Impaired CD4⁺ T-Cell Restoration in the Small Versus Large Intestine of HIV-1–Positive South Africans Receiving Combination Antiretroviral Therapy

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Background. Human immunodeficiency virus type 1 (HIV-1) infection is associated with a massive depletion of intestinal CD4⁺ T cells that is only partially reversed by combination antiretroviral therapy (cART). Here, we assessed the ability of nucleoside reverse-transcriptase inhibitor/nonnucleoside reverse-transcriptase inhibitor treatment to restore the CD4⁺ T-cell populations in the intestine of South African patients with AIDS.

Methods. Thirty-eight patients with advanced HIV-1 infection who had chronic diarrhea (duration, >4 weeks) and/or unintentional weight loss (>10% decrease from baseline) of uncertain etiology were enrolled. Blood specimens were collected monthly, and gastrointestinal tract biopsy specimens were collected before cART initiation (from the duodenum, jejunum, ileum, and colon), 3 months after cART initiation (from the duodenum), and 6 months after cART initiation (from the duodenum and colon). CD4⁺, CD8⁺, and CD38⁺CD8⁺ T cells were quantified by flow cytometry and immunohistochemistry analyses, and the HIV-1 RNA load was determined by the Nuclisens assay.

Results. CD4⁺ T-cell and HIV-1 RNA levels were significantly lower, whereas CD8⁺ T-cell levels, including activated CD38⁺CD8⁺ T cell levels, were higher in the duodenum and jejunum, compared with the colon. After 6 months of cART, a significant but incomplete recovery of CD4⁺ T cells was detected in the colon and peripheral blood but not in the duodenum. Failed restoration of the CD4⁺ T-cell count in the duodenum was associated with nonspecific enteritis and CD8⁺ T-cell activation.

Conclusions. Strategies that target inflammation and immune activation in the small intestine may be required to expedite CD4⁺ T-cell recovery and improve therapeutic outcomes.

Keywords. HIV-1; antiretroviral therapy; CD4 reconstitution; intestine; Africa; immune activation.

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Human immunodeficiency virus type 1 (HIV-1) infection is characterized by a profound depletion of CCR5⁺ memory CD4⁺ T cells in the lamina propria of the gastrointestinal tract [1–3]. This loss has been attributed to the direct killing of these cells by HIV-1 and to bystander apoptosis of uninfected cells [4, 5]. Depletion of CD4⁺ T cells in the gastrointestinal tract has been implicated in the immune dysfunction and loss of epithelial integrity that leads to increased microbial translocation, immune activation, and disease progression [6–8].
There are still many unanswered questions relating to the loss of intestinal CD4+ T cells and HIV-1 pathogenesis, particularly since intestinal CD4+ T cells are also depleted in nonpathogenic simian immunodeficiency virus (SIV) infection [9, 10]. Recent studies suggest differential depletion of T-helper CD4+ cells may account for this discrepancy as these cells are preferentially depleted in HIV-1 and pathogenic SIV infection and preserved in nonpathogenic SIV infection [11]. Importantly, T-helper 17 cells play a key role in regulating intestinal immunity and in maintaining the mucosal epithelium [12, 13]. Strategies aimed at restoring the CD4+ T-cell count in the gastrointestinal tract may be critical for reestablishing epithelial integrity and reducing chronic inflammation and immune activation.

Findings of investigations to determine whether combination antiretroviral therapy (cART) can restore the CD4+ T-cell level in the gastrointestinal tract have, thus far, been inconclusive. Most studies have been cross-sectional, have been performed at a single site, and have involved a small number of patients. Overall, these studies suggest that restoration of the CD4+ T-cell count in the gastrointestinal tract is incomplete and occurs more slowly than in blood, with many patients exhibiting limited restoration despite prolonged suppressive treatment [14–17]. Other studies have reported near-complete CD4+ T-cell count reconstitution in the jejunum of patients treated during acute infection [18] and in the ileum [19] and colon [19, 20] of patients receiving long-term (duration, >4 years) suppressive cART. Further investigation is needed to determine whether these discrepancies are due to differences in the timing, duration, and type of treatment or to tissue-specific differences in homing receptors, immune activation, and/or collagen deposition.

