Enteric Mucosa Integrity in the Presence of a Preserved Innate Interleukin 22 Compartment in HIV Type 1–Treated Individuals

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Background. Interleukin 22 (IL-22) is emerging as a key cytokine for gut epithelial homeostasis and mucosal repair. Gut disruption is a hallmark of human immunodeficiency virus (HIV) infection. Here, we investigated IL-22 production and gut mucosal integrity in HIV type 1 (HIV-1)–infected individuals receiving long-term antiretroviral therapy (ART).

Methods. Biopsy specimens from 37 individuals who underwent colonoscopy primarily for cancer screening and from 17 HIV-1–infected and 20 healthy age-matched controls were assessed.

Results. We found significant depletion of sigmoid IL-22–producing CD4+ T cells (T-helper type 22 [Th22] cells) even after prolonged ART, contrasting with the apparently normal compartments of regulatory and interleukin 17 (IL-17)–producing CD4+ T cells, as well as total mucosal CD4+ T cells. Despite the preferential Th22 cell depletion, IL-22 production by innate lymphoid cells (ILCs) was similar to that observed in HIV-1–seronegative subjects, and transcription of genes encoding molecules relevant for IL-22 production (ie, AHR, IL23, IL23R, IL1B, IL6, and TGFB1) was preserved. Remarkably, levels of transcripts of IL-22–target genes (ie, REG3G, DEFB4A, S100A9, MUC1, and MUC13) were unaltered, suggesting an adequate production of antimicrobial peptides and mucins. In agreement, enteric epithelial architecture was fully preserved.

Conclusions. Despite the reduced Th22 cell subset, innate IL-22–mediated mechanisms, essential for sigmoid mucosa integrity, were fully operational in long-term–treated HIV-1–infected individuals. Our data highlight IL-22 production by ILCs as an important target for therapies aimed at facilitating human mucosal reconstitution.

Keywords. gut associated lymphoid tissue; HIV/AIDS; mucosa reconstitution; IL-22; antiretroviral therapy.

The gut mucosa, the body’s largest interface with the external environment, provides the first microbial barrier. Furthermore, its interaction with the microbiome actively shapes the systemic immune system and determines human health [1]. Interleukin 22 (IL-22) has emerged as a key cytokine in these processes [2]. Signaling via the IL-22 receptor induces proliferative and anti-apoptotic molecules in gut epithelial cells [3], which have been shown to be critical for epithelial homeostasis, particularly in the context of mucosal insults. Additionally, IL-22 signaling triggers epithelial production of antimicrobial peptides, which are relevant both for the containment of the gut microflora and the response to infections [4].

While the IL-22 receptor is essentially expressed by nonhematopoietic cells, specifically the gut epithelia, IL-22 itself is produced, under tight regulation, by hematopoietic cells [2]. Of note, high rates of epithelial proliferation due to deregulated IL-22 production have been associated with colon cancer [5]. In the context of epithelial lesions, shedding of microbial products...
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 leads to the secretion of interleukin 23 by dendritic cells [6], which, in conjunction with interleukin 1β and interleukin 6, induces IL-22 production. On the other hand, interleukin 25 secretion by the intact/repaired epithelium is known to inhibit this process [7].

The main IL-22 producers in the gut mucosa are CD4⁺ T cells (T-helper type 22 [Th22] cells) [8] and a subset of innate lymphoid cells (ILCs), named ILC3 [9]. It is currently unknown whether factors governing IL-22 production impact distinctly on the above 2 populations or whether these populations differentially contribute to the preservation of mucosal integrity [10].

ILC3 were initially described as participating in the development of gut-associated lymphoid tissue (GALT) [11] and were later shown to be critical for the response to tissue injury in adult life in the gut, thymus, and lymph nodes [12, 13]. In addition to their nonredundant contribution to the control of attaching-effacing enteric infections [14], ILC3 were recently reported to suppress CD4⁺ T-cell activation in response to commensal bacteria [15], further emphasizing their fundamental role in intestinal homeostasis.

