross-sectional</td>
<td>SIV-uninfected sooty mangabeys SIV-infected sooty mangabeys SIV-uninfected rhesus macaques SIV-infected rhesus macaques</td>
<td>Highest LPS levels in lamina propria of chronically infected/AIDS animals Damage of epithelial barrier associated with infiltration of microbial products into the lamina propria Correlation between damage of the epithelial barrier and microbial translocation (highest in chronically infected/AIDS animals)</td>
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As intestinal Th17 cells have an important role in antimicrobial immunity, particularly at mucosal sites (88–90), as well as in sustaining enterocyte homeostasis (89, 90), the preferential deple- tion of Th17 cells in pathogenic HIV/SIV infections may be involved in the damage to the structural and functional integrity of the intestinal barrier and in the loss of control over microbial translocation. Indeed, Favre et al. elegantly demonstrated that the direct administration of LPS in a natural host of SIV (which lacks chronic immune activation and microbial translocation) results in increased immune activation and viral replication (91, 92).

However, while it is commonly accepted that the structural and functional damage of the intestinal mucosa barrier and immune function results in an increased permeability of the mucosal epithelium (93–96) and a consequent unchecked systemic translocation of bioactive microbial products that can trigger immune activation, it remains unclear to what extent this phenomenon contributes to HIV/SIV-associated chronic immune activation. For instance, one could also hypothesize a scenario in which chronic immune activation is the initial driver of mucosal immune dysfunction and that microbial translocation is a secondary amplification of a loop that further aggravates immune activation (9, 80).

While reciprocal interactions between immune activation and microbial translocation are most likely to occur during pathogenic HIV/SIV infections, evidence of microbial translocation as a factor driving pathological immune activation in HIV-infected

**MICROBIAL TRANSLOCATION AND IMMUNE ACTIVATION: WHAT IS THE CAUSE AND WHAT IS THE EFFECT? LESSONS LEARNED FROM THE ANIMAL MODEL**

Following multiple literature reports that strongly suggested an association between immune activation and microbial translocation parameters in HIV-infected patients (Table 2), two studies demonstrated that the direct administration of LPS in a natural host of SIV (which lacks chronic immune activation and microbial translocation) results in increased immune activation and viral replication (91, 92).