To assess how cART affects immune reconstitution in different regions of the gastrointestinal tract, we compared CD4+, CD8+, and CD38+CD8+ T-cell levels and HIV-1 RNA levels in intestinal biopsy specimens from South African patients with late-stage infection who had chronic diarrhea and/or weight loss. Restoration of the CD4+ T-cell levels during cART was impaired in the upper small intestine (ie, the duodenum and jejunum), compared with the colon and blood. Failed restoration of the CD4+ T-cell count was associated with nonspecific enteritis and sustained CD8+ T-cell activation. Treatment strategies that target inflammation and immune activation in the upper gastrointestinal tract may provide significant clinical and immunological benefit.

**MATERIAL AND METHODS**

**Study Participants**

Thirty-eight patients with advanced HIV-1 infection who had chronic diarrhea (duration, >4 weeks) and/or unintentional weight loss (>10% decrease from baseline) of uncertain etiology were recruited at the Comprehensive Care, Management, and Treatment Clinic in Pretoria, South Africa. Following gastrointestinal biopsy, they immediately initiated a standardized first-line drug regimen consisting of 2 nucleoside reverse-transcriptase inhibitors (NRTIs; stavudine plus lamivudine) and 1 nonnucleoside reverse-transcriptase inhibitor (NNRTI; either efavirenz or nevirapine), as recommended by South African guidelines [21]. Biopsy specimens were collected at enrollment (from the duodenum, jejunum, ileum, and colon), after 3 months of cART (from the duodenum), and after 6 months of cART (from the duodenum and colon). Monthly blood samples were collected for measurement of CD4+ and CD8+ T cell counts and viral load. Patients were eligible for the study if acid-fast stains of sputum smears and stool specimens were negative for *Mycobacterium tuberculosis* and enteric pathogens, respectively. Control biopsy specimens were obtained from the duodenum and colon of 5 HIV-1–seronegative controls who tested negative for pathogens and showed no evidence of disease during screening for colorectal cancer. Written informed consent was obtained from all study participants. The study was approved by the Ethics Committee of the University of Pretoria.

**Sample Collection**

Biopsy specimens from the duodenum and jejunum were collected using a Fujinon double-balloon enteroscope and Radial III biopsy forceps (Boston Scientific). Endoscopic biopsy specimens from the colon and terminal ileum were obtained through the anal route. A total of 8–10 samples were randomly collected from each site. Biopsy specimens were placed on ice in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% fetal calf serum and immediately processed for flow cytometry, fixed in 10% formalin for immunohistochemistry analysis, or snap frozen for tissue-associated HIV-1 RNA load analysis.

**Immunophenotyping**

Multiparameter flow cytometry was performed on mononuclear cells isolated from intestinal biopsy specimens. Tissue samples were digested (for 30 minutes at 37°C) with collagenase type IV (0.5 mg/mL; Sigma, St. Louis, MO) in RPMI 1640 medium and passed through a 70-μm cell strainer (Becton Dickinson Labware, Franklin Lakes, NJ). Residual tissue fragments were digested, and the pooled single cell suspensions were washed to remove collagenase. Mononuclear cells were stained (for 30 minutes at 4°C) with various combinations of anti-human CD4, CD8, CD3, CD45, CD45RO, CD27, and CD38 monoclonal antibodies (mAb; Beckman Coulter). The percentage of CD4+, CD8+, CD38+CD8+, CD45RO+CD4+ memory, and CD45RO+CD27+CD4+ naive T cells was determined by gating on the lymphocyte population defined by forward- and side-scatter characteristics (confirmed to be CD45+CD3+ cells by back gating), followed by sequential gating for either CD4 or CD8. A minimum of 5000 lymphocyte events were collected per tube on a Beckman Coulter FC500. Results were analyzed using Flow Jo (Tree Star, Ashland, OR).
Histological Analysis
Staining with hematoxylin-eosin, periodic acid-Schiff diastase, Ziehl-Neelsen, and Giemsa was performed to exclude fungal and parasitic organisms (Cryptosporidium, Isospora, and Microsporidia) and acid-fast bacilli. For T-cell subset quantification, sections of the duodenum and colon were incubated with murine anti-human CD4 or CD8 mAbs (Dako, Denmark), visualized with diaminobenzidine, and counterstained with hematoxylin. The number of CD4+ and CD8+ lymphocytes in 5 representative high-power fields of 0.80 mm² (original magnification, ×400) was counted, and the results are reported as the average number of cells/0.8 mm². All histological studies were performed in a blinded fashion by a histopathologist with extensive experience in the analysis of intestinal biopsy specimens.