IL-22 is likely to be implicated in the pathogenesis of human immunodeficiency virus (HIV) infection and AIDS, given the profound disruption of gut mucosa typically observed throughout disease [16]. Acute HIV type 1 (HIV-1) infection leads to a massive loss of mucosal CD4⁺ T cells, which persists into the chronic phase [17], and is inconsistently recovered upon long-term antiretroviral therapy (ART) [18]. Loss of epithelial integrity and high levels of circulating microbial products have been reported even in treated patients and are considered important contributors to HIV-associated pan-immune activation that underlies the pathogenesis of HIV infection and AIDS [19–21]. The heightened activation state of the immune system is also associated with an increased risk of noninfectious events in ART-treated individuals.

Most previous studies of the gut of HIV-1–infected patients focused on the loss of IL-17–producing cells [22]. However, given the overlap between the mucosal populations that produce IL-22 and/or interleukin 17 [10], it is possible that some of the reported disturbances were due to IL-22. Interestingly, although IL-22 was initially described as a Th17 cytokine, there are several factors supporting a unique identity for IL-22–producing cells [8, 10]. Production of these 2 cytokines is linked to expression of the transcriptional factor retinoic acid orphan receptor (ROR)γt and the homing receptor CCR6 [23]. However, aryl hydrocarbon receptor (AHR) ligands have a preferential impact on IL-22 production [24]. Additionally, transforming growth factor β (TGF-β) induces IL-17 secretion while inhibiting IL-22 production via c-Maf induction in CD4⁺ T cells [25]. Nevertheless, concomitant production of IL-17 may balance positive and negative impacts of IL-22, as suggested in inflammatory bowel disease [26].

The limited data available suggest that mucosal IL-22 production is reduced during chronic HIV-1 [27] and simian immunodeficiency virus (SIV) infections [28]. However, mucosal distribution of enteric IL-22–producing cell populations in humans is still poorly defined. Furthermore, their relationship with the expression of genes involved in epithelial structure homeostasis is yet to be determined.

We performed a comprehensive study of Th22 cells and ILC3 in the sigmoid mucosa of long-term-treated HIV-1–infected individuals with effective control of viremia and immune reconstitution. We found histological evidence of mucosal integrity, despite a significant depletion of IL-22–producing CD4⁺ T-cells, compared with findings for HIV-1–seronegative subjects. Strikingly, our study revealed preserved numbers of ILCs and normal levels of mucosal IL-22–induced molecules. Thus, our data indicate a dissociation of the mechanisms that maintain Th22 and ILC3 in the human gut, highlighting ILC3 as potentially important therapeutic targets in achieving mucosal integrity in HIV.

METHODS

Studied Cohorts

The study enrolled 37 individuals with a clinical indication to perform colonoscopy (35 of 37 were for colon cancer screening) who were found to be cancer free: 17 were HIV-1–infected individuals who had been receiving ART for a median duration of 8 years with evidence of effective virological and immunological response, and 20 were HIV-1–seronegative patients (Table 1). All individuals gave written informed consent for blood sample and gut biopsy specimen collection and processing. The study was conducted under the approval of the Ethical Boards of the Faculty of Medicine of University of Lisbon, Hospitals Santa Maria and São Bernardo.

Cell Isolation and Culture

Twelve sigmoid biopsy specimens per individual were collected from macroscopically normal mucosa and processed immediately. A total of 7–10 biopsy specimens were digested with collagenase B (10 mg/mL, Roche, Penzberg, Germany) at 37°C and were then mechanically macerated. Lymphocytes were separated by Percoll gradient and cultured for 16 hours at 37°C in complete medium [29]. Cytokine production was assessed after 4-hour stimulation with phosphor myristate acetate plus ionomycin, as previously described [29]. Blood specimens were collected in parallel from 10 HIV-1–infected and 9 control individuals, and peripheral blood mononuclear cells (PBMCs) were isolated by a Ficoll-Hypaque centrifugation gradient.

