Early microbial translocation blockade reduces SIV-mediated inflammation and viral replication

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Damage to the intestinal mucosa results in the translocation of microbes from the intestinal lumen into the circulation. Microbial translocation has been proposed to trigger immune activation, inflammation, and coagulopathy, all of which are key factors that drive HIV disease progression and non-HIV comorbidities; however, direct proof of a causal link is still lacking. Here, we have demonstrated that treatment of acutely SIV-infected pigtailed macaques with the drug sevelamer, which binds microbial lipopolysaccharide in the gut, dramatically reduces immune activation and inflammation and slightly reduces viral replication. Furthermore, sevelamer administration reduced coagulation biomarkers, confirming the contribution of microbial translocation in the development of cardiovascular comorbidities in SIV-infected nonhuman primates. Together, our data suggest that early control of microbial translocation may improve the outcome of HIV infection and limit noninfectious comorbidities associated with AIDS.

Introduction
The current paradigm of HIV/SIV pathogenesis is that chronic immune activation and inflammation are major determinants of disease progression to AIDS, independent of viral replication or CD4+ T cell counts (1). The activation state in HIV-infected patients involves increased T cell turnover, a high frequency of activated T cells, markers of coagulation, increased plasma levels of inflammatory cytokines, and polyclonal B cell activation (2).

Several mechanisms have been proposed to be involved in the increased immune activation/inflammation observed in HIV-infected patients (2). One mechanism is microbial translocation from the intestinal lumen to the general circulation, which results from the mucosal damage inflicted by HIV/SIV at its preferential site of replication and CD4+ T cell depletion (3–5). These translocated microbial products can stimulate directly multiple immune cell populations via toll-like receptors, thus inducing increased levels of systemic immune activation and inflammation (3, 6). However, while abundant correlative data support a role of microbial translocation in the pathogenesis of HIV infection (3, 6), there have been conflicting reports (7), and this hypothesis has never been tested directly.

We previously modeled microbial translocation experimentally by bypassing the intestinal mucosa through intravenous LPS administration to chronically SIV-infected African green monkeys (8, 9), which maintain a healthy mucosal barrier, control chronic immune activation, and do not progress to AIDS (10, 11). These interventions increased systemic immune activation, viral replication, and hypercoagulability (8, 9), consistent with a key role for microbial translocation in the pathogenesis of AIDS.

To directly address the effect of microbial translocation on driving immune activation and inflammation, here we used a complimentary approach, consisting of binding LPS in the gut of a nonhuman primate pathogenic host that consistently develops gut immune dysfunction and microbial translocation during SIV infection (9, 12, 13). To this end, we used SIVsab-infected pigtailed macaques (PTMs), a new animal model of pathogenic SIV infection developed in our laboratory. The advantage of SIVsab-infected PTMs over the conventional AIDS macaque models is that SIVsab infection in PTMs is more suited for the study of AIDS-related comorbidities, being characterized by a robust viral replication, consistent high levels of microbial translocation associated with dramatic increases in the immune activation/inflammation, and a high rate of cardiovascular comorbidities, i.e., hypercoagulopathy (9, 14).

To achieve the LPS binding in the gut, we used sevelamer, a LPS-sequestering, phosphate-binding drug that has been shown to reduce plasma LPS levels in patients with chronic kidney disease (15), in which the levels of microbial translocation are increased due to uremia-associated gut damage (16, 17). We hypothesized that early in vivo blockade of microbial translocation in SIVsab-infected PTMs would result in the control of chronic immune activation/inflammation.

Results and Discussion
As shown in Figure 1, the levels of plasma LPS were almost unchanged in the PTMs treated with sevelamer. Conversely, in the control group, the LPS levels significantly increased, especially after...
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Impact of sevelamer treatment on the microbial translocation in SIVsab-infected PTMs. Comparison between the levels of microbial translocation in axillary LNs of SIVsab-infected PTMs receiving sevelamer and untreated controls. Representative images (original magnification, ×50) of the LNs stained for LPS core antigen (brown). Note the extensive accumulation of microbial products within the macrophages located around the subcapsular and medullary sinuses and in the paracortical parenchyma of the lymphatic tissues in untreated controls and that there is almost no increase in the levels of LPS in the LNs of PTMs treated with sevelamer. dpi, day after infection.