Tissue-Associated Viral Load
Biopsy specimens (4–12 mg) were weighed, minced with a razor blade, and placed in lysis buffer (BioMerieux) overnight at room temperature. RNA was extracted using the Magnetic Extraction Reagent Kit from BioMerieux. Viral RNA in tissue extracts and plasma was quantified using the Nucliens Easy Q HIV-1 RNA v.1.2 kit and expressed as copies per gram of tissue or milliliters of plasma.

Statistical Analysis
All statistical analyses were performed in Prism 5 from GraphPad Software (La Jolla, CA). Paired observations were compared using the Wilcoxon matched pairs tests or the Friedman test with the Dunn posttest. Linear correlations were assessed using a Spearman rank correlation coefficient. Two-tailed P values of >.05 were considered statistically significant.

RESULTS
Patient Characteristics
The demographic and clinical characteristics of our patient population are summarized in Table 1. Symptoms leading to endoscopy were diarrhea in 27 patients (71.1%) and/or unexplained weight loss in 26 (68.4%). Infectious agents were identified in the duodenum and jejunum of 6 patients (49). Diarrhea was present in the duodenum and jejunum of 6 patients (49), and 80% of patients studied, 71% and 66% had samples collected 3 and 6 months after the initiation of cART, respectively. Ninety-two percent had good immunological responses (defined as a >3-fold increase in blood CD4+ T-cell counts), and 75% had complete virological responses after 6 months of cART (defined as a plasma viral load of <50 copies/mL). Only 5 patients continued to have chronic diarrhea at the 6-month time point.

CD4+ T-Cell Depletion Was More Severe in the Small Versus the Large Intestine
CD4+ T-cell percentages and absolute numbers were significantly reduced in the gastrointestinal tract of HIV-1–positive patients, compared with controls. Compared with levels of 36.8% ± 8.7% (mean ± standard deviation; in the duodenum) and 39.3% ± 10.4% (in the colon) in controls, CD4+ T-cell percentages in cART-naïve HIV-1–positive patients ranged from very low levels in the duodenum (3.8% ± 2.8%) and jejunum (2.6% ± 3.2%) to somewhat higher levels in the ileum (5.1% ± 5.4%). CD4+ T-cell percentages in the colon (10.3% ± 8.2%) of HIV-1–positive patients were significantly higher than in the duodenum (P = .002) and jejunum (P = .001) and comparable to those in blood (8.4% ± 5.7%; P = .547; Figure 1A). Memory cells made up the vast majority (>90%) of CD4+ T cells (Figure 1B). Histological analysis revealed a similar, statistically significant decrease in the absolute number of CD4+ T cells in HIV-1–positive patients.
patients, compared with controls, with the decrease being more severe in the duodenum (78 ± 10 vs 8 ± 5 cells/0.80 mm²; 
P < .001) than the colon (65 ± 10 vs 17 ± 6 cells/0.8 mm²; 
P < .001; Figure 1C and 1D). No significant correlations were 
detected between CD4+ T-cell counts or percentages in the gut 
as compared to blood. Compared with controls, CD4+ T-cell 
levels in the duodenum and colon of HIV-1–positive patients 
were reduced by 89.5% and 73.7%, respectively, when assessed 
by flow cytometry analysis and by 83.3% and 69.4%, respectively, 
when assessed by immunohistochemistry analysis (Figure 1E).

