Vol 3:1-6

JEMI-PEARLS

Targeting the Gut Microbiome in HIV/AIDS: New Therapeutic Opportunities

Zoe O'Neill [a]

[a]Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY Despite major advances in HIV antiretroviral therapy (ART), complete elimination of the disease has not been achieved. Recently, the importance of the gut-associated lymphoid tissues (GALT) and the resident microbiota in HIV pathogenesis has been highlighted. The GALT is the largest of the human lymphoid organs and is home to the highest number of CD4+ T cells in the body. Chronic HIV infection is associated with significant metabolic pathology, including changes to the microbial composition of the gut. However, the exact mechanism by which the microbiome contributes to HIV disease progression is still unclear. It is possible that an inflammatory cycle in which changes to the gut microbiota leads to impairment of the gut mucosal barrier function, resulting in leakage of bacterial members of the gut into systemic circulation. This article will explore the current understanding of how HIV changes the gut microbiota and what mechanisms may link these changes to immune cell loss and disease progression. Chronic immune activation is a key component of HIV-pathogenesis and may underscore the depletion of CD4+ T cells. Understanding this link highlights the possible role of a master regulator within the gut mucosa, thus uncovering a potential therapeutic target that may slow HIV progression.

INTRODUCTION

Despite major advances in the treatment and detection of HIV, complete eradication of the disease has not yet been achieved. Strict adherence to an antiretroviral regimen and an undetectable viral load can still leave patients with few options if they do not have sufficient immune recovery [1]. Approximately 20% of patients who achieve complete viral suppression will fail to reconstitute CD4+ T cell populations [2]. These patients are referred to as Immunological Non-Responders (INR). In recent years, the model of HIV infection in which immune dysfunction is the direct result of viral replication has been challenged with a more nuanced understanding of HIV pathogenesis. In this model, immunodeficiency is the result of immune dysregulation and massive CD4+ T cell activation [3].

Accepted: 23 July 2018
Published: 01 August 2018
Citation: O'Neill Z. 2018. Targeting the Gut Microbiome in HIV/AIDS: New Therapeutic Opportunities. JEMI PEARLS 3: 1-6
Editor: François Jean, University of British Columbia

