Viewpoint

Differences in HIV burden in the inflamed and non-inflamed colon from a person living with HIV and ulcerative colitis

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ABSTRACT

The greatest obstacle to an HIV cure is the persistence of latently infected cellular reservoirs in people living with HIV (PLWH) taking antiretroviral therapy (ART). However, no consensus exists on the direct link between local tissue inflammation and the HIV burden. Herein, we have compared the levels of local inflammation, epithelial integrity and HIV DNA between inflamed and non-inflamed colon tissue in a PLWH who underwent a colectomy due to ulcerative colitis. We have observed a 27-fold higher frequency of cells harboring HIV DNA in inflamed compared to non-inflamed colon tissue. Analysis of the expression of occludin-1 and claudin-3 confirmed our macroscopic characterization of inflamed and non-inflamed colon. Our results confirm that increased gut permeability and inflammation are associated with a higher frequency of infected cells and suggest that restoring gut barrier integrity may be used as a strategy to reduce inflammation and HIV persistence in the gut.

Introduction

The greatest challenge to an HIV cure is the persistence of latently infected cellular reservoirs in memory CD4$^+$ T cells and macrophages in people living with HIV (PLWH) taking antiretroviral therapy (ART). One of the main reservoirs lies in the gastrointestinal (GI) tract which contains a large proportion of T cells and macrophages.[1] HIV RNA, HIV DNA and infectious virus have been detected in the gut of ART-suppressed individuals.[2–4] Moreover, higher levels of *in vitro* proinflammatory cytokine production were found in gut biopsies of HIV-infected patients when compared with uninfected controls.[5] Persistent gut inflammation is associated with HIV replication, gut epithelial barrier dysfunction and microbial translocation, even in PLWH taking ART.[6] However, no consensus exists on the direct link between local tissue inflammation and HIV burden.

There is evidence that HIV infection breaches the gut barrier in a way similar to inflammatory bowel disease (IBD).[7] The exact cause of IBD remains unknown but an important role of CD4$^+$ T cells is suggested in its pathophysiology. Some studies showed that the incidence of IBD in individuals with HIV is increased, either due to local inflammation, or immune changes induced by HIV such as loss of CD4$^+$ T cell number and function which may facilitate its development.[8] On the other hand, it is purported that a progressive decline in the CD4$^+$ T cell count caused by HIV may reduce IBD disease activity and contribute to remission.[9]

As availability of tissue samples is sparse, comparison of different sections of the same tissue is challenging in living people. Most studies comparing HIV reservoir frequency in different organs use animal models or autopsy-obtained specimens. Herein, we have compared the levels of local inflammation, epithelial integrity and HIV DNA between inflamed and non-inflamed colon tissue in a woman living with HIV who underwent a colectomy due to treatment-resistant ulcerative colitis UC).

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Methods and materials

Participant

A 36-year-old, Canadian-born Caucasian woman was diagnosed with HIV in 2008 with a normal $CD4^+$ T cell count at $627/mm^3$, a CD4:CD8 ratio of 0.9 and low viral load at 1089 HIV-1 copies/mL. No ART was initiated due to the normal $CD4^+$ T cell count. After four years with a viral load below 5000 copies, viremia increased to 78011 copies and a rapid $CD4^+$ T cell count drop to 443 cells/mm^3 with a decrease of the CD4:CD8 ratio to 0.3 were observed. Treatment with tenofovir disoproxil 300 mg/emtricitabine 200 mg orally once daily and raltegravir 400 mg orally twice daily was initiated in September 2012. Her HIV viral load was below the detection level from November 2012. A few months later, she presented with abdominal cramps and melena; a colonoscopy examination was compatible with ulcerative colitis (UC). The biopsy results confirmed chronic idiopathic inflammatory bowel disease, while CMV was negative by immunohistochemistry. Due to the severity of her UC, she was initially treated with oral prednisone (50 mg daily). In the following years, she had several flare-ups and received the tumor necrosis factor-α blockers infliximab and adalimumab, $\alpha_4\beta_7$ integrin blocker vedolizumab and Janus kinase inhibitor tofacitinib.[10] In June 2018, due to recurrent episodes of intestinal bleeding and pain, a total colectomy with ileal pouch was suggested. Her $CD4^+$ T cell count was then at 531 cells/mm^3 with a viral load below the level of detection. She underwent surgery in June 2018 and after signing an informed consent, she agreed to have a portion of her resected colon used for research purposes including tissue biobanking and HIV reservoir research.