**CD8+ T-Cell Levels Were Higher in the Small Versus the Large 
Intestine**

The percentages of CD8+ T cells in the duodenum (71.1% ± 
14.4%) and jejunum (77.5% ± 17.3%) of cART-naive HIV-1–positive patients were significantly higher than in the colon 
(53.8% ± 18.3%; P = .0069 and P = .0006, respectively), with the 
ileum displaying intermediate levels (66.9% ± 7.1%; Figure 2A). 
CD8+ T-cell percentages in the colon (53.8% ± 18.3%) were 
comparable to those in blood (56.1% ± 8.4%). The absolute 
numbers of CD8+ T cells were also significantly higher in the 
duodenum (101 ± 9.4 cells per 0.80 mm²) as compared to the 
colon (74 ± 5.7 cells per 0.80 mm²; P = .012; Figure 2B and 2C).

Although not statistically significant, the percentages of activated 
CD38+CD8+ T cells were slightly higher in the duodenum 
(35.9% ± 21.42%) and jejunum (40.9% ± 23.1%) than in the 
colon (30.1% ± 17.75%; P = .10 and P = .09, respectively; 
Figure 2D). In the colon, but not in the duodenum, jejunum, or ileum, the percentage of activated CD38+CD8+ T cells was posi-
tively correlated with the amount of tissue HIV-1 RNA 
(r = 0.50; P = .04).

**HIV-1 RNA Levels Were Higher in the Colon Than in the 
Duodenum and Jejunum**

All 38 patients (100%) had detectable HIV-1 RNA in the duo-
denum, jejunum, ileum, and colon prior to the initiation of 
cART (range, 3.89–9.31 log₁₀ HIV-1 RNA copies/g of tissue). 
Mean HIV-1 RNA levels were significantly higher in the colon 
(7.2 ± 1.2 log₁₀ HIV-1 RNA copies/g of tissue) than in the duodenum 
(6.4 ± 1.0 log₁₀ HIV-1 RNA copies/g of tissue; P = .0067) and jejunum 
(6.1 ± 1.1 log₁₀ HIV-1 RNA copies/g of tissue; P = .0004), 
with intermediate levels in the ileum (6.7 ± 0.95 log₁₀ HIV-1 RNA copies/g of tissue; P = .1192; Figure 3A).

Normalization by absolute CD4+ T-cell count indicated that higher levels of HIV-1 RNA in the colon were associated with 
an increased frequency of CD4+ T cells rather than with an in-
crease in virus production per cell (Figure 3B).

**NRTI/NNRTI Treatment Resulted in Partial Restoration of CD4+ 
T-Cell Counts in the Blood and Colon but Not in the Duodenum**

To assess the efficacy of short-term cART on CD4+ T-cell 
count restoration, we compared CD4+ T-cell levels in peripheral 
and longitudinal biopsy samples (from the duodenum 
and colon) collected before and 3 and 6 months after cART ini-
tiation. These analyses were limited to 23 patients who had 
complete virological responses in plasma (defined as an HIV-1 
RNA load of <50 copies/mL) and no evidence of opportunistic 
infection. The percentages of CD4+ T cells in the blood and 
colon of cART-treated HIV-1–positive patients increased from 
8.4% ± 5.7% and 10.3% ± 8.2%, respectively, at baseline to 
17.8% ± 10.1% (a 2.1-fold increase; P = .01) and 15.8% ± 7.4% 
(a 1.5-fold increase; P = .0003), respectively, 6 months after the 
initiation of cART (Figure 4A). Blood CD4+ T-cell counts 
increased on average, 3.5-fold after 6 months of cART. Des-
pite these increases, the frequency of CD4+ T cells in these 
compartments remained lower than in uninfected controls 
(17.8% ± 10.1% vs 38.0% ± 9.4% in blood [P = .0042] and 
15.8 ± 7.4 vs 36.3% ± 10.4% in the colon [P = .0006]). CD4+ T-
cell depletion was more persistent in the duodenum, with no 
measurable increase detected after 6 months of cART (mean 
frequency, 3.7% ± 2.8% at baseline vs 3.9% ± 3.1% at month 6; 
P = .5623; Figure 4).