Address correspondence to Zoe O'Neill
zoeoneill95@gmail.com

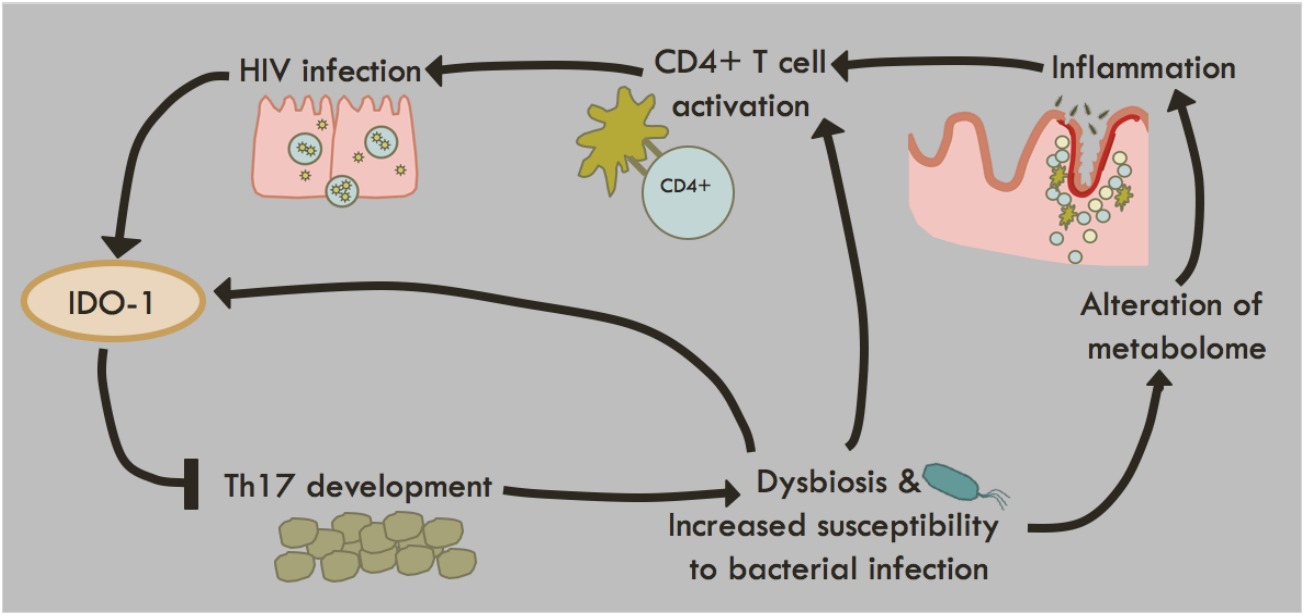

FIG. 1 IDO-1 enzyme activity driving immune activation, inflammation and immune cell loss. The self-amplifying nature of HIV infection, activation of IDO-1 and subsequent dysbiosis and inflammation.

The gut associated lymphoid tissue (GALT) is where the majority of CD4+ T cells reside. The GI tract is now recognized as a key player in immune system function and development. The gut is also an important site for viral replication in the acute stages of HIV infection [4]. HIV patients experience substantial decreases in CD4+ T cells at the GALT within weeks of infection. Immune cells in the GALT express elevated levels of CCR5, making them particularly vulnerable to infection by the virus in comparison to T cells in the peripheral blood [5]. Importantly, in chronically infected patients who are late to initiate ART, CD4+ T cell counts in the GALT never fully recover [6].

HIV has been shown to cause dramatic changes in the GI tract with significant alterations to the composition of the microbiota community [3]. The GI tract is constantly exposed to food and microbial antigens and plays a key role in regulating a balance of immune tolerance and activation [7]. This equilibrium is disrupted by HIV infection and local changes in the gut have far-reaching consequences. Changes in the gut community can lead to breaches of the gut mucosal lining, increased circulating microbial by-products and subsequent persistent immune activation. Given the accumulating evidence showing that immune activation is at the heart of HIV disease progression, understanding the mechanisms that underlie immune activation during the course of HIV infection will be crucial in developing new therapies in the future. This paper will investigate the links between the gut microbiota and HIV pathogenesis, propose a mechanism by which changes in the gut microbiota could be linked to the loss of CD4+ immune cells, and highlight the consequences of these findings for future treatment of HIV infection.

RESEARCH QUESTIONS

HIV disease remains a significant societal burden. In addition to impacting the quality of life of HIV infected individuals, the lifetime economic cost of those testing positive for HIV in Canada is estimated to be over $1,000,000 [8]. Elucidating the interaction between the gut microbiota and HIV infection may open doors for new therapeutic opportunities that help to reconcile this economic and psychological suffering. While antiretroviral therapy has been remarkably successful in controlling HIV viral replication, its success is limited by viral resistance, cost of treatment, adherence to treatment and significant drug-drug interactions [9]. These limitations indicate the work that still needs to be done in the fight against HIV. Research investigating the microbiota-immune axis is still in its infancy and complete understanding of its regulation and role in disease could provide novel targets for treatment.

Three questions will be addressed in order to illuminate the role of the gut in HIV infection. First, the way in which the gut microbiota is altered during the course of HIV infection will be reviewed. Then, possible mechanisms that link these changes to immune cell loss and poor immune recovery will be examined. Finally, a potential novel therapeutic target related to the gut-immune axis will be explored in detail.

PROJECT NARRATIVE

HIV infection changes the gut microbiota

In recent years, a growing number of studies have investigated HIV-induced changes in the gut microbiome and its association with systemic inflammation and immune activation. A healthy GI tract is colonized by a wide variety of microbes, which are shielded from the immune system by a robust mucosal barrier [10]. The microbiome has been implicated in numerous inflammation-associated pathologies such as those in diabetes, obesity and inflammatory bowel disease (IBD) [11]. It is now being proposed that HIV-related dysbiosis may contribute to inflammation in chronic HIV infection. Although the gut microbiome of HIV infected individuals varies, common patterns of change have emerged [12]. HIV infected individuals often display decreased microbial diversity as well as increases in potentially pathogenic bacteria such as Enterobacteriaceae [12, 13]. Interestingly, HIV infected individuals also show a simultaneous loss of protective commensals such as Lactobacillaceae, Ruminococcaceae and Lachnospiraceae [14].