Flow Cytometry

Cells were surface and intracellularly stained (Foxp3 Staining Buffer Set, Ebioscience) as previously described [30], using a panel of antibodies (Supplementary Table 1), and were acquired on Fortessa flow cytometer (BD Biosciences). After exclusion of
Table 1. Characteristics of Subjects in the Human Immunodeficiency Virus Type 1 (HIV-1)–Seronegative Control Cohort and the HIV-1–Infected Cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n = 20)</th>
<th>HIV-1 Infected (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Age, y</td>
<td>58 (49–63)</td>
<td>58 (52–60)</td>
</tr>
<tr>
<td>Length of follow-up, y</td>
<td>NA</td>
<td>12 (8–12)</td>
</tr>
<tr>
<td>Length of ART, y</td>
<td>NA</td>
<td>8 (6–11)</td>
</tr>
<tr>
<td>Nadir CD4+ T-cell count before ART initiation, cells/μL</td>
<td>NA</td>
<td>339 (162–389)</td>
</tr>
<tr>
<td>Viremia, HIV-1 RNA copies/mL, median</td>
<td>NA</td>
<td>&lt;40</td>
</tr>
<tr>
<td>CD4+ T-cell count, cells/μL</td>
<td>619 (566–739)</td>
<td>624 (326–774)</td>
</tr>
<tr>
<td>CD8+ T-cell count, cells/μL</td>
<td>375 (296–521)</td>
<td>884 (587–1197)</td>
</tr>
<tr>
<td>Percentage of naive (CD45RO+CCR7+) cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among CD4+ T cells</td>
<td>29.4 (23.2–33.4)</td>
<td>31.6 (26.9–43.8)</td>
</tr>
<tr>
<td>Among CD8+ T cells</td>
<td>16.1 (11.7–25.3)</td>
<td>15.3 (5.5–26.8)</td>
</tr>
<tr>
<td>Percentage of HLA-DR+CD38+ cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among memory CD4+ T cells</td>
<td>7.5 (6.3–8.7)</td>
<td>10.8 (7.1–15.4)</td>
</tr>
<tr>
<td>Among memory CD8+ T cells</td>
<td>21.1 (17.0–23.6)</td>
<td>29.3 (14.8–46.1)</td>
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<tr>
<td>Percentage of CD16+ cells among CD14bright monocytes</td>
<td>14.4 (12.8–14.7)</td>
<td>16.3 (11.9–28.2)</td>
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<td>Percentage of IL-22+ cells among memory CD4+ T cells</td>
<td>1.5 (0.9–1.5)</td>
<td>1.5 (0.8–1.7)</td>
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<tr>
<td>Percentage of IL-17+ cells among memory CD4+ T cells</td>
<td>0.7 (0.6–1.5)</td>
<td>0.9 (0.6–1.9)</td>
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<tr>
<td>Percentage of IFN-γ+ cells among memory CD4+ T cells</td>
<td>10.2 (6.8–38.8)</td>
<td>13.7 (9.2–18.4)</td>
</tr>
</tbody>
</table>

Data are median (interquartile range), unless otherwise indicated. Statistical analysis was performed with the Mann–Whitney U test.

Abbreviations: ART, antiretroviral therapy; NA, not applicable.

a Subjects were followed at the Departments of Infectious Diseases of the University Hospital de Santa Maria, Lisbon, and Hospital São Bernardo, Setúbal, Portugal.

b Triple therapy in all patients (protease inhibitors in 8 of 17).

c P < .05, compared with controls.

d Data are from peripheral blood analysis of 9 control and 10 HIV-1–infected subjects.

dead cells (Live/Dead-LD, Life Technologies, Carlsbad, CA), data analysis was performed with FlowJo (version 9.3.1, Tree Star, Ashland, OR) within gated populations containing ≥150 events (Supplementary Figure 1). Circulating lymphocyte populations were characterized using whole-blood staining and were analyzed for activation markers as previously described [31, 32]. The following antibody combinations were used to identify mucosal ILC and T-cell subsets: IL-17/IL-22/cKIT/IFN-γ/NKp44/LD/CD45/CD3 and IL-17/IL-22/CD8/IFN-γ/CD4/LD/FOXp3/CD3, respectively. Cytokine production by PBMCs was quantified using CCR7/IL-22/IL-17/IFN-γ/CD8/CD45RO/IL-2/CD3 within memory-effector T cells. Unstimulated cells were used to define cytokine cutoffs.