**Failed Restoration of CD4+ T-Cell Counts Was Associated With 
Persistent Inflammation and CD8+ T-Cell Activation**

To investigate whether cART resulted in decreased inflamma-
tion and CD8+ T-cell activation, we examined infiltration of 
mononuclear cells, including macrophages, lymphocytes, eo-
sinophils, and/or plasma cells, and assessed CD38+CD8+ T-cell 
levels in longitudinal duodenal and colonic biopsy specimens 
collected before cART initiation, 3 months after cART initia-
tion (from the duodenum), and 6 months after cART initiation 
(from the duodenum and colon). Before cART initiation, 67% 
and 77% of patients had mild-to-moderate levels of nonspecific 
inflammation in the colon and duodenum, respectively. After 6 
months of cART, only 44% of HIV-1–positive patients had sus-
tained nonspecific colitis (in the colon), compared with 72% 
with nonspecific enteritis (in the duodenum). Similarly, the 
proportion of activated CD8+ T cells in the colon decreased 
from 30.1% ± 17.8% at baseline to 18.9% ± 8.7% (P = .048) after 
6 months of cART. In the colon, the change in HIV-1 RNA 
load was positively correlated with the decrease in activated 
CD38+CD8+ T-cell percentage (r = −0.38; P = .03). Although 
not statistically significant, CD38+CD8+ T-cell percentages in 
the duodenum were somewhat higher after 3 months of cART 
(48.6% ± 23.4%) and 6 months of cART (44.1% ± 23.8%) than 
at baseline (35.9% ± 21.4%; P = .43 and 0.58, respectively). This 
occurred despite a marked decrease in the HIV-1 RNA load 
(from 6.4 ± 1.0 log₁₀ copies/g tissue at baseline to 4.2 ± 0.8 and 
4.9 ± 1.1 log₁₀ copies/g tissue 3 months [P = .0005] and 6 
months [P = .0134], respectively, after cART initiation). This 
decrease was comparable to that observed in the colon, where 
the HIV-1 RNA load decreased from 7.2 ± 1.2 log₁₀ copies/g of 
tissue at baseline to 5.15 ± 1.2 log₁₀ copies/g of tissue at month 
6 (P = .0005).


DISCUSSION

The impact of cART on CD4+ T-cell count reconstitution in the intestine of HIV-1–positive patients is a topic under considerable debate. Contributing to the confusion are the interstudy differences in sampling sites, treatment regimens, cART durations, and disease stages, as well as limitations inherent in cross-sectional studies. To our knowledge, this is the first longitudinal

Figure 1. Depletion of CD4+ T-cell counts in combination antiretroviral therapy (cART)–naive patients with late-stage human immunodeficiency virus type 1 (HIV-1) infection was more severe in the small intestine than in the large intestine. A, Proportion of T cells that express CD4, as measured by flow cytometric analysis of peripheral blood mononuclear cells or total cell suspensions isolated from the gastrointestinal tract of cART-naive patients with AIDS. Mean values and statistical significance among the different sites are indicated (P<.05, **P<.01, ***P<.001). B, The proportions of naive and memory CD4+ T cells in the various intestinal sites in a subset of 12 patients prior to the initiation of cART. Memory CD4+ T cells were defined as cells that express CD45RO, and naive cells were defined as CD45RO− and CD27+. C, Representative immunohistochemical staining for CD4 (brown) in the duodenum and colon of a cART-naive AIDS patient. D, Decrease in the absolute number of CD4+ T cells in the duodenum and colon of cART-naive patients with AIDS, relative to uninfected controls, as assessed by immunohistochemistry analysis (P<.01). E, Comparison of the decrease in CD4+ T-cell counts in cART-naive patients with AIDS versus uninfected controls as assessed by 2 different methods—flow cytometry (percentage of CD4+ T cells) and histological analysis (absolute number of CD4+ T cells).
study to examine the extent of CD4+ T-cell count depletion and restoration in the gastrointestinal tract of African patients with late-stage disease who had unexplained chronic diarrhea and/or weight loss. In the developing world, individuals presenting with CD4+ T-cell counts of <200 cells/mm³ are fast-tracked for treatment with a standardized NRTI-NNRTI regimen [21, 22].