Investigation into two opportunistic pathogens, *P. aeruginosa* and *C. albicans*, in the early stages of HIV infection showed that 92% of HIV infected patients were colonized by *P. aeruginosa* versus only 20% of healthy individuals [10]. *P. aeruginosa* also accounted for a larger proportion of the total microbiota in HIV infected individuals. Likewise, 100% of HIV-positive fecal samples were colonized by *C. albicans,* which was present at levels 10,000-fold higher than is found in healthy controls. Thus, impairment of the GI tract occurs early in infection and is associated with higher proportions of pathogenic microbes [10].

Interestingly, elite controllers (EC) of HIV infection, who show suppressed viral replication in the absence of ART, have a distinct microbiome profile in comparison to those individuals who are INR or not HIV infected. Overall, ECs show increased species richness and diversity of the microbiota, similar to that of healthy individuals [15]. Additionally, ECs display an abundance of the microbial genus, *Succinivibrio,* which has been associated with successful immune recovery under ART therapy [15]. *Succinivibrio* has been shown to accumulate pro-inflammatory mediators, sequestering compounds that would otherwise cause inflammation in the gut [16]. So, the loss of protective commensals may lead to increased gut inflammation. Fecal calprotectin, a protein secreted by neutrophils in the GALT, can be used as a marker for inflammation. HIV infected individuals tend to show higher fecal calprotectin levels in comparison to healthy controls, indicating breakdown of the intestinal barrier function [10].

Changes in the profile of metabolites produced by the gut microbial community have also been associated with immune cell recovery. Analysis of HIV-infected patients who are either viremic and untreated (VU), immunological ART responders (IR) or immunological ART non-responders (INR) shows that INR and VU tend to be most similar in their metabolome profiles, and different to IR. This suggests a correlation between adequate CD4+ T cell recovery and gut bacteria metabolic profiles [16].

Thus, loss of protective community members and increases in pathogenic gut microbes may promote intestinal inflammation during HIV.

Mechanisms that link changes in the gut microbiota to immune cell loss

Several mechanisms link changes in the gut microbiota to immune cell loss. While the virus itself certainly triggers immune activation, it is not antigenically diverse enough to cause the observed depletion in CD4+ T cell populations [3]. It is hypothesized that HIV induces changes in the GI tract, compromising gut mucosal immunity. This leads to increased translocation of intestinal microbes and their products into circulation, with subsequent systemic immune activation [17].

In a study using a SIV-infected RM model, co-localization of microbial products and levels of production of inflammatory cytokines demonstrated that damage to the integrity of the gut lining was associated with microbial translocation, and this was linked to local immune activation [18,19]. This observation is supported by findings that lipopolysaccharide binding protein (LBP), a marker of microbial gut translocation, is present at significantly higher concentrations in naïve HIV infected individuals versus EC and non-HIV infected individuals. Additionally, INR show persistently high levels of circulating LPS and bacterial DNA, indicating less efficient containment of microbial translocation across the gut lining [20]. Given the relatively limited viral replication in the gut lining, it is unlikely that viral replication is solely responsible for immune activation in the gut. Instead, damage to the gut barrier and subsequent microbial translocation leads to persistent immune activation, providing activated CD4+ T cell targets for the virus [21].

However, the question of what induces the initial damage to the gut epithelium remains. A subset of T-helper cells, Th-17 cells, is essential in protection against bacterial infection at mucosal surfaces like the GALT [22]. HIV has been demonstrated to alter the balance between intestinal Treg and Th17 cells [23]. Treg cells maintain tolerance to self and prevent autoimmune reactions. The loss of Th17 cells may account for the breakdown of gut mucosal immunity, providing the initial breach that allows for subsequent increased microbial translocation [23].

