Macrophages Accumulate in the Gut Mucosa of Untreated HIV-infected Patients

Kristina Allers,¹ Mira Fehr,¹ Kristina Conrad,¹ Hans-Jörg Epple,¹ Dirk Schümann,² Anika Geelhaar-Karsch,¹ Katina Schinnerling,¹ Verena Moos,¹ and Thomas Schneider¹

¹Department of Gastroenterology, Infectious Diseases, and Rheumatology, Medical Clinic I, Campus Benjamin Franklin, and ²Department of Internal Medicine, Division of Infectious Diseases and Pulmonary Medicine, Campus Virchow-Klinikum, Charité—University Medicine Berlin, Berlin, Germany

Background. Mucosal macrophages are involved in the maintenance of epithelial barrier integrity and the elimination of invading pathogens. Although an intestinal barrier defect and microbial translocation are hallmarks of human immunodeficiency virus (HIV) infection, recent data on gut mucosal macrophages in HIV infection are sparse.

Methods. Treatment-naive and treated HIV-infected patients and healthy controls were studied for frequencies and functional parameters of blood monocytes and macrophages in duodenal mucosa.

Results. We found mucosal enrichment of macrophages in untreated HIV infection associated with reduced monocyte counts in blood and increased monocyte expression of the gut-homing molecule integrin β7. Increased CCR2 density on integrin β7-expressing monocytes and mucosal secretion of CCL2 suggest that CCR2/CCL2-chemotaxis is involved in enhanced trafficking of blood monocytes to the gut. Secretion of macrophage-related proinflammatory molecules interleukin 1β, CCL5, CXCL9, and CXCL10 was increased in the gut mucosa of untreated patients. Moreover, mucosal macrophages of untreated patients showed reduced phagocytic activity.

Conclusions. These data suggest a role for gut mucosal macrophages in HIV immune pathogenesis: infiltrated macrophages in the intestinal mucosa may promote local inflammation and tissue injury, whereas their low phagocytic activity prevents the efficient elimination of luminal antigens that cross the damaged intestinal barrier.

Keywords. HIV; gut mucosa; mucosal immune system; mucosal macrophages; phagocytosis; HIV immune pathogenesis.

Tissue macrophages play a pivotal role in the initiation and regulation of innate and adaptive immune reactions against microbes, food antigens, and apoptotic cells. An effective host response involves proinflammatory and antiinflammatory functions of macrophages, both of which are tightly regulated by the local environment. Most of the body’s macrophages reside in the small and the large intestine [1]. Under homeostatic conditions, intestinal macrophages are located in the gut mucosa underneath the epithelium [2]. Here, they are strategically positioned for the rapid clearance of gut lumen–derived bacteria and foreign antigens that have crossed the epithelial layer. In contrast to macrophages located in other tissues, gut mucosal macrophages are functionally inert despite potent phagocytic and antibacterial activity; that is, they do not produce inflammatory effector molecules in response to pathogen-associated molecular patterns [3–7]. Factors in the intestinal environment are responsible for this functional behavior of gut mucosal macrophages. In humans, transforming growth factor β (TGF-β) produced by different cell types in the mucosa, including epithelial and mast cells, has been identified as an important factor for downregulation of macrophage inflammatory responses [5]. The inflammation anergy of gut mucosal macrophages is critical to prevent inflammation-induced tissue damage despite constant exposure to bacterial and other luminal stimuli. In addition, mucosal macrophages are important regulators of tissue repair mechanisms and crucial for maintaining the epithelial barrier integrity [8, 9].
In human immunodeficiency virus (HIV)–infected persons, the intestinal epithelial integrity is impaired [10]. Enhanced translocation of luminal antigens across the damaged epithelial barrier is thought to trigger systemic chronic immune hyperactivation [11, 12], which is a main driving force for HIV disease progression [13–15]. Although macrophages are the first line of defense against invaded microbes and antigens, and their functional propensity influences the epithelial integrity, little is known about the quantity and quality of mucosal macrophages in HIV-infected persons [16]. Interestingly, recent findings from chronically simian immunodeficiency virus (SIV)–infected rhesus macaques indicate impaired bacterial clearance function of gut mucosal macrophages [17].