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<td>40</td>
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<td>HIV-infected patients starting HAART with CD4+ counts of &lt;200 cells/µL</td>
<td>Persistence of high LPS and sCD14 levels despite suppressive HAART. Lack of probiotic Lactobacillaceae DNA in plasma both prior and after therapy in patients with CD4+ counts of &lt;200 cells/µL following HAART.</td>
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<td>64</td>
<td>Cross-sectional</td>
<td>HIV-infected patients, HAART naive, HIV-infected patients on HAART, HIV-negative controls</td>
<td>Negative correlation between total bacterial load and duodenal activated CD4+ and CD8+ T cells. Positive correlation between fecal Bacteroidales (rRNA) and activated, peripheral CD8+ T cells. Negative correlation between fecal Enterobacteriales (rRNA) and duodenal CD4+ T cells.</td>
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<td>65</td>
<td>Cross-sectional</td>
<td>HIV-infected patients, HAART naive, HIV-infected patients on HAART, HIV-negative controls</td>
<td>Correlation between gut HIV DNA, plasma LPS, and peripheral CD8+ CD38+ T cells in HAART-treated patients.</td>
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<td>66</td>
<td>Cross-sectional</td>
<td>HIV-infected patients, HAART naive, HIV-infected patients on HAART, HIV-negative controls</td>
<td>Depletion of gut Th17 cells, which is normalized by HAART. Persistence of high LPS levels despite HAART. Inverse correlation was observed between plasma LPS levels and gut Th17 frequencies. Correlation between plasma LPS levels and gut HIV proviral reservoir.</td>
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<td>38</td>
<td>Cross-sectional</td>
<td>HIV-infected patients on HAART, HIV-negative controls</td>
<td>Positive correlation between LPS and sCD14 and gut-homing CD4+ T cells in the blood. Inverse correlation between LPS and sCD14 and gut-homing CD4+ T cells in the gut. Positive correlation between peripheral CD4+ Ki67+ cells and circulating gut-homing CD4+ T cells.</td>
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*Studies are listed in chronological order.*
humans is quite strong although not necessarily conclusive. In the SIV model, Estes et al. provided elegant in vivo evidence of the presence of microbial products, LPS and E. coli, in the colon lamina propria of SIV-infected RM but not of SIV-infected sooty mangabeys (63). In particular, using immunohistochemistry and confocal fluorescence microscopy, those authors were able to show the presence of LPS/E. coli in the colonic mucosa since the earliest phases of infection, with a progressive increase throughout chronic disease that is associated with epithelial barrier dysfunction, as documented by the multifocal disruption of the epithelial GI barrier via staining of the tight junction protein claudin-3. In that same study, LPS was also identified within systemic lymph nodes and liver, the amount of which positively correlated with colonic LPS, therefore confirming the systemic passage of gut-derived microbial elements (63). In addition, the magnitude of epithelial breakdown was correlated with the extent of microbial translocation and intestinal immune activation, as shown by the colocalization of bacterial products and proinflammatory cytokines (IFN-α and IL-18) in the colon lamina propria. Those authors took a further step by investigating the timing and dynamics of macrophage phagocytosis and concluded that intestinal macrophages appear free from bacterial cells during chronic infection, whose cell-free component is, on the contrary, increased (97). The latter findings suggest that the progressive exhaustion of intestinal macrophage phagocytic function, with the extracellular accumulation of microbial components, is also involved in the pathogenesis of HIV/SIV-associated microbial translocation. Overall, the available data strongly suggest that in SIV infection of RM and, by extension, in HIV infection of humans, a complex series of events leads to mucosal immune dysfunction, damage to the intestinal epithelial barrier, microbial translocation, and chronic systemic immune activation, all ultimately contributing to disease progression. However, the reciprocal contribution of these events to the immunopathogenesis of AIDS remains incompletely defined, and further studies of both humans and nonhuman primates will be needed to better elucidate the chain of events that leads to the progressive demise of the host immune system during pathogenic HIV/SIV infections.

CHARACTERIZATION OF STOOL MICROBES AND TRANSLOCATING FLORA IN HIV-INFECTED PATIENTS

Numerous studies have demonstrated a major influence of the gut microbiome in the maintenance of immunity and health by exerting many immunologic, metabolic, and structural functions. Germfree animals are in fact characterized by a dramatic disruption of gut integrity and the intestinal immune system, with increased susceptibility to infections (98). In these experimental models, the replication of the gut microbiota results in a reconstitution of the different components of mucosal immunity (99). Indeed, the specific composition of the gut microbiome is essential to maintain both local and systemic immunity, as recently reviewed by Paiardini et al. (80) and Brenchley and Douek (100). While several human diseases have been associated with a disruption of the gut microbiome (i.e., dysbiosis), it has somehow been difficult to establish with certainty whether and to what extent such a dysbiosis is the cause or the effect of the disease, given the influence of a multitude of potential pathogenic factors. In the macaque model, microbial communities residing in the gut have been described to differ substantially in both quantity and composition between healthy and diseased animal (101).

Interestingly, Gori et al. demonstrated that HIV-infected individuals in early stages of disease are characterized by an impaired fecal flora, with a predominance of opportunistic pathogens (Pseudomonas aeruginosa and Candida albicans) and low levels of protective bacteria (bifidobacteria and lactobacilli) compared with historical data on healthy HIV-uninfected persons (102). Altered stool composition has also been reported, with increased levels of fecal calprotectin, which is secreted by recruited neutrophils in the intestinal lining and has been proposed to be a marker of intestinal inflammation (103). Taken together, these data demonstrate a substantial subversion of physiological gut microbiome and a correlation between pathogenic intestinal flora and gut inflammation in HIV disease (102). In a recent study, Ellis et al. (64) explored the intriguing hypothesis that the gut microflora composition affects both local and systemic immunity, in analogy with what was described in several clinical settings (104, 105). Those authors observed a greater representation of proinflammatory/inflammation-thriving order-level bacteria, such as Enterobacteriales and Bacteroidales (both Gram-negative, LPS-containing pathogens associated with several clinical conditions) in the gut of HIV-infected subjects than in healthy controls. Those authors next investigated correlations between the stool bacterial populations and CD4 T-cell loss and immune activation at the intestinal and peripheral blood levels. Most interestingly, the total bacterial load in the stools negatively correlated with cytophagocytic T-cell activation, and levels of Enterobacteriales and Bacteroidales fecal DNA were significantly associated with CD4 T-cell loss in the duodenum and peripheral CD8 T-cell activation, respectively (64). Therefore, by correlating specific features of the gut microbiome and markers of HIV disease progression, these data provide evidence for a direct role of the gut microbiome in driving local and systemic immune activation in HIV-infected patients (39, 45).