Indoleamine-2,3-Dioxygenase 1 (IDO-1) is the main inducible enzyme of Tryptophan metabolism through the kynurenine pathway. IDO-1 is found in dendritic cells and its activity is upregulated by interferons and toll-like receptor agonists [24]. Murine and *in vitro* studies have shown that IDO-1 activity leads to decreased Th17 development, tipping the Th17/Treg balance in the gut towards an immunosuppressive Treg pathway [23]. Increased IDO-1 activity during HIV infection indicates the existence of a positive feedback loop. Initial infection by HIV leads to upregulated IDO-1, which in turn suppresses Th17 development, dampening mucosal immunity and leading to breaches of the gut barrier and persistent immune activation [23]. RMs infected with SIV who do not progress to disease exhibit normal levels of Th17 cells [25]. The loss of normal protective factors in the gut leads to gut epithelial damage and initiates a vicious cycle of immune activation, which may lie at the heart of HIV disease progression.

Interestingly, enrichment of certain pathogenic microbes in the gut is correlated with differing levels of Kyn:Trp catabolism [26]. Tryptophan (Trp) is an amino acid that is converted to Kynurenine (Kyn) by IDO-1. Several bacterial species enriched in HIV infected patients have IDO-1 homologs capable of producing Kynurenine from Tryptophan. Thus, initial infection with HIV may activate IDO-1, supporting the outgrowth of these pathogenic microbes, which may in turn exacerbate IDO-1 mediated mucosal disruption by accelerating Tryptophan catabolism along the Kynurenine pathway [26]. This would create a feedback cycle in which dysbiosis augments IDO-1 activity leading to loss of Th17 protection, further alteration of the gut microbial community and microbial translocation.

Targeting IDO-1 to fight HIV

IDO-1 dependent tryptophan metabolism may be the essential link between immune activation and the decline of immune cells seen in HIV infection [23]. IDO-1 is highly expressed in the gut mucosa during the early stages of HIV infection and this is associated with a decreased capacity to generate Th17 cells [25]. ECs of HIV have similar IDO-1 enzyme activity to uninfected individuals [15]. SIV infected animal models have shown that using a pharmacological blocker of IDO-1, in combination with ART leads to decreased viral load in animals with incomplete suppression of viral replication [27]. Thus, directly targeting IDO-1 could prevent the initiation of a vicious cycle of immune activation and allow for more robust immune recovery.

In light of evidence suggesting that certain resident-gut bacteria may augment IDO-1 destruction of the gut mucosal barrier, targeting the gut microbial community to regulate IDO-1 activity presents a potential therapeutic opportunity [26]. Administration of *Lactobacillus johnsonii* in rats led to a reduction of serum Kynurenine, which has been shown to reduce IDO-1 activity *in vitro* [28,29]. Additionally, Kynurenine seems to prevent IL-2 signaling, impairing the survival of CD4+ T cells [30]. Thus, the introduction of certain microbes into

the intestinal tract may bolster the intrinsic immunity of the gut barrier and halt the cycle of inflammation in HIV disease progression.

Decreased microbial species richness has also been associated with increased IDO-1 activity. Of patients on HAART, those with the highest degree of dysbiosis show the most elevated levels of Kynurenine production and plasma IL-6 levels, markers of chronic inflammation and disease progression [26]. So, in addition to populating the gut with specific bacteria that could modulate the activity of IDO-1, the use of therapeutic interventions that increase species richness in the gut may be beneficial [15].

IDO-1 knockout studies also support the notion that this enzyme may be a master regulator of gut immunity and extremely important in HIV disease progression. Mice with an IDO-1 gene knockout show enhanced induction of protective antibodies against bacterial pathogens and an attenuated intestinal inflammatory response [31]. Additionally, absence of IDO-1 has also been shown to suppress viral replication in a retrovirus-infected mouse model [32]. IDO-1 is pro-inflammatory and its regulation could decrease gut inflammation and slow disease progression in HIV disease.

SUMMARY AND CONCLUSION

The role of the gut in HIV disease progression is an area of intense interest. While antiretroviral therapy has been extremely successful, it fails to consistently encourage immune recovery amongst all HIV-infected persons. Emerging evidence confirms that HIV infected patients show dramatic changes in the gut mucosa, including alterations of community members and increased inflammation. Loss of protective microbial community members and increased proportions of pathogenic gut microbes augments the activity of the IDO-1 enzyme. This ultimately upends the Treg/Th17 balance at the gut with loss of protective Th17 immune cells. Disruption of the mucosal barrier leads to microbial translocation and persistent systemic immune activation, activating CD4+ T cells and preventing immune recovery.