To gain further insight into the possible role of mucosal macrophages into the immune pathogenesis of HIV infection, we evaluated the number und functional parameters of macrophages in the duodenal mucosa of treatment-naive HIV-infected patients in comparison to healthy control subjects and HIV-infected patients receiving combination antiretroviral therapy (cART).

**METHODS**

**Subjects**

HIV-seropositive patients without previous antiretroviral therapy (treatment naive) or receiving cART for a minimum of 8 months underwent diagnostic endoscopy for various symptoms related to the gastrointestinal tract, such as nausea, abdominal pain, heartburn, diarrhea, and weight loss. With the patients’ informed consent for this procedure, additional duodenal biopsy specimens were collected for the purpose of the present study. Biopsy specimens were obtained from macroscopically unaffected areas in the first portion of the duodenum. Patients with diagnosis of a secondary infection in the intestine or with gastrointestinal neoplasia were excluded from the study. From 32 treatment-naive and 15 treated HIV-infected patients, 2 biopsy specimens per patient were obtained for immune histological examination and/or for short-term tissue cultivation. From 7 additional treatment-naive patients, 12 biopsy specimens were obtained for mucosal cell isolation and subsequent functional analysis. Heparinized blood samples were collected from 15 treatment-naive and 18 treated patients who did not undergo endoscopic biopsy.

HIV-seronegative, voluntary subjects without intestinal symptoms and with no current medication served as healthy controls. We obtained paraffin-embedded duodenal tissue sections and/or stored short-term duodenal tissue culture supernatants from 22 persons who underwent baseline endoscopy in a previous study (ie, before the study-related vaccination described elsewhere was administered [18]). Biopsy material sufficient for mucosal cell isolation was collected from 8 additional HIV-seronegative persons. Heparinized blood samples were obtained from 27 healthy donors who did not undergo endoscopic biopsy. Patient and healthy control characteristics are given in Table 1.

Quantitative and phenotypic flow cytometric analysis of peripheral monocytes cells was performed immediately after blood sampling. The study was approved by the Charité–Universitätsmedizin Berlin institutional review board, and all participants gave informed consent to participate in the study.

**Immunohistochemical Analysis**

All biopsy samples used for immunohistochemical analysis were embedded in paraffin. Immunostaining on paraffin sections was performed as described previously [19–21]. Antibodies were mouse anti-CD68 (PGM1, Dako) or mouse anti-CD163 (10D6, Novocastra). For detection of CD68 or CD163 labeling, a streptavidin alkaline phosphatase kit (Dako) was used according to the manufacturer’s instructions. Positive cells within the mucosa of duodenal tissue were quantified per high-power field (hpF: 0.237 mm²), and 10 hpF were averaged in each case. Immunohistochemical evaluations were performed in a blinded manner (ie, the researcher was unaware of the subject’s clinical characteristics). Negative controls were performed by omitting the primary antibody and by using the appropriate isotype control.

**Flow Cytometric Analysis**

Flow cytometric analysis was performed using antibodies against CD4 (clone RTA-T4; BD Biosciences [BD]), CD14 (M5E2; BD), integrin β7 (FIB504; BD), CCR2 (48607; BD), and CX3CR1 (2A9-1; Medical & Biological Laboratories). For staining of whole blood, antibodies were added to 50 µL of heparinized blood and incubated for 15 minutes at room temperature in the dark. Erythrocytes were then lysed and fixed with FACS Lysing Solution (BD). Absolute numbers of CD4⁺ T cells were determined in whole blood by the use of BD Trucount Tubes and BD TriTest CD4/CD8/CD3 according to the manufacturer’s protocol. Derived from absolute CD4⁺ T cell numbers and the percentages of CD4⁺ T cells and CD14⁺ monocytes within the total lymphocyte population, absolute CD14⁺ monocyte numbers were calculated. Antibodies were conjugated to fluorescein isothiocyanate, phycoerythrin, peridin chlorophyll protein, allophycocyanin, or Alexa Fluor 647. CCL2 concentration was quantified in thawed plasma samples with a flow cytometric bead array (BD) according to the manufacturer’s protocol. Data were acquired on the FACS Calibur (BD) and analyzed with CellQuest software (BD).