Before cART initiation, we detected a profound decrease in CD4+ T cell counts and a corresponding moderate increase in CD8+ T cell counts in all sites of the gastrointestinal tract, regardless of the method (ie, flow cytometry or immunohistochemistry analysis) used to quantify T-cell subsets. The severity of depletion exceeded that reported for patients with acute/early HIV-1 infection, presumably a reflection of the continued loss of CD4+ T cells during disease progression [14, 23]. One of the most striking findings was the profound depletion of CD4+ T cells in the small intestine (ie, duodenum, jejunum, and ileum) versus the large intestine (ie, colon) and peripheral blood. Other studies have reported that CD4+ T-cell count depletion is more severe in the duodenum [23], ileum [1, 24], and colon [4, 24] than in the blood of treatment-naive patients and in the duodenum, compared with the colon and rectum [25], of patients receiving suppressive cART.

To determine whether the distinct immune environments support different levels of HIV-1, we compared total HIV-1
RNA levels (encompassing both cell-associated and virion RNA) in the duodenum, jejunum, and ileum with those in the colon. HIV-1 RNA was readily detectable throughout the gastrointestinal tract of cART-naive patients, at levels exceeding those in blood (on the assumption that 1.0 g of tissue is equivalent to 1.0 mL of plasma). As observed for CD4+ T-cell counts, HIV-1 RNA levels were moderate in the duodenum and jejenum, intermediate in the ileum, and significantly higher in the colon. Similarly, in cART-treated patients, studies have shown that the amount of HIV-1 proviral DNA per CD4+ T cell increases when descending through the gastrointestinal tract toward the colon and rectum [25].

The positive correlation observed between activated CD38+CD8+ T-cell counts and HIV-1 RNA loads in the colon of treatment-naive patients, as well as the parallel decrease in both parameters in response to cART, suggest that CD8+ T-cell activation is closely linked to viral replication. Other investigators have reported that, in cART-treated patients with maximally suppressed viral replication, the degree of CD8+ T-cell activation in the colon correlates with the size of the residual HIV-1 DNA reservoir [2]. In the duodenum, the lack of a correlation between CD38+CD8+ T-cell counts and HIV-1 RNA loads and the persistence of activated CD38+CD8+ T cells during cART, despite a marked decrease in the HIV-1 RNA load, suggest that factors other than or in addition to HIV-1 infection (eg, coinfections, microbial translocation, disruption of the microenvironment, and persistent immune activation that is not fully reversed by cART) contribute to CD8+ T-cell activation in this tissue.

In agreement with a growing body of evidence [14, 26–28], our results also suggest that persistent inflammation and CD8+ T-cell activation are associated with lower increases in CD4+ T-cell counts. This view is supported by studies showing an inverse correlation between activated CD38+CD8+ T cells and CD4+ T-cell levels in blood [29, 30] and between the activated Ki67+CD8+ T cells and the severity of CD4+ T-cell depletion in the lower gastrointestinal tract [24]. Although the mechanisms are not fully understood, activated CD8+ T cells have the capacity to delay or prevent CD4+ T-cell recovery by producing proinflammatory cytokines and chemokines, suppressing CD4+ T-cell proliferation, and inducing CD4+ T-cell apoptosis [18, 31, 32]. Chronic inflammation and immune activation may also lead to increased collagen deposition and fibrotic damage [33, 34]. Fibrotic deposition of collagen occurs in Peyer’s patches of the ileum, and the extent of deposition/damage is predictive of CD4+ T-cell count restoration [35, 36]. To our knowledge, there are no studies of collagen deposition in the duodenum, jejenum, or colon. Other investigators have reported that genes involved in inflammation and cell activation are upregulated in the jejenum of patients who exhibit poor mucosal CD4+ T-cell responses [28]. Further studies are needed to determine whether these changes are confined to the small intestine.

Another factor that could contribute to poor reconstitution of the CD4+ T-cell count in the duodenum relates to the CCL25/CCR9 axis of CD4+ T-cell recruitment. This axis plays an important role in lymphocyte homing to the small intestine but not to the colon. CCL25 is a chemokine produced by epithelial cells of the small intestine [37, 38]. Its receptor, CCR9, is expressed on lymphocytes that traffic to the small intestine [39, 40]. HIV-1–positive patients produce less CCL25, and as a result many CCR9+CD4+ T cells remain in the circulation rather than repopulating effector sites in the small intestine [41]. Reduced homing of CCR9+CD4+ T cells has been associated with mucosal damage, increased microbial translocation, and systemic immune activation, changes that may contribute to poor immune restoration [42].

