Viewpoint

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

Xiaorong Peng a,b,c, Stéphane Isnard a,b,d, John Lin a,b, Brandon Fombuena a,b, Talat Bessissow e, Nicolas Chomont f, Jean-Pierre Routy a,b,g,*

a Infectious Diseases and Immunity in Global Health Program, Research Institute, McGill University Health Centre, Montréal, QC, Canada
b Chronic Viral Illness Service, McGill University Health Centre, Montréal, QC, Canada
c State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China
d CIHR Canadian HIV Trials Network, Vancouver, BC, Canada
e Division of Gastroenterology, McGill University Health Center, Montréal, QC, Canada
f Centre de Recherche du Centre Hospitalier de l’Université de Montréal, Montréal, QC, Canada
g Division of Hematology, McGill University Health Centre, Montréal, QC, Canada

ARTICLE INFO

Keywords:
HIV DNA
Reservoir
Colon
Ulcerative colitis
Inflammation
Gut barrier integrity

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. HIV RNA, HIV DNA and infectious virus have been detected in the gut of ART-suppressed individuals. Moreover, higher levels of in vitro proinflammatory cytokine production were found in gut biopsies of HIV-infected patients when compared with uninfected controls. Persistent gut inflammation is associated with HIV replication, gut epithelial barrier dysfunction and microbial translocation, even in PLWH taking ART. 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). 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 T cell number and function which may facilitate its development. 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.

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.

* Corresponding author. Infectious Diseases and Immunity in Global Health Program, Research Institute, McGill University Health Centre, Montréal, QC, Canada.
E-mail address: jean-pierre.routy@mcgill.ca (J.-P. Routy).

https://doi.org/10.1016/j.jve.2021.100033
Received 19 November 2020; Received in revised form 10 February 2021; Accepted 12 February 2021
Available online 15 February 2021
2055-6640/© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
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/mm³, 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/mm³ 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 were 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, α4β7 integrin blocker vedolizumab and Janus kinase inhibitor tofacitinib.11 In June 2018, due to recurrent episodes of intestinal bleeding and pain, a total colectomy was performed in the inamed section of the colon, 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 expression of claudin-3 is higher in the inflamed than non-inflamed gut tissue,15 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.

HIV-1 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 RT² 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 RT² 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 levels in PBMCs was 103 copies/10⁶ cells. In three biopsies of the inflamed part of the colon, we detected a median of 27 (range 26–97) copies/10⁸ cells of HIV DNA, while HIV DNA level in the adjacent non-inflamed gut tissue was only 1 copy (0–22)/10⁶ 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/10⁶ 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 epithelial integrity which decreases during inflammation. The expression of occludin-1 was decreased in inflamed tissue. As expected, the ratio of claudin-3:occludin expression was higher in the inflamed than non-inflamed gut sections.15 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,16,18 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

| Gut          | CD4⁺ | CD8⁺ | CD3⁺ | CD45⁺ | Total HIV DNA (median) normalized by CD4% |
|--------------|------|------|------|-------|------------------------------------------|
| PBMC         | 47   | 40   | 88   | 100   | 250                                      |
| Inflamed gut | 65.7 | 30.9 | 26.5 | 10.8  | 1482                                     |
| Non-inflamed | 59.6 | 38.8 | 42.2 | 19.6  | 27                                       |
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.\(^4\) There is solid evidence that HIV infection breaches the gut barrier in a way similar to UC.\(^5\)\(^,\)\(^6\)\(^,\)\(^7\)\(^,\)\(^8\) 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.\(^1\) 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.

Acknowledgments

The authors thank Angie Massicotte, Josée Giroud and Cezar Iovi for study coordination and assistance. They authors are grateful to Dr Patrick Charlebois for providing fresh tissues during surgery. The authors thank the patient for agreeing to participate in this research.

This work was funded by the Fonds de la Recherche du Québec-Santé (FRQ-S): Réseau 218 SIDA/Maladies infectieuses et Thérapie cellulaire; the Canadian Institutes of Health Research (CIHR; Grants HOP 103230 and PTo 166049); the Vaccines & Immunotherapies Core of the CIHR Canadian HIV Trials Network (CTN; Grant CTN 247); the Canadian Foundation for AIDS Research (CanFAR; Grant 02–512); CIHR-funded Canadian HIV Cure Enterprise (CanCURE) Team Grant HB2-164064 and China Scholarship Council (No.201906325018).