**Short-term Tissue Culture and Quantification of Mucosal Cytokine and Chemokine Secretion**

Short-term culture supernatants of duodenal biopsy specimens were prepared as described before [10, 22]. Briefly, biopsy specimens were washed, weighed, and incubated in 200–1000 µL fetal calf serum–free Roswell Park Memorial Institute 1640 medium (Gibco-BRL) containing 100 U/mL penicillin, 100 µg/mL streptomycin, and 2.5 µg/mL amphotericin (Seromed Biochrom).
KG) for 48 hours at 37°C in a humidified 5% CO₂/70% O₂ air atmosphere. Supernatants were stored at −70°C until assay. Cytokines and chemokines were quantified in the supernatants with flow cytometric bead arrays (BD or BenderMed systems) according to the manufacturer’s protocol and are given as weight of the respective molecule per tissue weight.

**Phagocytic Activity of Macrophages and Monocytes**

Mucosal mononuclear cells were isolated from duodenal biopsy specimens by collagenase type II (Sigma) digestion as described previously [23] and immediately used for analysis. The percentage of viable mononuclear cells in the cell preparations was >97% as assessed flow cytometrically, using propidium iodide for dead cell staining. Whole blood was analyzed within 2 hours of blood collection. Phagocytic activity of mucosal macrophages or blood monocytes was evaluated with the use of the pHrodo *Escherichia coli* BioParticles phagocytosis kit for flow cytometry (Invitrogen) according to the manufacturer’s protocol. Emission intensity of the fluorogenic dye pHrodo increases with acidification during the phagocytosis process. Mucosal mononuclear cell suspension of 3 × 10⁵/100 µL or 100 µL of whole blood was incubated with 50 µL or 20 µL of pHrodo dye-labeled *E. coli* BioParticles at 37°C, 5% CO₂, for 1 hour or 15 minutes, respectively. As control of attached but not phagocytically ingested particles, cells were incubated with pHrodo *E. coli* on ice (background control), and a sample without particles incubated at 37°C served as negative control. Phagocytic activity was evaluated as the mean fluorescence intensity (MFI) of pHrodo in the monocyte/macrophage gate after 37°C incubation divided by the MFI obtained with the corresponding background control and is expressed as the MFI ratio. In addition, the proportion of monocytes/macrophages that have phagocytized *E. coli* particles was identified as the percentage of highly fluorescent pHrodo⁺ cells.

