Microbial Translocation Correlates with the Severity of Both HIV-1 and HIV-2 Infections

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Microbial translocation has been linked to systemic immune activation during human immunodeficiency virus (HIV) type 1 infection. Here, we show that an elevated level of microbial translocation, measured as plasma lipopolysaccharide (LPS) concentration, correlates with the severity of HIV infection. This correlation was independent of HIV type, rising plasma LPS concentration also correlates with CD4+ T cell count and viral load independently of HIV type. Furthermore, elevated plasma LPS concentration was found to be comcomitant with defective innate and mitogen responsiveness. We suggest that microbial translocation may contribute to loss of CD4+ T cells, increase in viral load, and defective immune stimuli responsiveness during both HIV type 1 and HIV type 2 infections.

Primary human immunodeficiency virus type 1 (HIV-1) infection is followed by a burst of viremia and a rapid decrease in the number of CD4+ T cell targets, mainly those localized in the gut-associated lymphoid tissue [1]. However, after an increase in the innate and human immunodeficiency virus (HIV)–specific immune responses, the peripheral CD4+ T cell levels recover, the degree of viremia is reduced, and then the infection enters its chronic phase [2]. Systemic immune activation has been shown to serve as an independent marker of disease progression [1]. Furthermore, it has recently been suggested that immune activation in HIV-1–infected individuals results from microbial translocation into the peripheral blood after disruption of the mucosal barrier in the gut [3, 4]. In addition to a gradual decrease in the number of CD4+ T cells, HIV-1 pathogenicity is accompanied by an overall defective responsiveness of several innate and adaptive immune functions [1, 2].

Although HIV-1 and human immunodeficiency virus type 2 (HIV-2) are biologically similar, HIV-2 is less pathogenic and less transmissible [5]. The plasma viral load of HIV-2–infected individuals is also significantly lower than the viral load of HIV-1–infected individuals, a characteristic believed to contribute to the slower disease progression in individuals infected with HIV-2 [5]. Immune activation during HIV-2 infections has been reported, but it is unclear whether it differs from that observed during HIV-1 infection [6, 7]. In the present study, we evaluated the extent of microbial translocation during HIV-2 infection compared with that during HIV-1 infection by analyzing the lipopolysaccharide (LPS) concentration in the plasma of HIV-1–infected individuals and HIV-2–infected individuals living in Guinea-Bissau. We found that LPS levels were also related to CD4+ T cell count, viral load, innate immunity, and T cell mitogen responsiveness.

Methods. The participants in the present study and the methods used for blood sampling and laboratory diagnostics have been described elsewhere [8]. Briefly, blood samples were cross-sectionally obtained from 2 study groups in Guinea-Bissau: 1 occupational cohort of police officers [9] and 1 population-based study in which individuals were selected on the basis of HIV-1 and HIV-2 status [10]. Peripheral blood was collected at local health centers and police stations. The samples were subsequently analyzed for HIV infection status and CD4+ T cell count at the National Laboratory for Public Health, Guinea-Bissau [8]. AIDS was defined by a CD4+ T cell count of <200 cells/μL. The plasma viral loads of both HIV-1–infected individuals and HIV-2–infected individuals were analyzed by measuring reverse transcriptase activity with the use of the ExaVir Load kit (Cavidi) and are presented as the number of RNA copies per milliliter equivalent. Antiretroviral treatment was not available at the time of sampling. The study was ap-
Figure 1. Levels of plasma lipopolysaccharide (LPS) according to human immunodeficiency virus (HIV) status, CD4+ T cell count, viral load, and Toll-like receptor stimuli responsiveness. Shown are LPS levels in plasma in relation to HIV status (HIV type 1 [HIV-1] AIDS [n = 21], HIV type 2 [HIV-2] AIDS [n = 7], HIV-1 chronic infection [n = 43], HIV-2 chronic infection [n = 66], and HIV-negative [n = 66]) (A), CD4+ T cell counts (B), and plasma viral load (C) in HIV-1–infected individuals and HIV-2–infected individuals. Box plots denote the median, 25%–75% interquartile range, nonoutlier range, and outliers.

* P < .05; ** P < .01; *** P < .001; solid lines, HIV-1; dotted lines, HIV-2. Also shown are LPS levels in plasma in relation to interleukin 12 (IL-12) expression after R848 stimulation (P < .05; r = −0.22) (D) and interferon-α (IFN-α) expression after CpG oligodeoxynucleotide stimulation (P < .01; r = −0.26) (E) of whole blood from HIV-1–positive individuals and HIV-2–positive individuals.
proved by ethical committees of Guinea-Bissau, Lund University, and Karolinska Institute.