This self-perpetuating cycle of dysbiosis and inflammation highlights the possibility of targeting a potential master regulator, IDO-1, in HIV therapeutics. IDO-1 activity could be modulated with a pharmacological blocker or through the careful administration of gut microbes. Preventing the destructive activity of this enzyme would normalize the gut mucosal lining, decrease activation of the immune system and allow reconstitution of CD4+ T cell populations. It is possible that controlling the activity of IDO-1 could adjunct ART, providing the key to HIV eradication. Given the growing body of evidence indicating the importance of this enzyme in HIV disease progression, further investigation of the precise molecular mechanism through which it is regulated is certainly warranted.

This area of research is still in its infancy, and the proposed model is a relatively simple interpretation of an extremely complex system. Additionally, this model is limited as most research into the microbiome focuses exclusively on adults and is therefore unlikely to apply to pediatric or geriatric populations. As children account for a significant proportion of newly HIV-infected persons, more research into the role of the gut microbiome in HIV infected children must be done. Investigation into the role of the microbiome in chronic viral infections is becoming an increasingly exciting area of research. As we move into the post-metagenomics era, we will able to move beyond merely associating microbial species presence with cellular markers, and begin to investigate causation.

ACKNOWLEDGEMENTS

I would like to thank Dr. François Jean for inspiring perseverance and a passion for Virology. His expertise and feedback was vital in the development of my research proposal. I would also like to thank my MICB 406 peers for their valuable input.

REFERENCES

1. Gaardbo, Hans J. Hartling, Jan Gerstoft and Susanne D. Nielsen. Incomplete immune recovery in HIV infection: mechanisms, relevance for clinical care, and possible solutions. Clinical and Developmental Immunology. 2012. 2012:1-17.
2. Wilson and Irini Sereti. Immune restoration after antiretroviral therapy: the pitfalls of hasty or incomplete repairs. Immunol.Rev. 2013. 254: 343-354.