Given the relationship between microbial translocation and CD4 T-cell homeostasis, based on our previous report on higher levels of microbial rRNA genes specific to Enterobacteriaceae in patients with incomplete immune reconstitution (39), our group investigated whether the composition of microflora translocating in peripheral blood may influence the degree of immunological recovery during the course of ART. In a longitudinal study aimed at assessing the composition of microbial products translocating in peripheral blood prior to and during ART, we reported that patients with a poor immunological response to therapy exhibited a translocating bacterial microflora enriched in Enterobacteriaceae, with no evidence of probiotic Lactobacillales spp. (40), which are known to possess immunomodulatory and anti-inflammatory properties (106), including the suppression of proinflammatory cytokine production from E. coli LPS-activated monocytes (83). This change in the composition of the gut microbiome might result in a disproportionate antigenic challenge that is not contained by counterregulatory bacterially derived factors, overall exacerbating immune activation in this population of HIV-infected individuals. Collectively, these data suggest that early changes in the composition of the intestinal microflora may affect local and systemic immune activation and, by extension, HIV disease progression and the response to therapy.

While intriguing, these data need to be corroborated by further research specifically designed to investigate (i) whether and to what extent the bacterial load and composition in the GI tract correlate with the microbiome in peripheral circulation by exten-
sive genetic sequencing in both compartments and (ii) possible direct correlations between the microbiome at both sites and markers of T-cell depletion and immune activation. By defining putative pathological mechanisms that link the gut/systemic microbiome with immune activation in HIV disease, these studies might provide a rational basis for the investigation of therapeutic approaches aimed at recovering the symbiotic host-microorganism relationships as a way to reduce HIV-associated chronic immune activation and to improve the response to antiretroviral therapy.

CLINICAL IMPLICATIONS OF MICROBIAL TRANSLLOCATION IN HIV DISEASE

Microbial Translocation Predicts HIV Disease Progression and Poor Response to ART

The association of mucosal immune dysfunction and microbial translocation with systemic immune activation and disease progression in the course of HIV infection prompts a number of questions of major clinical relevance. A first crucial issue is whether microbial translocation is a predictor of HIV disease progression independently of other established markers such as CD4+ T-cell counts and viral load. If such an association is established, the next questions would be, (i) should markers of microbial translocation be measured as part of the routine clinical and laboratory monitoring of HIV-infected individuals (107); (ii) should the level of markers of microbial translocation be used to make management and therapeutic decisions, e.g., on when to start/modify antiretroviral treatment, and (iii) how reversible is microbial translocation once HIV replication is suppressed by ART?

The role of microbial translocation in predicting disease progression in the absence of antiretroviral therapy as well as the effects of HAART have recently been investigated in several studies. For a cohort of HIV-infected individuals from Rakai, Uganda, who were monitored longitudinally from preinfection to death for levels of proinflammatory cytokines and microbial translocation, Redd et al. failed to find significant associations between levels of LPS, sCD14, and endotoxin antibody and HIV disease progression (108). Moreover, circulating immunoreactive cytokine levels either decreased or remained virtually unchanged throughout the progression of the disease. However, subsequent data by Redd et al. found longitudinal increases in plasma levels of C-reactive protein, which correlated with levels of sCD14 (109).

