Longitudinal Analysis of Distribution and Function of Plasmacytoid Dendritic Cells in Peripheral Blood and Gut Mucosa of HIV Infected Patients

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Aberrant activation of plasmacytoid dendritic cells (pDCs) with excessive production of interferon alpha (IFNα) represents one of the hallmarks of immune activation during chronic phase of human immunodeficiency virus (HIV) infection. A number of studies have shown that disruption of mucosal integrity in the gut is a cause of persistent immune activation. However, little is known about the role that pDCs play in this process, and our current understanding comes from the simian immunodeficiency virus macaque model. Thus, in the present study we sought to investigate the frequency and function of pDCs in peripheral blood and gut samples from HIV-infected individuals before and 6 months after initiation of antiretroviral therapy (ART). We show that circulating pDCs were depleted in ART-naive HIV+ patients, and upregulated the gut-homing receptor CD103 compared with uninfected controls. By converse, pDCs accumulated in the terminal ileum of ART-naive HIV individuals compared with controls. Baseline levels of IFNα production and markers of immune activation in gut samples of ART-naive HIV subjects were elevated. All these parameters declined after 6 months of ART. Our results suggest that in chronic HIV infection, pDCs migrate from peripheral blood to the gut-associated lymphatic tissue, where they may contribute to immune activation.

Keywords. GALT; HIV; IFNα; immune activation; pDC.

The appearance of the proinflammatory cytokine, interferon alpha (IFNα), in the serum of human immunodeficiency virus (HIV)-infected individuals is associated with disease progression, and has been implicated as a cofactor in chronic immune activation [1, 2]. Plasmacytoid dendritic cells (pDCs) are innate immune cells that respond to microbial infection by producing large amounts of IFNα [3]. During HIV infection, pDCs decline in peripheral blood (blood) [4–7], and relocate to peripheral lymph nodes where they produce high levels of IFNα [8–10].

The gastrointestinal mucosa (gut-associated lymphatic tissue, GALT) represents the largest lymphoid organ and harbors the majority of the body’s lymphocytes [11]. Severe damage and abnormalities of the intestinal epithelial barrier have been described in HIV-infected patients, even in early stages of disease [12]. A recent study provided evidence for pDCs being rapidly recruited to the colorectum after pathogenic simian immunodeficiency virus (SIV) infection in rhesus macaques. These pDCs produced IFNα and other proinflammatory cytokines, causing immune activation in colorectal tissue [13]. The consequences of local mucosal damage...
of the intestine and the coherence with systemic immune activation in HIV-infected individuals have been described [14], particularly an increased permeability to microbial products such as lipopolysaccharide (LPS) that exit the gastrointestinal lumen and reach the bloodstream [15]. The levels of LPS found in plasma of HIV patients are sufficiently high to induce inflammatory responses [16, 17].

Indeed, the dynamics of pDCs, IFNα production, and chronic immune activation appear to be interrelated in HIV infection. Plasma levels of LPS and IFNα closely correlate in HIV patients [15].

So far, little is known about the distribution and function of pDCs in the intestinal mucosa of HIV-infected patients, and the majority of our current understanding is resulting from the SIV-macaque model. Therefore, our study sought to address the hypothesis that in the course of chronic HIV infection, peripheral pDCs migrate to the GALT where they contribute to immune activation. For the first time, we report a longitudinal study analyzing the frequency and steady-state IFNα secretion of pDCs as well as LPS levels in the GALT and blood of HIV-1 patients before and after the initiation of antiretroviral therapy (ART).

PATIENTS AND METHODS

Study Design

This prospective longitudinal study was carried out in 3 Departments of Internal Medicine at the University of Cologne and the Center for HIV and Hepatogastroenterology Düsseldorf, Germany. After informed consent, 30 HIV-infected adults meeting entry criteria were recruited. Inclusion criteria comprised: (1) age 18–65 years, (2) infection with HIV-1, and (3) naïve to ART. Exclusion criteria were: (1) hepatitis B or C co-infection, and (2) clinical contraindications for sedation or endoscopy. Endoscopic biopsies and blood samples were obtained before starting ART and 6 months thereafter. Standard treatment according to international guidelines was applied. In addition, 15 healthy HIV negative individuals served as controls. All individuals had to be free of inflammatory or lymphoproliferative bowel diseases on histopathologic examination. The study was approved by the Institutional Review Board of the University of Cologne, Germany (09–210). Clinical Trial Registry Number Identifier (ClinicalTrials.gov): NCT 01679067.