Plasma was diluted at 1:5 in endotoxin-free water, and plasma proteins were heat-inactivated at 70°C for 10 min. LPS levels were measured in plasma by the use of the limulus amebocyte lysate assay (Lonza) according to the manufacturer’s instructions and calculated in relation to an Escherichia coli endotoxin standard provided with the assay, after background subtraction.

Whole blood stimulation assay was performed as described elsewhere [8]. Briefly, whole blood was diluted at 1:10 in medium, and either of the following stimuli reagents were added: 1.5 μmol/L CpG oligodeoxynucleotide 2216 (CyberGene), 1 μmol/L R848 (PhamaTech), or 2.5 μg/mL phytohemagglutinin (BD Bioscience). Supernatants were harvested after 20 h of incubation at 37°C. Concentrations of interferon α (IFN-α), interleukin 12 (IL-12) p40/p70, macrophage inflammatory protein 1α (MIP-1α), and macrophage inflammatory protein 1β (MIP-1β) in the supernatants were measured using a Lumixen instrument (Bio-Rad) and Cytokine Antibody Bead kits (BioSource).

Statistical analyses were performed using univariate non-parametric tests, including the Mann-Whitney U test and the Spearman rank correlation test, whereas multivariate statistical analysis was performed according to linear regression models with Statistica software (version 7.1; StatSoft).

**Results.** To compare the level of microbial translocation during HIV-1 and HIV-2 infections, LPS levels were measured in the plasma of individuals infected with HIV-1 or HIV-2, either during the chronic infection phase or after AIDS development, and compared with plasma LPS levels in control participants who were HIV-negative. We found that both HIV-1–infected individuals with AIDS and HIV-2–infected individuals with AIDS had significantly higher plasma LPS levels than did HIV-negative individuals (P < .001) (Figure 1A). However, no significant difference was observed between HIV-1–infected individuals and HIV-2–infected individuals within the same disease stage, classified as either chronic infection or AIDS.

LPS levels were subsequently analyzed in relation to CD4+ T cell counts. We observed that plasma LPS levels correlated with CD4+ T cell counts among both individuals infected with HIV-1 (P < .05; r = −0.29; data not shown) and individuals infected with HIV-2 (P < .01; r = −0.32; data not shown). Similarly, when the infected individuals were divided according to different CD4+ T cell count intervals, we found that HIV-1–infected individuals with CD4+ T cell counts of <200 or 200–500 cells/μL had significantly higher plasma LPS levels than did HIV-1–infected individuals with CD4+ T cell counts of >500 cells/μL (P < .001 and P < .01, respectively) (Figure 1B). Likewise, HIV-2–infected individuals with CD4+ T cell counts of <200 cells/μL showed higher plasma LPS levels compared with HIV-2–infected individuals with CD4+ T cell counts of >500 cells/μL (P < .05) (Figure 1B).

We also noted correlations between LPS level and plasma viral load in both individuals infected with HIV-1 (P < .01; r = 0.44; data not shown) and individuals infected with HIV-2 (P < .001; r = 0.47; data not shown). This observation was supported by the analysis of viral load intervals showing that HIV-1–infected individuals with plasma viral loads of <1000 RNA copies/mL had lower plasma LPS levels than those with plasma viral loads of >10,000 RNA copies/mL (P < .01) (Figure 1C). Additionally, HIV-2–infected individuals with viral loads of <1000 RNA copies/mL had lower levels of plasma LPS than did HIV-2–infected individuals with viral loads of 1000–10,000 or >10,000 RNA copies/mL (P < .01 and P < .001, respectively) (Figure 1C). However, no significant differences in plasma LPS levels were found between HIV-1–infected individuals and HIV-2–infected individuals within the same CD4+ T cell count or viral load intervals.

Multivariate statistical analysis using type of HIV infection, CD4+ T cell count, viral load, age, sex, and cohort as variables further supported the correlations between LPS level and CD4+ T cell count, as well as viral load (P < .05 and P < .001, respectively) (Table 1). Moreover, the multivariate analysis showed that plasma LPS levels were not correlated with type of HIV infection (Table 1). Instead, sex tended to influence LPS levels (P = .059); women had higher concentrations of LPS in plasma, according to the multivariate statistical analysis (Table 1).