We obtained different results by investigating markers of microbial translocation (LPS, sCD14, and EndoCAb) in a cohort of 379 HIV-infected, ART-naïve patients with early HIV infection (approximately 3 years after seroconversion) and CD4+ T-cell counts of >200 cells/µl. In our study, circulating levels of LPS were a strong predictor of disease progression, measured through a combined endpoint of AIDS, death, CD4+ T-cell counts of <200 cells/µl, or the start of ART, independent of CD4+ T-cell counts and plasma viremia. Indeed, patients with heightened LPS levels showed substantially accelerated disease progression, with a median time to clinical event of 1.5 years, versus 4 years for patients with lower levels (41). As a possible complement to these findings, previous data strongly suggested a correlation between intestinal immune dysfunction and intestinal functionality, as suggested both by gene expression profiling that associated gut CD4+ T-cell depletion with downregulated expressions of genes involved in mucosal function (110, 111) and by earlier clinical data on malabsorption and nutritional complications in HIV-infected RM (111) and HIV-infected humans (112). Along the same lines, a nested-control study from the Strategies for Management of Anti-Retroviral Therapy (SMART) trial by Sandler et al. reported that plasma levels of sCD14 are independent predictors of overall mortality in HIV disease (37). Taken together, these data strongly suggest a negative effect of mucosal immune dysfunction and microbial translocation on HIV disease progression.

Circulating levels of LPS have been consistently reported to decrease after the initiation of ART, even though they did not return to the levels observed for healthy HIV-uninfected individuals (33, 39, 45, 62, 65). Since persistent immune activation has long been implicated as a factor impairing the immunological response to ART (13, 14), the question as to whether microbial translocation might affect immune recovery after ART has been investigated by different groups in the last few years. Following the original observation by Brenchley et al. of an inverse correlation between CD4+ T-cell reconstitution and microbial translocation (33), this finding was confirmed by several groups. Our group reported, in a cross-sectional study, increased plasma levels of LPS in immunological nonresponder (INR) ART-treated HIV-infected individuals (defined by CD4+ T-cell counts of ≥200 cells/µl despite long-term HIV RNA levels of ≤50 copies/ml while on HAART) compared to levels in subjects with full immunological recovery after ART (39).

Analogously, Jiang et al. showed that higher levels of DNA sequences encoding bacterial 16S rRNA genes are associated with greater T-cell activation and impaired CD4+ T-cell restoration after ART (45). Similar data were also reported for a South African cohort, confirming persistently elevated LPS and sCD14 levels in INR despite virologically suppressive ART (62). While these findings provide important insights into the mechanisms determining the immunological response to ART, they still fail to formally prove that the level of microbial translocation is a key factor regulating CD4+ T-cell reconstitution upon virologically suppressive ART. Given the well-described increased risk of AIDS- and non-AIDS-related morbidity and mortality in HIV-infected patients who do not experience full immunological recovery on ART (113, 114), further ad hoc-designed observational and randomized studies are warranted to investigate this crucial aspect of SIV/HIV pathogenesis.

Role of Microbial Translocation in Non-AIDS Comorbidities

Over the past few years, strong evidence has accumulated indicating that long-term-ART-treated HIV-infected individuals exhibit a higher-than-expected risk of degenerative diseases, including cardiovascular disease, cancer, osteoporosis, liver disease, and other end-organ illnesses, therefore resulting in a shortened life expectancy and also depicting a pattern of early and accelerated aging (115). Given the burden of these non-AIDS comorbidities in the clinical management of HIV infection, great scientific effort is now being put into the identification of possible immunopathogenetic factors. Among these, some evidence suggests that microbial translocation may play an important role in liver and cardiovascular diseases as well as, possibly, neurocognitive decline. Since LPS induces monocyte activation and enhances the trafficking of these cells to the brain, Ancuta et al. hypothesized a possible association between microbial translocation and HIV-associated neurocognitive dementia (HAND). For a cohort of HIV-infected individuals with CD4+ T-cell counts of <300 cells/µl and with
Microbial Translocation in Liver Disease

The liver has a crucial, physiological role in LPS detoxification from the circulation, as bacteriaally derived products from the intestine translocate into the portal system, where they are sensed from the circulation, as bacterially derived products from the intestine translocate into the portal system, where they are sensed by the liver. Microbial translocation has been proposed to exert a major role in the worsening liver disease in HIV-infected individuals. Moreover, Forysth et al. described a link between enterotoxin exposure markers and early Parkinson’s disease.