Phlebotomies (50 mL blood) were undertaken immediately before endoscopy. An amount of 1–2 × 10⁶ peripheral blood mononuclear cells (PBMCs) isolated by Ficoll-Hypaque density gradient were set aside for flow cytometry, while the remainder was divided and frozen at −80°C for subsequent DNA or RNA extraction. Plasma and serum were frozen until use.

Endoscopic biopsies were obtained from the terminal ileum and immediately placed in tissue culture medium or in formalin.

Isolation of Gut Cells

Six to 10 biopsies from each site were separated into single cells using the method by Shaklett et al [18] with minor modifications. Briefly, biopsies were subjected to 3 rounds of collagenase digestion, mechanical disruption (passage through a blunt 16-gauge needle), clarification (70-µm cell strainer), and washing. An amount of 1–2 × 10⁶ cells were set aside for flow cytometry; the remainder was divided and frozen at −80°C for subsequent RNA extraction.

HIV plasma viral load was quantified using Roche Amplicor kits (Roche Diagnostics) with a lower limit of detection of 40 copies/mL.

Levels of LPS in plasma and IFNα in serum were quantified by the limulus amebocyte assay (Hycultec, Netherlands) and enzyme-linked immunosorbent assay (PBL Biomedical Laboratories, Piscataway, NJ).

Immunophenotyping

In brief, fresh PBMCs and lymph node mononuclear cells (LNMCs) were incubated with the following antibodies and appropriate isotype controls: anti-CD123-APC (clone REA114) and BDCA2-FITC (clone AC144) (Miltenyi Biotec, Germany), CD3-APC (clone HIT3a), CD4-FITC (clone L200), CD8-FITC (clone HIT8a), CD38-PE (clone HIT2), HLA-DR-Percp (clone G46-6), and CD103-PE (clone Ber-Act8) (BD Bioscience). Cells and data were analyzed by flow cytometry (FACSCanto2) using FACSDiva software (BD Biosciences) and FlowJo software (Tree Star, Ashland, OR).

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted (Roche Diagnostics) from PBMCs and 1 × 10⁶ cells from the terminal ileum. Quantitative MxA expression (LightCycler, Roche Diagnostics) was performed as previously described [19]. The HIV RNA in gut tissue was quantified by real-time polymerase chain reaction (PCR) using the SuperScript III One-Step RT-PCR System (Invitrogen) on a LightCycler 480 (Roche). HIV RNA copies were measured in 10⁶ cells isolated from the GALT biopsy sample. To account for the variable number of HIV harboring CD4+ T cells in different samples, results were normalized based on the percentage of CD3+ CD4+ T cells/biopsy as determined by flow cytometry. HIV in plasma was quantified using Roche Amplicor kits (Roche Diagnostics; lower limit of detection: 40 copies/mL).

Immunohistochemistry

Fresh tissues were fixed in 4% formalin and embedded in paraffin. Sections (3 µm) were stained with hematoxylin and eosin. Immunohistochemistry (IHC) was performed using anti-CD3, -CD4 and -CD8 (Dako, Germany); anti-CD123 (eBioscience, San Diego, CA); and the appropriate secondary antibodies. Using a 40 × objective, positive cells of ileal mucosa were scored.
visually in 10 whole cross-sections of the tissue slice. Values are expressed as median of positive cells in 10 sections.

**Statistics**
GraphPad Prism (GraphPad Software, La Jolla, CA) was used to graph and analyze data for statistical significance. Significant differences were tested with 2-tailed Mann–Whitney test, and Wilcoxon matched-pairs test as applicable. All statistical analyses assumed a 2-sided significance level of 0.05. Spearman’s r was used to describe nonparametric correlations. All values are represented as the median [interquartile ranges (IQRs)] unless otherwise stated.

**RESULTS**
We obtained ileal biopsies from 30 HIV-infected patients and 15 healthy controls. The demographic and clinical characteristics of HIV-infected individuals and controls (all white individuals) are summarized in Table 1. All HIV-infected patients were ART naive at the time of sample collection, and ART was started after the colonoscopy. Eleven of the HIV-infected persons were examined by colonoscopy 6 months after initiation of ART. Out of these 11 patients, 7 had a protease inhibitor–based regimen, and 4 had a nonnucleoside reverse transcriptase inhibitor–based regimen.