**Table 1. Characteristics of Human Immunodeficiency Virus (HIV)–Infected Patients and Control Subjects in This Study and Numbers of Examined Samples**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Subjects</th>
<th>Treatment-Naive Patients</th>
<th>Treated Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenal samples, no. a</td>
<td>42 from 30 subjects</td>
<td>50 from 39 patients</td>
<td>20 from 15 patients</td>
</tr>
<tr>
<td>For immune histologic analysis b</td>
<td>14</td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td>Sex, male; female</td>
<td>14; 0</td>
<td>21; 7</td>
<td>7; 4</td>
</tr>
<tr>
<td>Age, y</td>
<td>40 (22–49)</td>
<td>43 (19–77)</td>
<td>52 (31–62)</td>
</tr>
<tr>
<td>CD4⁺ T-cell count, cells/µL</td>
<td>ND</td>
<td>192 (2–657)</td>
<td>512 (322–929)</td>
</tr>
<tr>
<td>Viral load, log₁₀ copies/µL</td>
<td>NA</td>
<td>5.1 (3.2–5.9)</td>
<td>Less than the detection limit</td>
</tr>
<tr>
<td>Esophageal or gastric diseases</td>
<td>0</td>
<td>10 had <em>Candida</em> esophagitis, 2 had <em>H. pylori</em> gastritis (1 patient had both diseases)</td>
<td>2 had <em>H. pylori</em> gastritis</td>
</tr>
<tr>
<td>For culture supernatants b,c</td>
<td>20</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Sex, male; female</td>
<td>20; 0</td>
<td>13; 2</td>
<td>7; 2</td>
</tr>
<tr>
<td>Age, y</td>
<td>36 (22–49)</td>
<td>41 (26–51)</td>
<td>51 (38–60)</td>
</tr>
<tr>
<td>CD4⁺ T-cell count, cells/µL</td>
<td>ND</td>
<td>297 (9–650)</td>
<td>611 (318–878)</td>
</tr>
<tr>
<td>Viral load, log₁₀ copies/µL</td>
<td>NA</td>
<td>5.1 (3.6–5.7)</td>
<td>Less than the detection limit</td>
</tr>
<tr>
<td>Esophageal or gastric diseases</td>
<td>0</td>
<td>4 had <em>Candida</em> esophagitis</td>
<td>3 had <em>H. pylori</em> gastritis</td>
</tr>
<tr>
<td>For phagocytosis tests</td>
<td>8</td>
<td>7</td>
<td>ND</td>
</tr>
<tr>
<td>Sex, male; female</td>
<td>5; 3</td>
<td>6; 1</td>
<td>. . .</td>
</tr>
<tr>
<td>Age, y</td>
<td>49 (20–83)</td>
<td>46 (20–75)</td>
<td>. . .</td>
</tr>
<tr>
<td>Viral load, log₁₀ copies/µL</td>
<td>NA</td>
<td>5.8 (2.1–6.15)</td>
<td>. . .</td>
</tr>
<tr>
<td>Esophageal or gastric diseases</td>
<td>1 had <em>H. pylori</em> gastritis</td>
<td>2 had <em>Candida</em> esophagitis</td>
<td>. . .</td>
</tr>
<tr>
<td>Total whole blood samples a</td>
<td>27</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Sex, male; female</td>
<td>25; 2</td>
<td>11; 4</td>
<td>16; 2</td>
</tr>
<tr>
<td>Age, y</td>
<td>39 (21–78)</td>
<td>47 (20–75)</td>
<td>47 (28–78)</td>
</tr>
<tr>
<td>CD4⁺ T-cell count, cells/µL</td>
<td>793 (476–1409)</td>
<td>334 (12–626)</td>
<td>571 (365–1320)</td>
</tr>
<tr>
<td>Viral load, log₁₀ copies/µL</td>
<td>NA</td>
<td>4.7 (2.1–6.2)</td>
<td>Less than the detection limit</td>
</tr>
</tbody>
</table>

Data are no. of patients or median value (range), unless otherwise indicated.

Abbreviations: *H. pylori*, *Helicobacter pylori*; NA not applicable; ND, not determined.
a In both control and patient groups, subjects who underwent endoscopic biopsy and subjects who were included in the whole blood analysis did not overlap.
b Twelve control subjects, 11 treatment-naive patients, and 5 treated patients were included in both the immune histological examinations and the culture supernatant experiments.
c Frozen plasma samples were obtained in parallel.
Statistical Methods
Data are represented as median values with interquartile ranges and were analyzed with the use of the Mann-Whitney U test. Bivariate correlations and statistical significance were determined by the Spearman rank correlation test. All data were statistically analyzed with Prism software, version 4.0 (Graph Pad Inc).

RESULTS

Macrophages Accumulate in the Gut Mucosa of Treatment-Naive HIV-Infected Patients, Whereas the Monocyte Count in the Peripheral Blood Is Decreased

Absolute numbers of macrophages in the duodenum and CD14+ blood monocytes were assessed by immunohistochemistry and quantitative flow cytometry, respectively. Using the human tissue macrophage markers CD68 and CD163, we found 1.7- and 1.8-fold higher numbers of mucosal macrophages in treatment-naive HIV-infected patients than in healthy controls (Figure 1A).