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Figure 2
Impact of sevelamer treatment on the microbial translocation in SIVsab-infected PTMs. Comparison between the levels of microbial translocation in axillary LNs of SIVsab-infected PTMs receiving sevelamer and untreated controls. Representative images (original magnification, ×50) of the LNs stained for LPS core antigen (brown). Note the extensive accumulation of microbial products within the macrophages located around the subcapsular and medullary sinuses and in the paracortical parenchyma of the lymphatic tissues in untreated controls and that there is almost no increase in the levels of LPS in the LNs of PTMs treated with sevelamer. dpi, day after infection.

Figure 1
Sevelamer treatment reduces microbial translocation during early SIVsab infection of PTMs. (A) Comparison between plasma LPS levels in SIVsab-infected PTMs receiving sevelamer (red) and untreated controls (blue). (B) Comparison between plasma sCD14 levels in SIVsab-infected PTMs receiving sevelamer (red) and untreated controls (blue). The P values were assessed as long-term differences in temporal dynamics and obtained using mixed-effects models.

Acute infection (P = 0.0001 for difference in levels of control vs. treated) (Figure 1A), likely due to the fact that the major CD4+ T cell depletion and mucosal breaches occur between 3 and 4 weeks after SIV infection (18). Similar to the levels of LPS, the levels of soluble CD14 (sCD14), which is a surrogate marker of microbial translocation and monocyte activation (19), were not significantly increased from the baseline in the sevelamer-treated group, while being significantly increased (P = 0.0003) in untreated controls (Figure 1B). These results were confirmed by the immunohistochemical assessment of the LPS levels in the peripheral LNs, which were virtually unchanged in the PTMs treated with sevelamer and dramatically increased in controls (Figure 2). These results demonstrate clearly that sevelamer blocks microbial translocation.

We found decreased immune activation of peripheral CD4+ T cells in sevelamer-treated PTMs compared with that in untreated PTMs (Figure 3). Later in the infection, Ki67+ CD4+ T cells continued to decrease in treated macaques but increased in controls (P < 0.0001) (Figure 3A). Ki67+ CD8+ T cells had somewhat lower levels in the treated group, but these differences did not reach significance (P = 0.24) (Figure 3B). There was also a lower frequency of HLA-DR+ CD38+ CD8+ T cells in this group (P = 0.0001) (Figure 3C), while the differences in HLA-DR+ CD38+ CD4+ T cell frequencies, although an average of 4-fold, reached only a marginal statistical significance (P = 0.0341), because one of the controls was an outlier (Figure 3D).

Furthermore, we identified important differences in the levels of plasma proinflammatory cytokines (Figure 3E) and C-reactive protein (CRP) (Figure 3F) between the two groups. The latter mimicked the dynamics of Ki67+ CD4+ T cells, with gradual decreases in treated animals compared with increases in the control group (P = 0.0075).

Lower levels of immune activation and inflammation in sevelamer-treated PTMs were associated consistently with lower plasma levels of D-dimer (P = 0.009, Figure 3G). This observation is particularly important, as D-dimer is a biomarker associated with the clotting cascade, and elevated D-dimer levels are strongly associated with cardiovascular and all-cause mortality in antiretroviral-treated, HIV-infected individuals (20) and with cardiovascular disease and thrombosis in SIV-infected nonhuman primates (9). These results strongly support the position that microbial translocation from the intestinal lumen is a key mediator of immune activation and inflammation that drive cardiovascular comorbidities and progression to AIDS in HIV-infected patients.

No significant difference in memory CD4+ T cell changes in either the periphery or the intestine were observed between sevelamer-treated PTMs and controls (data not shown). Note, however, that the frequency of CD4+ T cells was assessed during the acute infection and after acute infection, when they are massively depleted by high viral replication. The follow-up was too short to allow CD4+ T cell restoration, which, in untreated monkeys, only occurs after several months after infection.