**CD4+ T-cell Depletion in Peripheral Blood and GALT Is Only Partially Reversible in HIV-Infected Individuals Upon 6 Months of ART**
We evaluated the presence of CD4+ and CD8+ T cells in blood and gut tissues from HIV-infected patients prior and post 6 months on ART by flow cytometry and immunohistochemistry, respectively. CD4+ T cells were significantly depleted in both compartments in HIV-infected individuals compared with HIV-negative individuals (GALT HIV vs control: P < .001, Figure 1A–C). Upon initiation of ART, median CD4+ T-cell count in blood of HIV-infected individuals increased from 265 cells/µL (IQR, 68–383) to 410 cells/µL (IQR, 380–463; P = .017), with the percent fraction recovering from 15% (IQR, 8–24) to 25% (IQR, 15–41; P = .007). In the GALT, the CD4+ T cells increased from 10% (IQR, 3–13) to 14% (IQR, 10–16) compared with 32% (IQR, 26–36) in healthy individuals. Thus, the frequency of CD4+ T cells in blood and in the GALT increased after 6 months of ART, but these values did not normalize to levels observed in healthy individuals.

Median viremia of HIV-infected patients was 136 500 copies of HIV-RNA/mL (IQR, 25 858–574 674). All 11 patients who completed the 6-month study achieved virologic control in plasma to less than 40 copies/mL. In contrast, only 6 of 11 patients achieved clearance of viremia in the GALT, with HIV-RNA decreasing from 4.2 (IQR, 3.3–4.5) log_{10} copies/100 000 CD4+ T cells to less than 1.6 log_{10} copies/100 000 CD4+ T cells (Figure 1F).

The median frequency of mucosal CD8+ T cells decreased from 25% (IQR, 18–31) to 18% (IQR, 10–23; P = .1250) compared with 17% (IQR, 9–20; P = .006) in healthy individuals (data not shown). Immunohistochemistry revealed that CD8+ T cells were significantly increased in HIV-infected individuals in comparison to healthy controls (median, 26 [IQR, 21–43] vs 17 [IQR, 13–24] cells per 10 visual fields; P = .0348, Figure 1D). The CD4/CD8 ratio in the GALT from controls was 1.8 (IQR, 1.3–3.5). In HIV-infected subjects, the CD4/CD8 ratio in the GALT increased from 0.4 (IQR, 0.1–0.7) to 0.9 (IQR, 0.4–1.5) after 6 months of ART (P = .04, Figure 1E).

Due to insufficient biopsies, we were unable to assess absolute numbers of immune cells in microscopic immunohistochemistry sections for most of our patients post ART. Thus, the interpretation of our data is focused on the longitudinal changes in the frequency of respective cells as determined by flow cytometry.

**pDCs Relocate From Peripheral Blood to the Gut Mucosa During Chronic HIV Infection**
We analyzed the frequency of pDCs in GALT and blood from HIV-infected individuals compared with healthy controls. HIV-1–positive subjects show a significantly higher percentage of pDCs in GALT than in blood (P = .0014), whereas in controls the converse occurs (P < .0019). The median percentage of pDCs in GALT of HIV-infected patients was 0.5 (IQR, 0.2–0.8) versus 0.3 (IQR, 0.2–0.6) in controls (see Figure 2A). Comparing their frequency in blood of HIV-infected patients before and after 6 months on ART, pDCs increased significantly (P = .008) up to levels similar to those of healthy individuals.

| Table 1. Baseline Clinical Characteristics of HIV-Infected Patients |
|----------------|----------------|----------------|----------------|
|                | HIV Median   | HIV IQR       | Control Median | Control IQR |
| Gender (M:F)   | 25:5         | 8:7           |                |              |
| Age (years)    | 41           | 35–50         | 50             | 42–70        |
| CD4+ T cells   | 265          | 68–383        | 930            | 745–1070     |
| CD4+ T cells (%) | 15           | 8–24          | 46             | 42–48        |
| CD8+ T cells   | 745          | 450–1255      | 640            | 422–777      |
| CD8+ T cells (%) | 56           | 51–66         | 29             | 21–46        |
| CD4/CD8       | 0.2          | 0.2–0.4       | 1.8            | 1.4–3.5      |
| CD4+ T cells nadir (µL) | 205          | 60–310        | n.a            |              |
| HIV RNA (copies/mL) | 136 500     | 25 858–574 674 | n.a            |              |