Immunohistochemistry Staining
One sigmoid biopsy specimen and 1 ileum biopsy specimen were embedded in paraffin after preservation in 4% formaldehyde. Three-micrometer sections were stained with antibodies (Supplementary Table 1) after paraffin removal with xylene, rehydration with alcohol, and antigen retrieval by heat. Cells were counted manually in 10 images (original magnification ×400) acquired with a Leica DM2500 bright-field microscope, and the lamina propria area was assessed using ImageJ 1.47i (National Institutes of Health, Bethesda, MD) in blinded samples by 2 researchers. Staining with hematoxylin-eosin, Masson’s trichrome, and periodic acid Schiff–Alcian blue (PAS-AB) was concurrently performed, and stained specimens were blindly evaluated by a trained pathologist. Samples were scored according to inflammatory alterations, type of infiltrate, presence or absence of fibrosis, and number of mitotic figures at the crypt base, with scores ranging from 0 to 3.

Immunofluorescence Staining
One sigmoid biopsy specimen and 1 ileum biopsy specimen were frozen in OCT (VWR, Radnor, PA) after 6-hour preservation in 4% paraformaldehyde, followed by 24 hours in 18% sucrose to preserve biopsy structure. Three-micrometer tissue sections were stained by sequential antibody incubations (Supplementary Table 1) after protein blocking. DAPI was used for nuclear counterstaining. Images were acquired with a Zeiss LSM 710 confocal microscope (Carl Zeiss, Oberkochen, Germany), using a Plan-Apochromat 20×/0.8 objective; the surface area was measured with ImageJ 1.47i; and positive cells were counted using in-house software, written in MATLAB (Mathworks, Natick, MA), allowing automatic generation of individual report images for cell-counting verification (Figure 1).
RNA Extraction and Analysis

One sigmoid biopsy specimen and 1 ileum biopsy specimen were stored in RLT buffer (Qiagen, Valencia, CA) immediately after collection. RNA was extracted using the Allprep RNA/DNA mini kit (Qiagen), and 250 ng was used to synthesize complementary DNA (SuperScript III, Life Technologies). Expression levels of IL22, IL17A, IL22RA1, IL23, IL23R, IL25, TGFB1, IL1B, IL6, MUC1, MUC13, S100A9, REG3G, DEFB4A, AHR, and IDO1 were measured after preamplification with the TaqMan Preamp Master Mix, using TaqMan gene expression assays with an Applied Biosystems 7500 Fast Real-Time PCR System (all from Life Technologies). Results are expressed as ΔCT normalized to the medium CT levels of GAPDH and r18S. When gene expression was undetectable, a CT value of 40 was used for statistical analysis.

Statistical Analysis

Results are expressed as medians and interquartile ranges (IQRs). Comparisons between 2 groups were made using Mann–Whitney U test, using Graph Prism, version 5.0 (GraphPad Software, San Diego, CA). Nonadjusted regression analysis or Spearman correlation was used to identify associations between several parameters, and multivariate linear regression analysis was used to adjust results for age or sex, using Stata, version 12.1 (StataCorp, College Station, TX). Analysis and presentation of distributions of IL-22–producing cells was performed using SPICE, version 5.1 (available at: http://exon.niaid.nih.gov). Comparison of cytokine-producing subset distributions was performed using the Wilcoxon signed rank test and partial permutation test [33]. P values of <.05 were considered statistically significant.

RESULTS

IL-22–Producing Cells in the Sigmoid Mucosa

Mucosal IL-22 production was evaluated in sigmoid biopsies from 17 long-term-treated HIV-1–infected individuals with effective suppression of viremia and evidence of immunological reconstitution and 20 HIV-1–seronegative patients (Table 1). The total amount of IL-22 production in the sigmoid mucosa was quantified within hematopoietic cells, defined as CD45+ cells (Supplementary Figure 1).