Appendix A. Supplementary data

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

References

1. Mowat AM, Viney JL. The anatomical basis of intestinal immunity. Immunol Rev. 1997;156:145–166.
2. Yukl SA, Gianella S, Sinclair E, et al. Differences in HIV burden and immune activation within the gut of HIV-positive patients receiving suppressive antiretroviral therapy. J Infect Dis. 2016;202(10):1553–1561.
3. Telwate S, Lee S, Somsock M, et al. Gut and blood differ in constitutive blocks to HIV transcription, suggesting tissue-specific differences in the mechanisms that govern HIV latency. PLoS Pathog. 2018;14(11), e1007357.
4. Goselin A, Wiche Salinas TR, Planas D, et al. HIV persists in CCR6\(^+\)-CD4\(^+\) T cells from colon and blood during antiretroviral therapy. AIDS. 2017;31(1):35–48.
5. Di Stefano M, Favia A, Momo L, et al. Intracellular and cell-free (infectious) HIV-1 in rectal mucosa. J Med Virol. 2001;65(4):637–643.
6. Inazad S, Ramendra R, Dupuy FP, et al. Plasma levels of C-type lectin REG3alpha and gut damage in people with HIV. J Infect Dis. J Infect Dis. 2019;221(1):110–121. https://doi.org/10.1093/infdis/jiz423, 2020 Jan 1.
7. Alazhar Jal, Hussain T, Simar D, et al. Inflammatory and immunometabolic consequences of gut dysfunction in HIV: parallels with IBD and implications for reservoir persistence and non-AIDS comorbidities. EBioMedicine. 2019;46:522–531.
8. Sharpstone DR, Duggal A, Gazzard BG. Inflammatory bowel disease in individuals seropositive for the human immunodeficiency virus. Eur J Gastroenterol Hepatol. 1996;8(6):575–579.
9. Skamnelos A, Tatsioni A, Katsanos KH, Tsianos V, Christodoulou D, Tsianos EV. CD4 count remission hypothesis in patients with inflammatory bowel disease and human immunodeficiency virus infection: a systematic review of the literature. *Ann Gastroenterol*. 2015;28(3):337–346.

10. Costiniuk CT, Bessisow T, Isnard S, Routy J-P. Use of various immunotherapies for refractory ulcerative colitis in a person living with HIV: a case report. *Oxford Med Case Rep*. 2021;1:2021.

11. Cattin A, Wiche Salinas TR, Gosselin A, et al. HIV-1 is rarely detected in blood and colon myeloid cells during viral-suppressive antiretroviral therapy. *AIDS*. 2019;33(8):1293–1306.

12. Vandergeeten C, Fromentin R, Merlini E, et al. Cross-clade ultrasensitive PCR-based assays to measure HIV persistence in large-cohort studies. *J Virol*. 2014;88(21):12385–12396.

13. Poritz LS, Harris 3rd LR, Kelly AA, Kolunn WA. Increase in the tight junction protein claudin-1 in intestinal inflammation. *Dig Dis Sci*. 2011;56(10):2802–2809.

14. Poles MA, Boscardin WJ, Elliott J, et al. Lack of decay of HIV-1 in gut-associated lymphoid tissue reservoirs in maximally suppressed individuals. *J Acquir Immune Defic Syndr*. 2006;43(1):65–68.

15. d’Ettorre G, Paiardini M, Zaffiri L, et al. HIV persistence in the gut mucosa of HIV-infected subjects undergoing antiretroviral therapy correlates with immune activation and increased levels of LPS. *Curr HIV Res*. 2011;9(1):48–53.

16. Landy J, Ronde E, English N, et al. Tight junctions in inflammatory bowel diseases and inflammatory bowel disease associated colorectal cancer. *World J Gastroenterol*. 2016;22(11):3117–3126.

17. Yu LC. Microbiota dysbiosis and barrier dysfunction in inflammatory bowel disease and colorectal cancers: exploring a common ground hypothesis. *J Biomed Sci*. 2018;25(1):79.

18. Ouyang J, Isnard S, Lin J, et al. Treating from the inside out: relevance of fecal microbiota transplantation to counteract gut damage in GVHD and HIV infection. *Front Med*. 2020. https://doi.org/10.3389/fmed.2020.00421.

19. Wacleche VS, Goulet JP, Gosselin A, et al. New insights into the heterogeneity of Th17 subsets contributing to HIV-1 persistence during antiretroviral therapy. *Retrovirology*. 2016;13(1):59.

20. Raehzi KD, Barrenas F, Xu C, et al. African green monkeys avoid SIV disease progression by preventing intestinal dysfunction and maintaining mucosal barrier integrity. *Sci Rep*. 2020;16(3), e10063033.