All participants were naive to ART and initiated therapy after the colonoscopy. Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range.
Figure 1. CD4⁺ T-cell depletion and HIV-infected persistence in the gut mucosa of HIV-infected individuals is seen despite effective ART. If not indicated differently, the following legend applies for the dot plots: Each data point (dot) represents an individual. Horizontal lines indicate median values; whiskers represent IQR. The 11 patients (dots) who were evaluated pre-ART and 6 months post-ART are marked in red and so are the statistical numbers that apply for this group, whenever comparing pre- and post-ART parameters (2-tailed Wilcoxon matched-pairs test). Two-tailed Mann–Whitney test was used to compare HIV-infected and -uninfected individuals. A, Frequencies of CD4⁺ T cells (%) in peripheral blood and in the terminal ileum of HIV-infected individuals before (n = 30) and after 6 months of ART (n = 11), and uninfected individuals (n = 15) as determined by flow cytometry. B, IHC detection of intestinal CD4⁺ T cells (brown staining) in a representative HIV-infected individual and an uninfected control are shown; original magnification × 100. C and D, Absolute numbers of CD4⁺ and CD8⁺ T cells in the terminal ileum of HIV-infected individuals (n = 12) as well as of uninfected individuals (n = 6). Horizontal lines indicate median values/10 visual fields in IHC-stained tissue. E, CD4/CD8 ratio in blood and in the terminal ileum of HIV-infected individuals before (n = 30) and after 6 months of ART (n = 11) and uninfected individuals (n = 15). Distribution of cells (%) was determined by flow cytometry. F, HIV-RNA in CD4⁺ T cells of the terminal ileum in HIV-infected individuals before (n = 30) and after 6 months of ART (n = 11). Here, horizontal lines represent mean; and whiskers, SEM. Abbreviations: ART, antiretroviral therapy; GALT, gut-associated lymphatic tissue; HIV, human immunodeficiency virus; IHC, immunohistochemical; IQR, interquartile range; LNMCs, lymph node mononuclear cells; PBMCs, peripheral blood mononuclear cells; SEM, standard error of margin.
contrast, the percentage of pDCs in the GALT remained unchanged (0.5 [IQR 0.3–0.7]; \( P = .327 \)).

To further validate the evidence that during HIV-1 infection pDCs relocate from blood to the GALT, we assessed the expression of CD103 on peripheral pDCs in HIV-positive and -negative individuals. CD103 has been previously shown to mediate trafficking and retention of T cells in the gut mucosa [20, 21]. Circulating pDCs of HIV-infected patients express higher levels of the gut-homing receptor CD103 compared with controls (geometric mean fluorescence intensity, GMFI; 15 [IQR, 12–21] vs 10 [IQR, 7–15]; \( P = .007 \); Figure 2D). In addition, we sought to visualize the accumulation of pDCs in the gut mucosa during HIV infection. To this end, we performed immunohistochemistry of CD123+ cells in ileal biopsy sections [22]. Microscopic analysis revealed that pDCs were significantly increased in HIV-infected individuals in comparison to healthy controls (median, 6 [IQR, 4–6] vs 3 [IQR, 3–4] cells per 10 visual fields; \( P = .0192 \); Figure 2C).

**High Frequency of pDCs in the GALT Correlates With IFNα Expression and a Proinflammatory Phenotype of CD8+ T-cells**

As pDCs are the main source of IFNα in humans, we analyzed the interferon-induced MxA protein in blood and GALT and IFNα in the blood of HIV-positive and -negative individuals. Expression of MxA is exclusively induced by type I interferons and persists long after IFNα expression wanes [23–25]. MxA mRNA analysis was performed at steady state; that is, in the absence of any ex vivo stimulation. Compared with healthy