We recently reported that advanced infections with either HIV-1 or HIV-2 may cause defective innate stimuli responsiveness [8]. To investigate whether defective innate stimuli responsiveness was associated with plasma LPS levels, we analyzed the expression of IL-12 and IFN-α in whole blood after stimulation with the Toll-like receptor 7/8 (TLR7/8) agonist R848 and the Toll-like receptor 9 (TLR9) agonist CpG oligodeoxynucleotide, respectively, in relation to LPS concentrations in HIV-infected individuals. We found an inverse correlation between plasma LPS levels and expression of IL-12 (P < .05; r = −0.22) and IFN-α (P < .01; r = −0.26) after stimulation with R848 and CpG oligodeoxynucleotide, respectively (Figure...
considered to be associated with a decrease in the CD4+ T cell count and increased viral load, independently of the type of HIV infection.

We found that HIV-1–infected individuals with AIDS displayed elevated levels of plasma LPS compared with uninfected individuals, which is in agreement with the findings of previous studies [3]. To our knowledge, we report for the first time that individuals with AIDS caused by HIV-2 infection also have higher plasma LPS levels than those of HIV-negative individuals. Furthermore, our study revealed that elevated plasma LPS levels correlate with low CD4+ T cell counts and high plasma viral loads among both HIV-1–infected individuals and HIV-2–infected individuals. Despite the fact that HIV-2–infected individuals display significantly lower plasma viral loads than do HIV-1–infected individuals within the same CD4+ T cell range [5, 8], we noted elevated levels of plasma LPS along with a decrease in the CD4+ T cell counts. A recent study demonstrated that microbial translocation, as detected by the presence of bacterial 16S ribosomal DNA, correlated with plasma viral load among untreated HIV-1–infected individuals [4]. Similarly, our study suggests that an elevated level of circulating virus coincides with microbial translocation during pathogenic infection of both HIV-1 and HIV-2. Nonetheless, in contrast to our study, a recent longitudinal study of African HIV-1–infected individuals with known disease course suggested that microbial translocation is not linked to disease progression [11]. However, the study by Redd et al [11] did not consider correlations between the plasma LPS level and CD4+ T cell count or plasma viral load.

Microbial translocation has been correlated with markers of systemic immune activation [3]. Moreover, immune activation has been described in both HIV-1–infected individuals and HIV-2–infected individuals in regard to disease progression [1, 5]. In agreement with our findings, another study has suggested that immune activation in HIV-2–infected individuals is linked to CD4+ T cell loss [7]. Instead, Sousa et al [7] did not observe any correlation between T cell activation marker expression and HIV-2 plasma viral load. However, ~20% of the HIV-2–infected individuals included in our study displayed plasma viral loads above those detected by Sousa et al [7] (data not shown). An alternative explanation for the different findings may be that the relationship between plasma LPS levels and T cell activation marker expression differs in immune activation caused by HIV-2. Interestingly, we also observed that women tended to have higher plasma LPS levels than did men, which would suggest that HIV-infected women may experience elevated immune activation, compared with men. In line with this, a recent study by Meier et al [12] suggested that CD8+ T cells of HIV-1–infected women display increased expression of activation markers compared with CD8+ T cells of men.

Recently, we demonstrated that defective Toll-like receptor stimuli responsiveness is paralleled by advanced disease caused by either HIV-1 or HIV-2 infection [8]. Here, we show that defective innate stimuli responsiveness (ie, reduced expression of IL-12 and IFN-α after TLR7/8 and TLR9 stimulation, respectively) correlates with elevated plasma LPS levels. Although it is known that cells involved in innate immunity are reduced in number and dysregulated in the peripheral blood of HIV-1–infected individuals [13], a direct correlation between defective innate immunity and microbial translocation has not been described previously, to our knowledge. Interestingly, a study of chronically immune-activated patients with intestinal parasites demonstrated that TLR9 expression and function was defective in these individuals [14]. Thus, our findings and those of Ayash-Rashkovsky et al [14] suggest that Toll-like receptor stimuli responsiveness may be linked to chronic immune activation caused by different infectious agents that affect the intestinal tract. We also noted that the production of MIP-1β chemokine after T cell mitogen stimulation was reduced in individuals with elevated plasma LPS levels. In agreement with this finding, a recent study reported on elevated plasma levels of neopterin in HIV-1–infected individuals in relation to defective cell proliferation after mitogen stimulation [15].

Taken together, our findings suggest that microbial translocation is associated with pathogenesis, including loss of CD4+ T cells, elevated viral load, and defective immune stimuli responsiveness, in individuals with HIV-1 or HIV-2 infection. Further studies on the impact of microbial translocation during the follow-up of HIV-1 and HIV-2 infection disease courses may reveal important implications for the clinical outcome of HIV-infected individuals.

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