The strengths of our study, in comparison to previous studies, relate to the homogeneity of our patient cohort (with respect to treatment, stage of infection, and, in the majority of patients, the absence of overt opportunistic infections) and the...
repeat sampling of multiple tissue sites from the same patients before and after the initiation of cART. We focused on the effects of short-term cART because we were interested in identifying early predictors of immunological failure. Although there is no consensus as to the best time to assess immunological responses [43, 44], studies performed on peripheral blood after 3–6 months of cART have been shown to be predictive of immune restoration in the long-term [45]. Limitations of our study relate to the small size of our control group and the limited number of immune and virological correlates examined. Furthermore, despite efforts to exclude patients with enteric coinfections (stool cultures and histological staining for acid-fast bacilli and fungal and parasitic infections), we cannot exclude the possibility that other coinfections may have affected our results. In addition, cell numbers did not allow us to determine the antigen specificity and clonal diversity of our CD8+ T cells. Further studies are also required to determine whether our results can be generalized to patients without diarrhea and weight loss and to patients of non-African origin.

Our study demonstrates that, despite the late stage of infection, gradual restoration of CD4+ T-cell levels is possible in the colon and blood of African patients with AIDS during short-term cART. It also underscores the distinct differences in the small versus large intestine with regard to CD4+ T-cell depletion and reconstitution and highlights the need for detailed comparative studies to better delineate the mechanisms leading to

Figure 4. Impaired CD4+ T-cell restoration and sustained CD8+ T-cell activation in the duodenum, but not in the colon, of patients with AIDS who were treated with nucleoside reverse-transcriptase inhibitors or nonnucleoside reverse-transcriptase inhibitors. A, Spaghetti plots of the kinetics of CD4+ T-cell restoration, HIV RNA clearance and CD8+ T-cell activation in the duodenum, colon, and blood. Each line represents an individual patient. Analysis was limited to patients with a complete virological response at month 6 after cART initiation (plasma viral load, < 50 copies/mL; n = 23). B, Summary statistics of the kinetics of CD4+ T-cell restoration, human immunodeficiency virus type 1 (HIV-1) RNA clearance, and CD8+ T-cell activation in the duodenum, colon, and blood (n = 23). Results are reported as mean and SD.
immunological failure in the upper small intestine. An understanding of these mechanisms may lead to the development of highly targeted treatment strategies that enhance recovery of the CD4+ T-cell count. If inflammation and immune activation in the small intestine are driven by local factors not directly related to HIV-1 (eg, tissue damage, loss of epithelial integrity, and nonspecific activation of CD8+ T cells), it is unlikely that drugs targeting HIV replication will be maximally effective with respect to immune recovery. This view is supported by studies showing that treatment intensification with raltegravir, a potent integrase inhibitor, did not have any significant effect on CD4+ T-cell levels or immune activation in the gastrointestinal tract or on HIV-1 RNA levels in plasma [46–48]. cART protocols that include immune modulating and antiinflammatory agents may offer an attractive alternative approach.

Notes

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E. C. and T. R. designed the study protocol. E. C. conceived and performed flow cytometric analyses, interpreted the data, and authored the first draft of the manuscript. T. R. and S. v. d. M. obtained specimens, supervised the collating of clinical data, and cared for the patients. S. M. performed some of the manuscript. E. C. and T. R. designed the study protocol. E. C. conceived and performed flow cytometric analyses, interpreted the data, and authored the first draft of the manuscript. T. R. and S. v. d. M. obtained specimens, supervised the collating of clinical data, and cared for the patients. S. M. performed some of the manuscript. T. R. and S. v. d. M. obtained specimens, supervised the collating of clinical data, and cared for the patients. S. M. performed some of the manifold analyses, including quantification of CD4+ and CD8+ T-cell levels in LM tissue sections. E. C., S. M., and P. M. performed viral load analyses. G. P. provided immunological expertise and participated in discussions of the results. C. S. provided expertise in database management. S. C. and C. S. obtained funding.

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