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**Figure 2.** pDCs relocate from peripheral blood to the gut mucosa during chronic HIV infection. The following legend applies for the dot plots: Each data point (dot) represents an individual. Horizontal lines indicate median values; whiskers represent IQR. The 11 patients (dots) who were evaluated pre- and 6 months post-ART are marked in red and so are the statistical numbers that apply for this group, whenever comparing pre- and post-ART parameters (2-tailed Wilcoxon matched-pairs test). Two-tailed Mann–Whitney test was used to compare HIV-infected and uninfected individuals. A, Frequencies of pDCs (%) in peripheral blood and the terminal ileum of uninfected individuals \( (n = 15) \) and HIV-infected individuals before \( (n = 30) \) and after 6 months of ART \( (n = 11) \) were determined by flow cytometry. B, Immunohistochemical detection of intestinal pDCs (brown staining) in a representative HIV-infected individual and an uninfected control; original magnification \( \times 100 \). C, Absolute numbers of pDCs per visual field in HIV-infected individuals \( (n = 12) \) and uninfected individuals \( (n = 6) \). D, Expression of CD103 on pDCs in blood of HIV-infected individuals before ART \( (n = 18) \) and uninfected individuals \( (n = 14) \). Expression of CD103 was measured as GMFI by flow cytometry. Abbreviations: ART, antiretroviral therapy; GALT, gut-associated lymphatic tissue; GMFI, geometric mean fluorescence intensity; HIV, human immunodeficiency virus; IQR, interquartile range; LNMCs, lymph node mononuclear cells; PBMCs, peripheral blood mononuclear cells; pDCs, plasmacytoid dendritic cells.
controls, we found 5-fold and 2-fold higher MxA mRNA levels in ileal biopsies and PBMCs, respectively, of ART-naive HIV-infected patients (Figure 3B). However, MxA expression normalized upon initiation of ART (HIV+ naïve vs treated: \( P = .012; \) treated vs controls: \( P = .3412 \) Figure 3B). Finally, we found a positive correlation between plasma IFN\( \alpha \) levels and frequency of ileal pDCs (\( r = 0.53; \) \( P = .01 \) Figure 3G), suggesting that GALT-homed pDCs of HIV-infected patients secrete excessive amounts of IFN\( \alpha \).

High frequency of CD4\(^+\) and CD8\(^+\) T cells expressing the immune “activation” markers CD38 and human leukocyte antigen (HLA)–DR is associated with poor prognosis [26, 27]. We assessed the coexpression of CD38 and HLA-DR on CD8\(^+\) T cells of the GALT and compared it to blood. In all groups, the frequency of HLA-DR \( ^+\) CD38 \( ^+\) CD8\(^+\) T cells was higher in blood compared with the GALT (blood vs GALT naïve: \( P = .018; \) ART: \( P = .015; \) control: \( P = .012).\) In HIV-infected naïve individuals, the frequency of HLA-DR \( ^+\) CD38 \( ^+\) CD8\(^+\) T-cells was higher in both compartments compared to controls and HIV-1–positive subjects on ART (Figure 3A). Interestingly, MxA mRNA in the GALT correlated positively with the expression of CD38 and HLA-DR on CD8\(^+\) T cells in the GALT (\( r = 0.49; \) \( P = .023 \); Figure 3F).

Translocation of microbial products (eg, LPS) as well as high IFN\( \alpha \) serum levels are associated with systemic immune activation observed during HIV-1 infection [15]. Thus, we assessed the levels of LPS and IFN\( \alpha \) in blood of HIV-infected subjects and controls. Median plasma concentration of LPS was higher in therapy-naive HIV-infected individuals than in uninfected controls (4.5 EU/mL [IQR, 2.8–11] vs 3 EU/mL [IQR, 2.5–3.2]; \( P = .02).\) We also measured higher levels of IFN\( \alpha \) in serum of HIV-1–positive subjects compared with uninfected individuals (74.02 pg/mL [IQR, 46.6–86.43] vs 52.8 pg/mL [IQR, 33.4–57.2]; \( P = .0087).\) Of note, we found a positive correlation between plasma LPS concentrations with MxA expression in the GALT (\( r = 0.45; \) \( P = .02 \); Figure 3E) only in HIV-infected naïve patients. Upon initiation of ART, the concentrations of LPS and IFN\( \alpha \) declined (treatment-naive vs treatment experienced \( P = .03 \) and \( P < .001, \) respectively; Figure 3C and 3D). Indeed, the plasma levels of LPS returned to those observed in uninfected individuals (\( P > .05; \) Figure 3C), whereas serum IFN\( \alpha \) levels dropped significantly below those found in healthy controls (\( P = .02; \) Figure 3D).