Subdividing the treatment-naive study group into patients with and those without infections in the esophagus or/and stomach (Table 1) revealed no difference in the median number of macrophages in the duodenum (for CD68+ macrophages, 73/hpf [interquartile range (IQR), 53–82/hpf] and 62/hpf [IQR, 53–77/hpf], respectively [P = .5150]; for CD163+ macrophages, 93/hpf [IQR, 75–101/hpf] and 81/hpf [IQR, 70–101/hpf], respectively [P = .6048]). Also, after exclusion of patients with esophageal or gastric infections from the analysis, the increase in mucosal macrophages in treatment-naive HIV-infected patients remained significantly increased (P < .0001 and P < .0001 for CD68+ macrophages/hpf and CD163+ macrophages/hpf, respectively, when compared with healthy controls; P = .0122 and P = .0156 for CD68+ macrophages/hpf and CD163+ macrophages/hpf, respectively, when compared with treated patients). This demonstrates that infections of the esophagus or stomach had no significant influence on the duodenal macrophage density in our study patients.

In treated HIV-infected patients, mucosal macrophage numbers were not significantly different from those in healthy controls (Figure 1A). In peripheral blood, the numbers of monocytes were 1.8-fold lower in untreated HIV-infected patients than in healthy controls and treated patients (Figure 1B). In untreated HIV-infected patients, plasma viral load did not correlate with peripheral blood monocytes or gut macrophages, and in both untreated and treated patients, as well as in healthy controls, there was no correlation of monocyte or macrophage numbers with peripheral CD4+ T-cell counts or age.

Consistent with the fact that macrophages are typically located subepithelially, we found in duodenal tissue of our healthy controls and treated HIV-infected patients a uniform distribution of macrophages close to the epithelial layer. In untreated HIV-infected persons, in contrast, mucosal macrophages were dispersed throughout the whole lamina propria (Figure 1C).

Loss of Monocytes From Peripheral Blood Is Associated With Altered Expression of Integrin β7, CCR2, and CCL2

Our results of macrophage accumulation in the gut mucosa together with the loss of monocytes from peripheral blood indicate enhanced trafficking of blood monocytes in the gut. To investigate a potential trafficking mechanism, we analyzed blood monocytes for expression of integrin β7, a cell adhesion molecule that is known to mediate trafficking of lymphocytes to the gut [24]. Additionally, we analyzed the expression of the chemotactic homing receptors CCR2 (C-C motif receptor 2), which has been shown to be involved in monocyte migration to inflamed tissues including the gut mucosa, and CX3CR1 (C-X3-C motif receptor 1), which is involved in monocyte recruitment to noninflamed tissues (Figure 2A) [25, 26].

In all comparison groups, CCR2 expression density was greater on integrin β7–expressing monocytes than on the integrin β7–nonexpressing monocyte fraction, whereas CX3CR1 expression did not differ significantly between both monocyte fractions (Figure 2B), indicating that the cell adhesion molecule integrin β7 and the chemokine CCR2 may act together in monocyte trafficking.

Expression of the adhesion molecule integrin β7 within monocytes was higher in treatment-naive HIV-infected patients than in healthy controls or treated patients (Figure 2C). Moreover, on the integrin β7–expressing monocyte fraction, chemokine receptor CCR2 but not CX3CR1 expression was increased in treatment-naive HIV-infected patients, compared with healthy controls or treated patients (Figure 2B). In addition, mucosal secretion of the main ligand for CCR2, the potent monocyte attracting chemokine CCL2 (C-C motif ligand 2; also known as monocyte chemotactant protein 1 [MCP-1]), was 3-fold increased in treatment-naive HIV-infected patients, whereas there was no significant difference in the CCL2 plasma concentration between healthy controls and the HIV-infected patient groups (Figure 2D), indicating that attraction of CCR2-expressing monocytes to the gut may be enhanced in untreated HIV infection.