At >6 weeks after infection, the viral loads were 1.1 log lower in sevelamer-treated PTMs compared with controls (P = 0.024) (Figure 3H). While 2 PTMs from the control group (40%) experienced a rapid progression to AIDS (<120 days after infection), none of the PTMs that received sevelamer were rapid progressors.

Our study design consisted of sevelamer administration early in infection, at the time when mucosal damage is produced and a
rapid intervention may alleviate the pathogenic consequences of acute HIV infection, even in the absence of virus control. While this design allowed us to determine the effect of sevelamer in the absence of most confounding factors, we recognize that most interventions in HIV-infected patients are performed in more chronic stages of disease. Therefore, studies in which sevelamer administration occurs later during infection seem warranted.

Our results demonstrating that early therapeutic interventions aimed at limiting microbial translocation may substantially affect levels of immune activation and inflammation corroborate recent results confirming the major benefits of early and sustained administration of antiretrovirals to HIV-infected patients (21). In patients with chronic kidney diseases, microbial translocation is associated with hyperphosphatemia (22–24), which may represent a confounding factor for assessing the therapeutic effect of the sevelamer (i.e., reduction of inflammation due to its phosphate binding properties and not to a direct effect on microbial translocation). However, such a confounding factor is not present in the HIV-infected patients. In contrast to the patients with renal failure, up to 35% of the HIV-infected patients and SIV-infected macaques present with hypophosphatemia (25, 26), making it unlikely that the observed therapeutic effects reported here are due to a direct effect of sevelamer on phosphorus levels. Furthermore, we have demonstrated previously that LPS administration to chronically SIV-infected nonprogressive hosts resulted in increased immune activation and inflammation (8, 9). As such, our studies directly validate microbial translocation as a major cause of persistent immune activation and inflammation in HIV-infected patients.

Our results strongly support a role for microbial translocation–reducing therapies in improving the prognosis of HIV infection.
infection. In addition to direct binding of microbial products with sevelamer, such strategies should alleviate gastrointestinal tract inflammation and damage; reduce the microbial burden, thus enhancing gastrointestinal immune function; and increase reconstitution of mucosal CD4+ T cells, potentially having a beneficial impact on morbidity and mortality. These strategies may include administration of antibiotics, antiinflammatory drugs, or symbiotic-probiotic supplementation of antiretroviral treatment (27). Clinical trials assessing each of these strategies are underway. Nevertheless, while each of these approaches may prove effective in achieving the overall goal, it is conceivable that only the combination of all of the above will prove effective in controlling the deleterious consequences of immune activation during HIV infection.

Methods

Animals and infection. Nine PTMs (Macaca nemestrina) were intravenously infected with plasma compatible to 300 million virus particles of simian immunodeficiency virus (SIVmac239) collected from 4 RMs and administered for 3 months. Remaining PTMs were used as untreated controls. During the follow-up, one of the PTMs in the study group died at day 53 after infection, due to causes unrelated to SIV infection or treatment.

Sample collection. Blood was collected from all PTMs prior to infection, biweekly for 2 weeks, weekly for 4 weeks, and bimonthly thereafter. Intestinal biopsies were collected prior to infection, during acute infection, at the set point, and during chronic infection, as described previously (11, 28, 29). Samples were processed as described previously (11, 28, 29).

Laboratory assessment. Plasma SIVsabBH66 viral RNA loads were quantified by real-time PCR, as described previously (30, 31). Whole blood and mononuclear cells isolated from intestinal biopsies were analyzed by flow cytometry, as described previously (9).

Cytokine and chemokine testing and D-dimer testing were performed as previously described (9). CRP was tested, as described previously (20), using a monkey CRP ELISA Kit (Life Diagnostics).

Statistics. GraphPad Prism 5 (GraphPad Software) was used for statistical analysis. Differences in late temporal dynamics were analyzed using mixed-effects models, with each macaque as the grouping factor to account for the repeated measurements made in that animal. Models with fixed effects for time and treatment, with or without interactions, were tested. When an interaction was significant, we describe this difference in the text. Assumption on the distribution of residuals and appropriateness of the fitted values were checked by visual inspection of residual and fitted plots. The best model for the data was chosen by comparing the log likelihood.

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