**DISCUSSION**

This is the first prospective, longitudinal study and comparative analysis of pDC distribution and function in the GALT and blood of HIV-infected individuals before and after initiation of ART. Our main findings are that pDCs relocate from blood to the GALT in chronic HIV infection. The high frequency of pDCs in the GALT correlates positively with IFN\( \alpha \) expression and an activated phenotype of CD8\(^+\) T cells, indicating an immune-inductive environment.

In a previous study, we concluded that the repeatedly reported decline of pDCs in blood of HIV patients [5, 7, 28] is due to trafficking and redistribution of pDCs from peripheral blood to lymph nodes rather than to systemic loss during the chronic phase of HIV infection [10]. Because the same study revealed that circulating pDCs of HIV-infected patients express higher levels of the gut-homing marker CD103, we hypothesized that pDCs would also accumulate in the GALT, the largest lymphoid tissue. Indeed, biopsies from the GALT of therapy-naive HIV-infected subjects showed a significantly higher frequency of pDCs compared with biopsies from healthy controls. As described previously [5, 7, 28], circulating pDCs were reduced in our HIV-infected therapy-naive group. These data from HIV-1–positive individuals are in line with 2 recent animal studies in a pathogenic SIV infection model also showing higher frequencies of pDCs in GALT compared with blood of infected animals [13, 29].

After 6 months on ART, the frequency of pDCs in blood normalized to levels of healthy individuals. In contrast, the percentage of pDCs in the GALT did not decline to normal values, indicating an incomplete reconstitution of the gut mucosa. Upon ART treatment, Kwa et al observed a decline of pDCs in the colorectum of SIV-infected rhesus macaques. Their specimens, however, were primarily obtained from the colorectum, whereas we analyzed biopsies from ileum where the density of Peyer’s patches is much higher than in the colorectum.

We thought of incomplete virological suppression of HIV in GALT as a possible reason for persistence of pDCs in the GALT. Though, when differentiating between “gut virological responders” and “gut nonresponders,” there was no association of response and frequency of pDCs (data not shown). Moreover, an expanded enteric virome as it has been described in pathogenic SIV infection [30] may also contribute to the migration of pDCs to the GALT.

Consistent with the evidence that pDCs of HIV-1 subjects relocate from blood to the GALT, we show that circulating pDCs of HIV-infected patients express higher levels of CD103 (also known as integrin \( \alpha_E \beta_7 \)), which—in complex with integrin \( \beta_7 \)—mediates cell redistribution to the intraepithelial sites and lamina propria of the GALT. Of note, expression of CD103 declined upon initiation of ART. Kwa et al observed upregulation of integrin \( \beta_7 \) on pDCs in SIV infection, but no increase in CD103 expression [13]. Instead, they found coexpression of CD49 (\( \alpha_4 \)), indicating the heterodimer expressed was \( \alpha_4 \beta_7 \) and not \( \alpha_E \beta_7 \). Moreover, they found upregulation of \( \beta_7 \)–integrin on pDCs derived from chronically HIV-infected patients. This observation—taken together with our finding of significant upregulation of integrin \( \alpha_E \) on pDCs from our therapy-naive individuals—suggests that, in humans, \( \alpha_E \beta_7 \) has a possible role in trafficking of peripheral pDCs to the GALT. The amount of
pDCs obtainable from blood and ileal biopsies was limited based on ethical consideration regarding the volume of blood and the number of biopsies. Thus, we were not able to analyze the expression of β7 integrin on pDCs in our study. However, our comparative analysis of GALT biopsies from healthy donors and HIV-infected individuals before and after initiation...
of ART suggests a role for pDC recruitment to the GALT in disease progression.

A possible causative link between increased IFNα expression, chronic immune activation, and HIV disease progression was suggested by the fact that after ex vivo exposure to IFNα, CD8+ T cells of HIV subjects express significantly more CD38 compared with uninfected individuals [2]. In our study, we show relocation of pDCs from blood to the GALT. In the GALT, these pDCs secrete excessive amount of IFNα, which is biologically active as indicated by an increased expression of MxA. This activity correlates positively with an activated phenotype of CD8+ T cells in the GALT. As also reported by others [31–33], we found that the frequency of HLA-DR+ CD38+ CD8+ T cells was higher not only in the GALT but also in blood of HIV-infected naive individuals compared with controls and longitudinally declined upon initiation of ART. This activated phenotype correlated with overexpression of MxA in the GALT and blood as well as with plasma levels of LPS in HIV-infected patients. The positive correlation of IFNα/MxA expression in the GALT with microbial translocation, the presence of LPS in blood, indicates that the mucosal barrier in HIV-positive naive subjects is not intact. The role of LPS in serum as a surrogate marker for the loss of mucosal integrity [34, 35] is reviewed in [36].