Taken together, these results suggest that enhanced integrin β7 monocyte expression and chemotaxis via the CCR2/CCL2 axis could be involved in an increased trafficking of monocytes to the gut in untreated HIV infection.

Mucosal Secretion of Effector Molecules Related to Proinflammatory Macrophage Activation Is Increased in Untreated HIV-Infected Patients

We measured mucosal secretion of cytokines and chemokines that are known to induce macrophage activation and/or are typically produced by activated macrophages, namely interleukin 1β (IL-1β), CCL5 (also known as regulated on activation
Figure 1. In untreated human immunodeficiency virus (HIV) infection, macrophages accumulate in the gut mucosa, whereas monocyte numbers in peripheral blood decrease. A, Mucosal macrophages were quantified in duodenal mucosa from healthy controls and from treatment-naive and treated HIV-infected patients by immunohistochemical stain of the human macrophage markers CD68 or CD163. Positive cells within the mucosa were counted per high-power field (hpf; 0.237 mm²), and 10 hpf were averaged in each case. B, Absolute numbers of CD14⁺ monocytes in fresh whole blood from controls and from treatment-naive or treated HIV-infected patients were quantified by flow cytometry. C, Representative immunohistochemically stained macrophages in duodenal mucosa from healthy controls and from untreated or treated HIV-infected patients; mucosal macrophages were detected as mononuclear cells expressing CD68 or CD163. Original magnification ×200. Data are given in medians with interquartile ranges and were analyzed using the 2-tailed Mann–Whitney U test. Abbreviation: cART, combination antiretroviral therapy.
Figure 2. Expression of integrin β7 and CCR2 on monocytes and mucosal secretion of CCL2 are increased in untreated human immunodeficiency virus (HIV) infection. A, CD14+ monocytes in fresh whole blood were analyzed for surface expression of integrin β7 and coexpression of CCR2 or CX3CR1. B and C, The density of the chemokine receptor CCR2 or CX3CR1 on integrin β7–expressing and integrin β7–nonexpressing monocytes (B) and the percentage of integrin β7–expressing monocytes (C) in healthy controls and in treatment-naive and treated HIV-infected patients. D, Mucosal secretion or plasma levels of CCL2, the main ligand of CCR2, in healthy controls and treatment-naive or treated HIV-infected patients. Data are represented as medians with interquartile ranges and were analyzed using the 2-tailed Mann–Whitney U test. Abbreviations: cART, combination antiretroviral therapy; MFI, mean fluorescence intensity.
normal T-cell expressed and secreted [RANTES]), CXCL9 (C-X-C motif ligand 9; also known as monokine induced by interferon γ [MIG]), and CXCL10 (also known as interferon γ–induced protein 10 [IP-10]), for the proinflammatory response, and interleukin 10 (IL-10) and interleukin 13 (IL-13), for the antiinflammatory response [27, 28]. In addition, the regulatory cytokine TGF-β, which acts as a potent inducer of inflammatory energy of mucosal macrophages [5, 29], was measured in its active form. Despite the increased density of macrophages in the duodenum of treatment-naïve HIV-infected patients (Figure 1A), mucosal secretion of TGF-β remained within the range of secretion by healthy controls (Figure 3A), which may indicate a less efficient TGF-β–mediated downregulation of macrophage inflammation in untreated HIV infection. In parallel, we found a 4–16-fold higher secretion of the proinflammatory molecules IL-1β, CCL5, CXCL9, and CXCL10 in duodenal tissue from treatment-naïve HIV-infected patients, compared with healthy controls (Figure 3B). In contrast, secretion of the typical antiinflammatory cytokines IL-10 and IL-13 was not increased in the tissue samples from these patients (Figure 3C).

Phagocytic Activity of Mucosal Macrophages Is Impaired in Untreated HIV Infection

To gain further insight into the function of mucosal macrophages and peripheral monocytes in HIV infection, we analyzed their phagocytic activity. The fluorogenic dye pHrodo, which increases in fluorescence with acidification during phagocytosis, was used to measure the uptake of E. coli particles.