Tissue samples

Blood samples were collected from the participant before surgery to isolate peripheral blood mononuclear cells (PBMCs) and plasma, which were then stored in liquid nitrogen and at −80 °C, respectively. Quantification of HIV plasma viral load (VL) was done using the Abbott Real Time HIV-1 assay (Abbott Laboratories, Abbott Park, Illinois, USA). $CD4^+$ and $CD8^+$ T cell counts were measured in blood using 4-colour flow cytometry. Immediately after surgery, a pathologist examined the specimen, and separated inflamed sections from non-inflamed tissue (Supplementary Fig. 1). Both tissue sections were kept in medium (RPMI + HEPES + Glucose, Wisent, Canada) and processed within 1 hour post-surgery. Small tissue sections were flash frozen in liquid nitrogen and stored at −80 °C until use. Cells were extracted from fresh tissue with multiple liberase treatments as previously described.[11] Percentages of $CD8^+$ and CD8 negative considered as $CD4^+$ T cells among $CD45^+CD3^+$ hematopoietic T-cells were assessed by flow cytometry as previously described.[11]

HIV-DNA quantification

The frequency of cells harboring HIV DNA in peripheral blood and gut tissue was measured using a PCR-based assay for the *gag* gene using a technique previously described that can detect a single copy of viral genome per PCR reaction.[12]

Markers of inflammation measured by real-time quantitative PCR

RNA was extracted from flash frozen pieces of tissue by using the RNeasy Mini Kit (Qiagen) and reversed transcribed by using the RT^2 First Strand Kit (Qiagen). Transcripts for GAPDH, tumor necrosis factor α (TNF-α), IL-6, CD4, occludin and claudin-3 were measured by using the RT^2 SYBR Green Mastermixes (Qiagen). All measurements were done in triplicate on a BioRad CFX384 and analyzed using CFX Maestro.

Study results

No difference in CD4$^+$ T cell frequency between inflamed and non-inflamed colon tissue

In order to assess the infiltration of $CD4^+$ T cells in both the inflamed and non-inflamed parts of the colon, $CD4^+$ T cell frequency was measured by flow cytometry; the expression of CD4 was assessed by real-time quantitative PCR. A low percentage of $CD4^+$ T cells was detected in both the inflamed (1.8%) and non-inflamed (4.9%) gut sections (Table 1) by flow cytometry. Furthermore, levels of CD4 transcripts were similar in the inflamed and non-inflamed tissues (Fig. 1C).

Higher levels of HIV DNA in the inflamed compared to non-inflamed colon

HIV DNA level in PBMCs was 103 copies/10^6 cells. In three biopsies of the inflamed part of the colon, we detected a median of 27 (range 26–97) copies/10^6 cells of HIV DNA, while HIV DNA level in the adjacent non-inflamed gut tissue was only 1 copy (0–22)/10^6 cells (Fig. 1A). HIV DNA levels normalized to the $CD4^+$ T cell percentage in each type of gut tissue also showed higher levels in the inflamed section with 1482 (1400–5142) compared to 27 (0–451) HIV copies/10^6 $CD4^+$ T cells in the non-inflamed section (Fig. 1B).

Decreased occludin-1 and increased claudin-3 expression in inflamed colon

Claudin-3 is a marker of gut epithelium which is increased during inflammation. We detected a higher expression of claudin-3 in the inflamed gut tissue compared to the non-inflamed part (Fig. 1C). Conversely, occludin-1 is a marker of epithelium integrity which decreases during inflammation. The expression of occludin-1 was decreased in inflamed tissue. As expected, the ratio of claudin:occludin expression was higher in the inflamed than non-inflamed gut sections.[13] However, the expression of IL-6 and TNF-α in inflamed and non-inflamed gut tissues was not different, possibly due to the long-term immune suppression with anti-TNF-α medication and prednisone.

Discussion

In an ART-treated adult woman who underwent colectomy for UC, we have observed a higher frequency of cells harboring HIV DNA in the inflamed than non-inflamed colon tissue. Previous studies have shown that HIV DNA levels per million $CD4^+$ T cells were on average 2–5 times higher in the gut than blood in ART-suppressed PLWH,[14,15] a trend we have also observed in this participant.

To confirm our macroscopic evaluation of inflamed and non-inflamed colon sections, we have performed RT-qPCR to quantify markers of inflammation and epithelial integrity. The gut barrier is composed of a single layer of epithelial cells joined at their apical side by tight junctions (TJs). The TJ is constituted by transmembrane proteins such as occludin, tricellulin and claudins.[16] TJ dysfunction can lead to the disruption of the intestinal barrier integrity, manifested by epithelial hyperpermeability as observed in patients with IBD. Moreover, increased macromolecular flux in the intestine has been suggested as a predictor for inflammatory relapse in UC patients in remission.[17] As expected, we have detected a lower expression of occludin-1 in the inflamed compared to the non-inflamed colon sections. Although the

Table 1
The percents of $CD4^+$ T cell in PBMC and different gut tissues.