Brenchley et al showed a positive correlation of IFNα levels, LPS plasma levels, and the presence of activated CD8+ T cells (CD38+ HLA-DR+) in chronically infected HIV patients [15]. A microarray analysis of in vivo–activated CD4+ T cells from chronically infected HIV patients showed that hyperproliferating CD4+ T cells had an IFNα signature [37]. Furthermore, Estes et al demonstrated that IFNα colocalizes with bacterial components in lymphoid tissues of SIV-infected macaques [38]. Taken together, these data imply that not only the loss of CD4+ T cells but also the persistent release of proinflammatory IFNα by pDCs in the GALT of patients with chronic HIV infection contributes to enteropathy with the loss of mucosal integrity, thereby leading to microbial translocation, which in turn promotes chronic immune activation.

Aside from the infiltration of pDCs, the composition of immune cells in the GALT changes remarkably in the course of HIV infection [32, 39]. We found a significant depletion of CD4+ T cells in the GALT compared with HIV-negative individuals. In contrast, the infiltration of CD8+ T cells in the GALT was markedly higher in untreated chronic HIV-infected patients compared with HIV-negative subjects. After 6 months on ART, blood CD4+ T cells increased from 15% to 25%, whereas CD4+ T cells in GALT only increased from 10% to 14%. Also, the CD8+ T-cell frequency did not decline to values observed for healthy controls. Moreover, among the patients who completed the 6-month study, only 6 out of 11 achieved virologic control in GALT (<40 copies/CD4+ T cells). In healthy, HIV-uninfected individuals, approximately 40% of lymphocytes in GALT are CD4+ T cells [40, 41]. In accordance with a very recent publication, the ileal reconstitution of CD4+ T cells after 6 months on ART is insufficient [42]. Possible reasons for such partial immune recovery in the GALT of HIV-infected subjects on ART include damaged tissue architecture and deposition of collagen [42, 43] as well as low-level HIV-1 replication in the GALT [44–46].

This is the first study in humans reporting that in untreated HIV infection pDCs relocate from peripheral blood to the gut mucosa, the primary site of virus replication. Moreover, we report increased MxA expression—a surrogate marker of IFNα production—in the GALT of untreated HIV-1 subjects compared with uninfected individuals. After 6 months on ART, we observed only partial restoration of normal pDC frequency in GALT of infected individuals, whereas MxA expression declined to normal levels. The composition of immune cells in the GALT is also characterized by predominance of CD8+ T cells exhibiting an activated phenotype. We propose that these events promote local inflammation of the gut mucosa, leading to a loss of mucosal integrity and subsequent bacterial translocation. The latter drives systemic immune activation among other mechanisms by the proinflammatory effects of LPS [47]. And finally, the excessive local release of IFNα in the GALT refuels serum levels of biologically active IFNα, thereby directly contributing to immune activation. However, a distinct cause-effect relationship between IFNα, microbial translocation, and chronic immune activation remains to be established.

Notes

Acknowledgments. The authors are grateful to all the donors who participated in this study. They thank Tim Kümmerle, Christoph Wyen, and Kathrin Ehren for recruiting patients; Katja Rolshofen, Ute Sandaradura de Silva, and Désirée Captein for excellent technical support; Rolf Kaiser for microbiostatistical analysis; Ellen Rund and Gisela Kremer for administrative support. C. L. designed and performed experiments; K. F. and N. K. performed experiments; U. D. performed the immunohistochemistry analysis; L. L., S. M., and H. M. S. obtained GALT biopsies and discussed results; G. F. and J. F. selected patients and discussed results; N. J., E. R., and G. F. contributed to the design of the study and data interpretation; and C. L. and P. H. designed the research, analyzed data, and wrote the manuscript.

Financial support. This work was supported in part by Abbvie; C. L., N. J., G. F. and P. H. are supported by the Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF) grant 01K11017.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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