In both the mucosal macrophage population and the peripheral monocyte population, we observed a shift in fluorescence intensity after incubation with the dye-conjugated particles, compared with the respective background control (of attached but not ingested particles), as well as a cell a fraction with high phagocytic activity (Figure 4A).

Similar to previous findings [30], we found a lower percentage of phagocytic monocytes in treatment-naïve HIV-infected patients, compared with healthy controls or treated HIV-infected patients (Figure 4B). In addition, the percentage of phagocytic mucosal macrophages was reduced in untreated HIV-infected patients, compared with healthy controls (Figure 4B). Analysis of the phagocytic activity of the whole monocyte/macrophage populations (expressed as the MFI ratio) also revealed impaired phagocytosis of both mucosal macrophages and peripheral monocytes in untreated HIV infection (Figure 4C). These results indicate that monocyte-derived macrophages in the gut of untreated HIV-infected patients do not recover their phagocytic activity in the mucosal microenvironment.

DISCUSSION

In the present study, we demonstrate alterations in the number and function of macrophages in the gut mucosa of untreated HIV-infected patients. For quantitative analysis, mucosal macrophages were detected by the expression of CD68, which belongs to the lysosome-associated membrane protein family and is present in most macrophages, and by the expression of the scavenger receptor CD163, which is restricted to monocytes and macrophages, with neither dendritic cells nor granulocytes expressing significant levels of this molecule [31–35]. In humans, CD163 expression increases with differentiation of monocytes to mature tissue macrophages and is associated with both proinflammatory and antiinflammatory activation [32, 34–37]. Using these specific markers for human tissue macrophages, we found an approximately 2-fold increased frequency of macrophages in the gut mucosa of untreated HIV-infected subjects. In contrast to this finding, one study from the early 1990s reported no difference in the duodenal CD68⁺ macrophage density between HIV-infected patients and HIV-seronegative controls [16]. However, patients in this previous study were not differentiated by treatment status, and, importantly, the CD68 antibody used to identify mucosal macrophages was later shown to be less specific than the clone used in our study [38]. Our results from CD68⁺ macrophage enumerations were confirmed by the use of CD163 as an additional macrophage marker. Moreover, as monocyte numbers were reduced in blood of untreated HIV-infected patients, our findings indicate increased recruitment of peripheral monocytes to the gut mucosa. One possible mechanism behind this altered monocyte trafficking was demonstrated by increased surface expression of integrin β7 on peripheral monocytes, an adhesion molecule that is involved in trafficking of T cells to the gut mucosa [24] and also appears to be associated with gut trafficking of natural killer cells and plasmacytoid dendritic cells [39, 40]. Receptor-mediated chemotactic response usually enhances cell migration into tissues. Our results indicate that, in HIV infection, the CCL2–CCR2 chemotactic axis is involved in mucosal infiltration by monocyte-derived macrophages, because increased expression of CCR2 on integrin β7–expressing monocytes of HIV-infected patients occurred together with increased mucosal secretion of its main ligand, the chemokine CCL2. CCR2-dependent macrophage infiltration of gut mucosa in response to CCL2 has recently been recognized during experimental colitis, as well as in patients with inflammatory bowel disease [25, 41]. Moreover, CCL2 production is induced by several infections, and CCR2-dependent recruitment of monocytes to tissue sites has been demonstrated to be essential for the defense against bacterial and protozoal infections [42–46]. During HIV infection, quantities of microbial components that cross the intestinal epithelium are thought to increase because of an impaired mucosal barrier function [10–12]. Therefore, high levels of lumen-derived stimuli in the lamina propria may change the release of chemokines by epithelial cells and/or other intestinal cells of HIV-infected patients and, in this way, promote the attraction of monocytes to the gut mucosa as part of the innate host response. Additionally, accumulation of innate cells,
including macrophages, may be exerted by HIV itself, which massively replicates within the intestinal mucosa. Interestingly, in SIV-infected rhesus macaques, mucosal enrichment of