We observed a significant depletion of IL-22–producing cells in the sigmoid mucosa of treated HIV-1–infected individuals as compared to age-matched controls, in the context of a preserved frequency of IL-17+ and interferon γ (IFN-γ)+ cells (Supplementary Figure 1). In agreement, the median number of IL-22–positive cells, as determined by immunohistochemistry studies performed using SPICE, version 5.1 (available at: http://exon.niaid.nih.gov). Comparison of cytokine-producing subset distributions was performed using the Wilcoxon signed rank test and partial permutation test [33]. P values of <.05 were considered statistically significant.
revealed similar numbers of IL-22+ cells in the ileum of HIV-1–infected patients when compared to HIV-1–seronegative subjects (Supplementary Figure 2). Our data are in line with previous studies demonstrating a distinct impact of HIV-1 infection of the colon and ileum [34].

Overall, both the frequency and total numbers of IL-22–producing cells were significantly reduced in the sigmoid mucosa in HIV-1 infection even after long-term ART.

**Mucosal Th22 Cells**
The immunofluorescence staining of IL-22 and CD3 showed a significant depletion of IL-22–producing T cells in HIV-1–positive patients as compared to HIV-1–seronegative subjects (Figure 1).

**Figure 2.** Interleukin 22 (IL-22)–producing CD4+ T cells in the sigmoid mucosa. A, Representative dot plots of IL-22, interferon γ (IFN-γ), interleukin 17 (IL-17), and FOXP3 expression within gated mucosal CD4+ T cells from human immunodeficiency virus type 1 (HIV-1)–infected and control individuals. Cytokine production was assessed upon phorbol myristate acetate–ionomycin stimulation of cells isolated from biopsy specimens of macroscopically normal mucosa. Numbers inside quadrants represent the frequency of positive cells. B, Frequency of IL-22+ cells among CD4+ T cells from the HIV-1–positive (n = 13) and seronegative (n = 13) cohorts and its association with age. C, Results of multiple linear regression analysis used to calculate R coefficients, including a quadratic term for age. During 22–producing CD4+ T cells in HIV-1–positive and seronegative cohorts, determined using SPICE software 5.0. E, IL-17+, FOXP3+, and IFN-γ+ cells among CD4+ T cells within the HIV-1–positive and control cohorts. Each dot represents an individual, and bars represent median. Groups were compared using the Mann–Whitney U test, and P values are shown. Abbreviation: Th22, T-helper type 22 cells.
sigmoid mucosa, though an expansion of CD8+ T cells was still observed (Supplementary Figure 1).

The levels of mucosal Th22 cells were not related to peripheral blood CD4+ T-cell counts (R = −0.031; P = .89; n = 22) or to the frequency of circulating IL-22-producing CD4+ T cells (R = 0.1; P = .72; n = 17), with the latter values being similar in the HIV-1–infected and control cohorts (Table 1). Importantly, there was no association between mucosal Th22 frequency and nadir CD4+ T-cell count before ART or by length of therapy.

Interestingly, aging was associated with a progressive increase in IL-22–producing cells within mucosal CD4+ T cells in healthy subjects (Figure 2C). This relationship was not observed in the HIV-1–infected cohort (Figure 2C), supporting a disturbed homeostasis of this subset in the sigmoid mucosa, even after long-term receipt of ART.

Nevertheless, the proportion of Th22 cells that concomitantly produce IL-17 and/or IFN-γ revealed no significant imbalances in HIV-1–infected patients as compared to healthy individuals (Figure 2D), with all IL-22–producing CD4+ T-cell subsets underrepresented in the HIV-1–infected cohort (Supplementary Figure 1). Mucosal Th22 cells had significantly more coexpression of IL-17 and/or IFN-γ than the circulating counterparts in both cohorts (data not shown). Thus, despite the major Th22 cell depletion found in HIV-1–infected patients, the factors governing the balance of these Th22 cell subpopulations seemed to be operating in a similar manner.