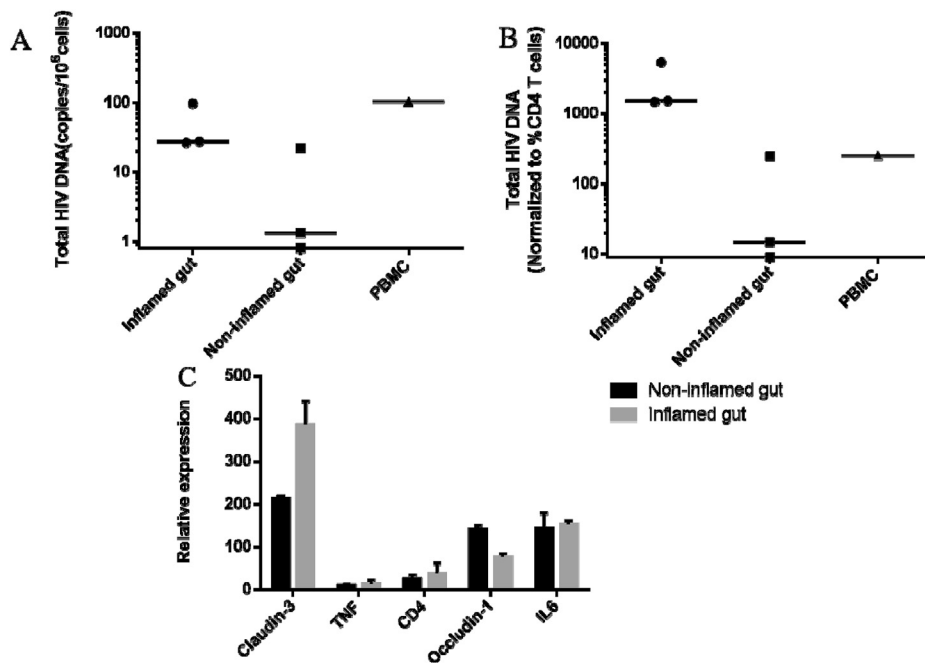|	$CD4^+$ %	$CD8^+$ %	$CD3^+$ %	$CD45^+$ %	Total HIV DNA (median) normalized by CD4 percents
PBMC	47	40	88	100	250
Inflamed gut	65.7	30.9	26.5	10.8	1482
Non-inflamed gut	59.6	38.8	42.2	19.6	27

Fig. 1. Total HIV DNA and relative expression of claudin-3, CD4, IL-6, TNF and occludin-1 in inflamed and non-inflamed gut tissue. Median of HIV DNA levels in 3 inflamed and non-inflamed tissue samples. PBMCs were measured by nested qPCR. A. HIV DNA copies per million cells. B. HIV DNA levels normalized to the percentage of CD4$^+$ T cells detected in both tissues and PBMCs. Unpaired nonparametric test (Mann-Whitney tests) was used for statistical analysis. C. Normalized expression of IL-6, TNF-α and occludin-1 according to GAPDH expression.

influence of UC on claudin-3 expression is unclear, we have found a higher ratio of claudin-3 over occludin-1 expression, as previously described.[13] There is solid evidence that HIV infection breaches the gut barrier in a way similar to UC.[6,7,18]

There are several explanations for the differences observed in the frequency of infected cells between inflamed and non-inflamed gut tissue. Local inflammation in the context of HIV infection would activate mucosal CD4$^+$ T cells and recruit new cells that become infected and remain on site as tissue residents. This is particularly documented for Th17 CD4$^+$ T cells, a population of helper T cells resident in the gut.[19] Through the expression of CCR6, they can migrate into the intestinal mucosa. CCR6 is also a marker for colon and blood CD4$^+$ T cells enriched for replication-competent HIV DNA.[4] Th17 cells could contribute to the difference in levels of HIV reservoir between inflamed and non-inflamed tissue.

In this well-documented case, our results confirm that increased gut permeability and inflammation could be associated with a higher frequency of HIV infected cells. Moreover, when gut epithelial integrity is preserved, there was no evidence of increased microbial translocation in a study of early SIV infection in natural hosts' infection.[20] This study and our results both suggest that a gut epithelial damage enhances the persistence of HIV reservoirs and increases inflammation-associated comorbidities.

Conclusions

Taken together, our results suggest that local gut inflammation may facilitate the persistence of a pool of infected cells as compared to non-inflamed colon tissue. Our work provides further evidence of the heterogeneous distribution of HIV infected cells within the same organ. Novel therapies that restore gut barrier integrity may reduce inflammation and HIV reservoir size in the gut of ART-treated PLWH.[18]

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jve.2021.100033.

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