Figure 4. Phagocytic activity of mucosal macrophages is impaired in untreated human immunodeficiency virus (HIV) infection. A, Representative flow cytometric analysis of monocyte/macrophage phagocytosis. The mean fluorescence intensity (MFI) ratio represents the MFI of pHrodo Escherichia coli at 37°C (filled histogram) divided by the MFI obtained with the corresponding on-ice control (open histogram). Percentages represent the proportion of monocytes/macrophages that have phagocytized E. coli particles. B and C, Percentage of phagocytic cells (B) and phagocytic activity of the whole monocyte/macrophage population (C, expressed as MFI ratio) in HIV-infected patients, compared with healthy controls. This assay was not performed for mucosal macrophages from treated HIV-infected patients.
plasmacytoid dendritic cells, one cell type that act together with macrophages in innate host immunity, has recently been demonstrated [40].

Innate immune response receptors, including CD163 on macrophages, mediate bacterial binding with subsequent release of proinflammatory cytokines [37]. In healthy intestinal mucosa, inappropriate inflammatory responses of macrophages are potentially downregulated to avoid activation-induced tissue injury and immune dysfunction [5]. The local microenvironment determines this functional hyporesponsiveness of mucosal macrophages, whereby TGF-β has been shown to have an important role in inducing inflammatory anergy of human macrophages [5, 29]. In untreated HIV infection, we found that the mucosal TGF-β level does not increase despite the mucosal macrophage infiltration, indicating a lack of efficient local downregulation of inflammatory macrophage responses. Consistent with this, mucosal secretion of effector molecules related to proinflammatory macrophage activation, such as IL-1β, CCL5, CXCL9, and CXCL10, was increased in untreated HIV infection, while secretion of IL-10 and IL-13, which are typically related to antiinflammatory macrophage activation, remained unchanged compared with findings for healthy controls. This alteration in the mucosal cytokine/chemokine milieu is characteristic for enhanced proinflammatory macrophage activity [27, 28] and likely contributes to inflammatory tissue injury and local immune dysfunction. For example, CXCL9, CXCL10, and CCL5 are potent chemotaxants for activated T cells and natural killer cells, which may further drive proinflammatory processes in the gut. Moreover, increased CCL5 secretion in the gut mucosa leads also to an enhanced attraction of activated CCR5+ CD4+ T cells, the primary targets of HIV infection, and may thus support viral replication within the gut mucosa.

The high phagocytic activity of mucosal macrophages contributes to the maintenance of intestinal homeostasis by clearing invaded microorganisms from the tissue [5]. However, we found reduced phagocytic activity of mucosal macrophages in untreated HIV-infected patients. Thus, low phagocytic function of mucosal macrophages in HIV infection may play a role in the inefficient defense against pathogens and antigens that cross the epithelial layer. Consistent with this, in situ and ex vivo data obtained from chronically SIV-infected rhesus macaques and from a humanized mice model of HIV infection, respectively, indicate insufficient elimination of translocated luminal products by tissue macrophages [17, 47].

In summary, our results demonstrate an accumulation of macrophages in the gut mucosa of HIV-infected patients. These mucosal macrophages appear to be less efficiently downregulated in their proinflammatory activity and have reduced phagocytic activity. Thus, mucosal macrophages may contribute to local inflammation-induced tissue injury without increasing their capacity to eliminate luminal products that cross the damaged epithelial layer. Assuming that enhanced luminal antigen translocation promotes chronic immune hyperactivation in HIV infection, these alterations of mucosal macrophages may play a central role in the pathogenesis of HIV infection.

Notes

Acknowledgments. We thank the patients and healthy subjects, for their participation in this project; and Diana Bösel, Martina Seipel, and Nadine Gehrmann, for excellent technical assistance.

Financial support. This work was supported by the Deutsche Forschungsgemeinschaft (grant SCHN 616/1-1).

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.

References