The frequency of the other main CD4+ T-cell subsets, namely IL-17+, IFN-γ+, and FOXP3+, were similar in the 2 cohorts (Figure 2E), supporting a preferential depletion of Th22 cells. Accordingly, immunohistochemistry analysis revealed similar median numbers of IL-17+ cells in HIV–infected and HIV–1-seronegative cohorts (123 cells/mm² [IQR, 60–202 cells/mm²; n = 15] vs 82 cells/mm² [IQR, 77–149 cells/mm²; n = 15]; P = .74) and FOXP3+ cells (37 cells/mm² [IQR, 26–87 cells/mm²; n = 17] vs 54 cells/mm² [IQR, 14–87 cells/mm²; n = 17]; P = .89).

In conclusion, long-term-treated HIV–1–infected individuals exhibited a significant depletion of Th22 cells in the sigmoid mucosa.

Levels of Molecules Known to Regulate IL-22 Production in the Sigmoid Mucosa

Next, we investigated possible alterations in the expression levels of molecules known to modulate IL-22 production in the gut. We found no alterations in the mRNA expression levels of IL23, IL23R, IL6, IL1B, AHR, IL25, IDO1, and TGFβ1 (Figure 3). Moreover, the levels of these transcripts were not significantly associated with the frequency of IL-22–producing cells, either within total hematopoietic cells or CD4+ T cells (P > .3). Similar findings were obtained in ileum biopsies (Supplementary Figure 2).

Thus, the expression levels of the main regulators of mucosal IL-22 production were preserved in treated HIV–1–positive individuals, suggesting they feature a normal IL-22–permissive environment.

IL-22–Producing Non-T Cells and ILC3 in the Sigmoid Mucosa

Next we investigated IL-22 production by non-T cells, and found that it was comparable in the 2 cohorts (Figure 4A). Notably, HIV–1–infected and control individuals featured a similar profile of concomitant production of IL-17 and/or IFN-γ by non-T cells, with a marked prevalence of cells producing only IL-22 in both cohorts.

Alternatively, we performed a triple immunofluorescence staining for CD3, RORγt, and IL-22 in the sigmoid mucosa and confirmed that the numbers of CD3+RORγt+ cells and CD3+RORγt+IL-22+ cells were similar in the 2 cohorts (Figure 4B).

The main source of innate IL-22 in the gut is a subset of ILCs that express the natural cytotoxic receptor NKP44: ILC3. HIV-1–infected individuals showed a preserved frequency of mucosal ILC3 cells, defined by the expression of cKIT and NKP44 within CD3− cells, as compared to HIV–1–seronegative patients (Figure 5A). These cells expressed RORγt (Figure 5B), which is essential for their development and function [35]. Moreover, similar to healthy subjects, the mucosal ILCs of HIV–1–positive individuals also expressed high levels of the IL-7 and interleukin 2 receptor α chains (CD127 and CD25, respectively; Supplementary Figure 1) [36]. In agreement with previous data [37], we found that the ILC3 subset was the main producer of...
IL-22 in both healthy and HIV-1–infected individuals, with no skewing toward IFN-γ production (Figure 5B).

Of note, a multivariate regression analysis including HIV status and sex as cofactors confirmed that sex did not bias our results regarding the frequencies of ILC (P = .42), IL-22–producing non-T cells (P = .49), or CD4+ T cells (P = .18).

Hence, in contrast to Th22 cell depletion, treated HIV-1–infected patients featured a preserved frequency of innate IL-22–producing cells.

### Epithelial Preservation in Long-Term-Treated HIV-1–Positive Individuals

Finally, we investigated the degree of sigmoid epithelial disruption in the context of marked Th22 cell depletion but preserved ILC3.

Initially, we quantified expression of IL-22–target genes, which are likely to help maintain epithelial integrity. We found that the genes for mucins (MUC1 and MUC13) and antimicrobial peptides (REG3G, S100A9, and DEFB4A)
were similarly expressed in the HIV-1–infected and control cohorts (Figure 6A). The strong association we observed between the levels of transcripts for IL22RA1 and MUC13 (Figure 6B) is worth emphasizing, as it supports the role of IL-22 in the induction of this particular molecule. Ileum biopsy specimens from both cohorts also featured similar levels of these transcripts (Supplementary Figure 2). Furthermore, there was no evidence of compromised tight junction integrity, as assessed by levels of expression of zonula occludens 1 (ZO-1; Figure 6C).