Microbial Translocation in Cardiovascular Disease

HIV-infected patients are at an increased risk of cardiovascular events compared to age- and sex-matched healthy controls. The reasons for this phenomenon are likely complex, although recent studies suggested that chronic inflammation and immune activation/senescence may contribute to the initiation and progression of atherosclerosis in HIV infection. LPS represents an important source of inflammatory stimuli and has been investigated in the setting of atherosclerosis. The Bruneck study by Wiedermann et al. in 1999 provided the first epidemiological evidence in support of a clinical association between levels of LPS and cardiovascular risk. Consistent with these findings, the role of bacterial endotoxin as a proinflammatory mediator of atherosclerosis has been widely reported for the HIV-uninfected population. Studies on the role of bacterial bio-products (i.e., LPS) and macrophage activators (sCD14) in the pathogenesis of atherosclerosis in HIV disease are under way, and discordant results have been generated so far. Merlini et al. reported higher levels of sCD14 in ART-treated HIV-infected individuals with increased carotid intima media thickness (cIMT) in an unadjusted analysis; however, this effect was lost after adjusting for classical cardiovascular predictors. Similarly, Kelesidis et al. recently showed that in HIV-infected subjects with low cardiovascular risk, sCD14 and LPS levels are independently associated with increases in the yearly rate of change in cIMT. Intriguing data were recently presented by Pandrea et al., who identified a nonhuman primate model for the role of microbial translocation in cardiovascular comorbidity. In that study, SIVagm-infected pigtail macaques showed elevated circulating levels of LPS in all stages of infection that correlated well with increased levels of coagulation markers, i.e., D-dimer and the thrombin-antithrombin complex. Most interestingly, and in analogy with what was observed for HIV-infected patients, these animals...
showed histopathological signs indicative of cardiovascular co-morbidity, such as renal thrombotic microangiopathy. Furthermore, the reduction of microbial translocation-driven immune activation also resulted in the reduction of coagulation markers, thus pointing to a role of microbial translocation in the onset of cardiovascular disease (92).

Collectively, these data seem to support the hypothesis that microbial translocation, by inducing substantial immune activation, might contribute to the pathogenesis of several age-associated degenerative clinical conditions described for ART-treated, HIV-infected individuals. However, although interesting, the studies presented above have to be considered hypothesis-generating studies, and the possible associations between microbial translocation and non-AIDS comorbidities need further investigation within larger studies. The demonstration that microbial translocation is indeed a major determinant of non-AIDS comorbidities would provide a strong rationale for therapeutic interventions aimed at reducing the overall morbidity and mortality of ART-treated, HIV-infected patients.

**TREATMENT OF HIV/SIV-ASSOCIATED MICROBIAL TRANSLOCATION**

The detrimental effects of microbial translocation on HIV disease progression, the response to ART, and the development of non-AIDS comorbidities provide a strong rationale for the exploration of novel therapeutic interventions aimed specifically at preventing and reducing microbial translocation, its determinants, and its downstream effects. Several strategies are being investigated, including (i) modification of the intestinal microbiome, (ii) enhancement of the enterocyte barrier, (iii) reduction of local inflammation, and (iv) clearance of microbial bioproducts translocated from the gut.