3. Brenchley, David A. Price and Daniel C. Douek. HIV disease: fallout from a mucosal catastrophe? Nat.Immunol. 2006. 7:235.
4. Brenchley and D. C. Douek. HIV infection and the gastrointestinal immune system. Mucosal immunology. 2008. 1: 23.
5. Poles, Julie Elliott, Philip Taing, Peter A. Anton and Irvin SY Chen. A preponderance of CCR5 CXCR4 mononuclear cells enhances gastrointestinal mucosal susceptibility to human immunodeficiency virus type 1 infection. J.Virol. 2001. 75: 8390-8399.
6. Guadalupe, Elizabeth Reay, Sumathi Sankaran, et al. Severe CD4 T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J.Virol. 2003. 77:11708-11717.
7. Cecchinato, Christopher J. Trindade, Arian Laurence, et al. Altered balance between Th17 and Th1 cells at mucosal sites predicts AIDS progression in simian immunodeficiency virus-infected macaques. Mucosal immunology. 2008. 1: 279.
8. Kingston-Riechers. The Economic Cost of HIV/AIDS in Canada. Canadian AIDS Society. 2011.
9. Desai, Geetha Iyer and R. K. Dikshit. Antiretroviral drugs: critical issues and recent advances. Indian journal of pharmacology. 2012. 44: 288.
10. Gori, Camilla Tincati, Giuliano Rizzardini, et al. Early impairment of gut function and gut flora supporting a role for alteration of gastrointestinal mucosa in human immunodeficiency virus pathogenesis. J.Clin.Microbiol. 2008. 46:757-758.
11. Guinane and Paul D. Cotter. Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. Therapeutic advances in gastroenterology. 2013. 6: 295-308.
12. Gootenberg, Jeffrey M. Paer, Jesus-Mario Luevano and Douglas S. Kwon. HIV-associated changes in the enteric microbial community: potential role in loss of homeostasis and development of systemic inflammation. Curr.Opin.Infect.Dis. 2017. 30: 31.
13. Nowak, Marius Troseid, Ekatarina Avershina, et al. Gut microbiota diversity predicts immune status in HIV-1 infection. AIDS. 2015. 29: 2409-2418.
14. McHardy, Xiaoxiao Li, Maomeng Tong, et al. HIV Infection is associated with compositional and functional shifts in the rectal mucosal microbiota. Microbiome. 2013. 1: 26.
15. Vesterbacka, Javier Rivera, Kajsa Noyan, et al. Richer gut microbiota with distinct metabolic profile in HIV infected elite controllers. Scientific reports. 2017. 7: 6269.
16. Serrano-Villar, David Rojo, Monica Martinez-Martinez, et al. Gut bacteria metabolism impacts immune recovery in HIV-infected individuals. EBioMedicine. 2016. 8: 203-216.
17. Price, Jason M. Brenchley and Daniel C. Douek. HIV disease: fallout from a mucosal catastrophe? Nat.Immunol. 2006. 7: 235-239.
18. Estes, Levelle D. Harris, Nichole R. Klatt, et al. Damaged intestinal epithelial integrity linked to microbial translocation in pathogenic simian immunodeficiency virus infections. PLoS pathogens. 2010. 6: e1001052.
19. Picker, Shoko I. Hagen, Richard Lum, et al. Insufficient production and tissue delivery of CD4 memory T cells in rapidly progressive simian immunodeficiency virus infection. J.Exp.Med. 2004. 200: 1299-1314.
20. Merlini, Francesca Bai, Giusi Maria Bellistri, Camilla Tincati, Antonella d'Arminio Monforte and Giulia Marchetti. Evidence for polymicrobic flora translocating in peripheral blood of HIV-infected patients with poor immune response to antiretroviral therapy. PloS one. 2011. 6: e18580.
21. Ancuta, Anupa Kamat, Kevin J. Kunstman, et al. Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. PloS one. 2008. 3, pg e2516.
22. Mills. Induction, function and regulation of IL-17-producing T cells. Eur.J.Immunol. 2008. 38: 2636-2649.
23. Favre, Jeff Mold, Peter W. Hunt, et al. Tryptophan catabolism by indoleamine 2, 3-dioxygenase 1 alters the balance of T H 17 to regulatory T cells in HIV disease. Science translational medicine. 2010. 2: 32-36.
24. Mellor and David H. Munn. IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nature Reviews Immunology. 2004. 4: 762.
25. Cecchinato, Christopher J. Trindade, Arian Laurence, et al. Altered balance between Th17 and Th1 cells at mucosal sites predicts AIDS progression in simian immunodeficiency virus-infected macaques. Mucosal immunology. 2008. 1: 279-288.
26. Vujkovic-Cvijin, Richard M. Dunham, Shoko Iwai, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Science translational medicine. 2013. 5: 193-91.
27. Boasso, Monica Vaccari, Dietmar Fuchs, et al. Combined effect of antiretroviral therapy and blockade of IDO in SIV-infected rhesus macaques. The Journal of Immunology. 2009. 182: 4313-4320.
28. Kennedy, J. F. Cryan, T. G. Dinan and G. Clarke. Kynurenine pathway metabolism and the microbiota-gut-brain axis. Neuropharmacology. 2017. 112: 399-412.
29. Zelante, Rossana G. Iannitti, Cristina Cunha, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity. 2013. 39: 372-385.
30. Dagenais-Lussier, Mouna Aounallah, Vikram Mehraj, et al. Kynurenine reduces memory CD4 T-cell survival by interfering with interleukin-2 signaling early during HIV-1 infection. J.Virol. 2016. 90: 7967-7979.
31. Harrington, Chittur V. Srikanth, Reuben Antony, et al. Deficiency of indoleamine 2, 3-dioxygenase enhances commensal-induced antibody responses and protects against Citrobacter rodentium-induced colitis. Infect.Immun. 2008. 76: 3045-3053.
32. Hoshi, Kuniaki Saito, Akira Hara, et al. The absence of IDO upregulates type I IFN production, resulting in suppression of viral replication in the retrovirus-infected mouse. The Journal of Immunology. 2010. 185: 3305