Additionally, blinded examination of specimens stained with hematoxylin-eosin, Masson’s trichrome, and PAS-AB revealed no significant differences between the 2 cohorts with respect to crypt morphology, number and type of cell infiltrates, and number of epithelial mitotic events (Figure 6D). Mucin production was apparently preserved in all samples, and significant collagen deposition was only found in biopsy specimens from 2 HIV-1–infected individuals (data not shown).

Interestingly, our long-term-treated HIV-1–infected cohort showed no significant increase in the expression of activation markers in circulating CD4+ and CD8+ T-cell subsets or an expansion of CD16+ cells within CD14bright monocytes (Table 1). The lack of upregulation of these markers, which have been associated with microbial translocation, was in agreement with preserved epithelial integrity, as suggested by our histological data and the levels of antimicrobial peptides.

Altogether, our study of long-term-treated HIV-1 infection provides evidence for full epithelium integrity in the context of significant Th22 cell depletion and preserved ILC3.

**DISCUSSION**

Maintenance of intestinal homeostasis is linked to IL-22 production in the lamina propria [26]. This study provided evidence favoring distinct regulation of the 2 main IL-22 producers in the human gut, CD4+ T cells (Th22 cells) and ILC3. We showed that IL-22–mediated processes were functioning in long-term-treated HIV-1–infected patients, despite evidence of Th22 cell depletion. Conversely, innate IL-22 production was unaltered, suggesting a role for ILC3 in ensuring epithelial integrity.

Gut disruption is a hallmark of HIV-1 infection, and the restoration of intestinal integrity upon ART initiation is still controversial [18]. Using a comprehensive approach combining tissue structure analyses, cell counts, and relative proportion of functionally characterized populations, as well as quantification

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**Figure 5.** Innate lymphoid cells in the sigmoid mucosa. A, Representative dot plots of cKIT and NKp44 expression within gated CD3− cells from human immunodeficiency virus type 1 (HIV-1)–infected and control individuals. Numbers inside quadrants represent frequency of positive cells. Graphs show the frequency of cKIT−NKp44− and cKIT−NKp44+ cells among CD3− cells from HIV-1–infected (n = 11) and control (n = 12) cohorts. Each dot represents an individual, and bars indicate medians in the graphs. Analysis was performed using the Mann–Whitney U test; P values are shown.

B, Representative analysis of RORγt expression, as well as interleukin 22 (IL-22), interleukin 17 (IL-17), and interferon γ (IFN-γ) production within cKIT−NKp44− and cKIT−NKp44+ gated CD3− cells from the sigmoid mucosa of HIV-1–infected and control subjects.
of expression of key genes implicated in gut homeostasis, we demonstrated the absence of major GALT disturbances in patients starting ART even in advanced stages of immunodeficiency. In summary, we documented: (1) an absence of CD4+ T-cell depletion, reaching normal absolute counts in the lamina propria despite the persistence of increased CD8+ T-cell numbers; (2) normal numbers, as well as balance, of regulatory T cells and CD4+ T cells that produce the proinflammatory cytokines IL-17 and/or IFN-γ; (3) evidence of control of local inflammation provided by lack of overexpression of relevant genes (eg, IL6, IL1B, TGFB1, and IDO1); (4) preserved epithelial structure in the sigmoid and ileum; and (5) absence of systemic