**Modification of GI Microbiome**

Strategies aimed at restoring the normal composition of the intestinal microbiome include antibiotics and probiotics. Ideally, the most suitable antibiotics in such a context would be broad-spectrum, nonabsorbed antibiotics available for oral administration. Rifaximin is a semisynthetic, rifamycin-based, nonsystemic, broad-spectrum antibiotic, meaning that very little of the drug will pass the gastrointestinal wall into the circulation. Given its unique properties, it is widely used for the treatment of hepatic encephalopathy and as a gut-sterilizing treatment prior to abdominal surgery, while contrasting results have been obtained thus far on its efficacy in the treatment of inflammatory diseases (100). Rifaximin is now being intensively tested in HIV/SIV infection. Most recently, the efficacy of 3 months of rifaximin in association with the anti-inflammatory agent sulfasalazine was tested by Pandrea et al. in the acute phase of SIVagm infection of pigtail macaques. In that study, the rifaximin-sulfasalazine combination resulted in significant reductions in levels of circulating CD14 cells, activated CD38+ HLA-DR+ T cells, D-dimer, proinflammatory cytokines, and chemokines in treated animals compared to untreated controls. A slight reduction in the magnitude of the CD4+ T-cell loss at the GI mucosa was observed for rifaximin-sulfasalazine-treated macaques versus controls, with no changes in circulating CD4+ T-cell counts (92). In HIV-infected humans, rifaximin is under investigation within the ACTG A5296 trial that is specifically aimed at testing the efficacy of oral sevelamer carbonate (which is effective in reducing inflammation and endotoxemia in the context of renal failure) to decrease endotoxemia in HIV-infected individuals naïve to antiretrovirals with CD4+ T-cell counts of ≥400 cells/µL (https://actgnetwork.org/) (153).

**Interventions To Improve the Enterocyte Barrier**

Given the dramatic structural and functional changes in the intestinal epithelial barrier during HIV infection, the possibility of positively supporting enterocyte homeostasis by means of immunomodulatory molecules is quite intriguing. In this context, two possible candidates are IL-22 and IL-17, due to their role in mucosal immunity and the association between the loss of IL-17- and IL-22-secreting lymphocytes and mucosal immune dysfunction during SIV infection (86).

Of note, encouraging results are provided by data on a murine model of ulcerative colitis, where the administration of IL-22 reduced intestinal inflammation (151). More recently, data have been presented to support a possible role of recombinant human IL-7 (rhIL-7) in the restoration of gut mucosal T-cell homeostasis in HIV-infected individuals. Indeed, the administration of rhIL-7 to a cohort of subjects with low-level CD4+ T-cell recovery on ART was efficacious in expanding both circulating and gut-residing naïve and memory CD4+ T cells, with parallel reductions in plasma levels of sCD14 and D-dimer (152).

**Clearance of Microbial Bioproducts Translocated from the Intestinal Lumen**

An intriguing therapeutic option is to neutralize microbial bioproducts translocated in peripheral blood as shown in Fig. 1 and 2. One such option is currently being investigated within the ACTG A5296 trial that is specifically aimed at testing the efficacy of oral sevelamer carbonate (which is effective in reducing inflammation and endotoxemia in the context of renal failure) to decrease endotoxemia in HIV-infected individuals naïve to antiretrovirals with CD4+ T-cell counts of ≥400 cells/µL (https://actgnetwork.org/) (153).
CONCLUSIONS

Chronic microbial translocation is a feature of progressive HIV and SIV infections. It is now widely accepted that microbial translocation arises from a substantial immunological and structural disruption at the level of the gastrointestinal mucosa, which starts during acute infection and continues through chronic disease. This mucosal dysfunction includes the depletion of CD4+ Th17 cells, with a preferential loss of Th17 cells; the establishment of local mucosal hyperactivation/inflammation; the exhaustion of intestinal macrophage phagocytic function; and structural epithelial damage (apoptosis of enterocytes and disruption of tight junctions, etc.). Both in vivo and in vitro data have documented that the unchecked continuous passage of immunostimulatory microbial molecules such as LPS from the intestinal lumen to the systemic circulation is able to sustain a substantial activation of innate and adaptive immunity, leading to the paradigm that microbial translocation is a crucial determinant of systemic immune activation in HIV/AIDS. Most interestingly, microbial translocation has been shown to be a clinically significant event, as it is associated with HIV clinical progression, suboptimal CD4+ T-cell recovery on ART, and the early onset of non-AIDS comorbidities such as liver disease progression, atherosclerosis and cardiovascular disease, and neurocognitive impairment. These findings have emphasized the need for therapeutic approaches that...
would mitigate microbial translocation and its effects on immune homeostasis, possibly contributing to improving HIV prognoses and life expectancy. These interventions could include modification of the intestinal microbiome, enhancement of the enterocyte barrier, reduction of local inflammation, and clearance of microbial bioproducts translocated from the gut.

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