Figure 6. Levels of expression of interleukin 22 (IL-22)–induced genes and sigmoid mucosa integrity. A, Messenger RNA (mRNA) levels of mucins and antimicrobial molecules induced by IL-22 in biopsy specimens from human immunodeficiency virus type 1 (HIV-1)–infected and control cohorts. Numbers indicate the total number of samples tested and those with levels above the detection threshold of the respective gene. Results are expressed in relative units, normalized to the mean CT levels of GAPDH and r18S, with bars representing medians and interquartile range. Comparisons were made using the Mann–Whitney U test, and P values are shown. B, Association between levels of expression of IL22RA1 and MUC13 mRNA. Spearman correlation was used to calculate the r coefficient and P values. C, Illustrative immunofluorescence staining of zonula occludens 1 (ZO-1) in 3 different HIV-1–infected and seronegative individuals. Findings were used to quantify the median percentage of the epithelial area staining positive for ZO-1 in HIV-1–infection (21% [interquartile range, 16%–30%]; n = 7) and control (22% [interquartile range, 20%–28%]; n = 5) groups. D, Representative hematoxylin-eosin (H-E), Masson’s trichrome, and periodic acid Schiff–Alcian blue (PAS-AB) staining of sigmoid biopsy specimens from HIV-1–infected and control individuals. Findings were used to score the histological alterations. The mean value was 1.1 for HIV-1–positive subjects (n = 13) and 1.4 for HIV-1–seronegative patients (n = 15). Bars = 50 μm.
markers associated with microbial translocation, in our long-term-treated HIV-1–infected cohort.

Importantly, our study revealed the maintenance of several processes that are known to rely on IL-22, namely ZO-1–associated tight junction integrity and production of regulins, S100, defensins, and mucins, all of which are essential for preserving intestinal homeostasis [38]. This central role of IL-22 in tissue repair is in line with several reports in other clinical contexts and models of disease [26].

Strikingly, we documented mucosal integrity despite the significant Th22 cell depletion in the sigmoid mucosa of long-term-treated HIV-1–infected individuals. To our knowledge, there is only 1 report that evaluated IL-22 production in the human sigmoid mucosa [27]. This cross-sectional study described Th22 cell depletion associated with disruption of epithelial integrity that was apparently recovered in treated patients. However, this recovery should be cautiously interpreted because only 8, much younger controls were evaluated. This is particularly critical given our finding of a positive correlation between the frequency of Th22 cells among CD4+ T cells and age in HIV-1–seronegative subjects. Thus, the use of a large cohort of age-matched controls strengthens our observation of Th22 cell depletion after long-term ART in HIV-1 infection.

This preferential Th22 cell depletion could not be linked to an altered expression of factors that distinctly govern IL-22 production. In the gut, IL-22 production is in line with several reports in other clinical contexts and models of disease [26]. Importantly, ILC3 were not depleted in the treated HIV-1 cohort, in contrast with published data on untreated SIV infection in nonhuman primates [39].

Importantly, ILC3 were not depleted in the treated HIV-1 cohort, supporting a role for distinct factors governing the homeostasis of the 2 main IL-22–producing subsets in the gut mucosa. Given the lower frequency of these cells and the limited amount of tissue obtained during human studies, data on the relative contribution of ILC3 and Th22 cells to IL-22 production are still scarce. In contrast, murine models of colitis have generated data in favor of distinct regulation of these 2 subsets during disease [40]. Moreover, ILCs were shown to maintain epithelial preservation in the absence of IL-22 production by T cells [40]. Thus, it is plausible that the relative proximity to epithelial cells, rather than the actual frequency in lamina propria, may determine the role played by ILCs in maintaining epithelial integrity and may also help preserve epithelial stem cells [3]. Nevertheless, although we were able to identify ILC3 in the human gut, the structure of the frozen tissue biopsy specimens was insufficiently preserved to allow us to assess their relative localization in relation to Th22 cells. Notably, in addition to IL-22 production, ILC3 were recently reported to control mucosal inflammation by modulating local CD4+ T-cell activation [15]. Thus, ILC3 may represent an important population to target in the context of HIV-1 infection, since a hyperactivated state is believed to underlie mucosal damage.

Overall, mucosal homeostasis, including IL-22–mediated mechanisms essential for epithelial integrity, could mainly rely on ILCs, as suggested by our data on long-term-treated HIV-1–infected patients. Thus, the therapeutic targeting of ILCs may represent an important strategy to promote intestinal recovery.

### Supplementary Data

**Supplementary materials** are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

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