“Go”, “No Go,” or “Where to Go”: does microbiota dictate T cell exhaustion, programming, and HIV persistence?


Purpose of review
People living with HIV who fail to fully reconstitute CD4⁺ T cells after combination antiretroviral therapy (i.e., immune nonresponders or INRs) have higher frequencies of exhausted T cells that are enriched in a small pool of memory T cells where HIV persists and have an abundance of plasma metabolites of bacterial and host origins. Here, we review the current understanding of critical features of T cell exhaustion associated with HIV persistence; we propose to develop novel strategies to reinvigorate the effector function of exhausted T cells with the aim of purging the HIV reservoir.

Recent findings
We and others have recently reported the role of microbiota and metabolites in regulating T cell homeostasis, effector function, and senescence. We have observed that bacteria of the Firmicute phyla (specifically members of the genus Lactobacilli), associated metabolites (β-hydroxybutyrate family), and bile acids can promote regulatory T cell differentiation in INRs with a senescent peripheral blood gene expression profile.

Summary
The cross-talk between immune cells and gut microbes at the intestinal mucosa (a major effector site of the mucosal immune response), regulates the priming, proliferation, and differentiation of local and distant immune responses. This cross-talk via the production of major metabolite families (like serum amyloid A, polysaccharide A, and aryl hydrocarbon receptor ligands) plays a key role in maintaining immune homeostasis. HIV infection/persistence leads to gut dysbiosis/microbial translocation, resulting in the local and systemic dissemination of microbes. The ensuing increase in immune cell-microbiome (including pathogens) interaction promotes heightened inflammatory responses and is implicated in regulating innate/adaptive immune effector differentiation cascades that drive HIV persistence. The exact role of the microbiota and associated metabolites in regulating T cell-mediated effector functions that can restrict HIV persistence continue to be the subject of ongoing studies and are reviewed here.

Keywords
Co-inhibitory receptors, HIV, metabolites, microbiome, senescence, T cell exhaustion

INTRODUCTION
Immune health is the state in which the immune system is under homeostasis and fully recovers from any intrinsic or extrinsic perturbations. The successful achievement of disease clearance depends on the proper immune activation, expansion, and contraction of innate and adaptive immune cells. The quality of the immune responses is dependent on a timely cascade of upstream signals stemming from other immune cells and the host environment. Among them is the impact of the microbiome and its bioproducts (metabolites) on cells of the innate immune system is now widely recognized as being critical for the development of a protective immune state.

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**KEY POINTS**

- This review highlights the link between microbiome/metabolites in T cell senescence.
- Microbiome and metabolites are thought to influence T cell exhaustion function and having potential implications in sustained HIV reservoir.
- Targeting T exhaustion pathway through metabolites might serve as a desired therapeutic intervention in treating HIV patients.
- The presence of pro- and anti-inflammatory cytokines and metabolites after microbial dysbiosis can impact immune reconstitution resulting in HIV reservoir persistence.

response. A masterpiece of this puzzle is the priming of the immune cells in the gut, one of the principal scenarios for HIV infection and spreading. In this review, we will focus in the three signals associated with the priming of the immune response and the co-adjuvants responsible for an ideal/defective disease clearance and homeostasis reestablishment. Major impaired immune status is exacerbated inflammation, exhaustion, immune senescence, and Anergy. We will focus on the interplay between microbiome, metabolome, and their downstream signals to cells of the immune system during HIV infection, immune reconstitution, and HIV reservoir persistence.

The induction of protective T cell response against pathogens or cancers, first, requires the binding of the T cell receptor (TCR) on CD4$^+$ and CD8$^+$ T cells to antigens presented on major histocompatibility complexes by antigen presenting cells (APCs). However, a T cell does not get activated until a co-stimulatory signal (e.g. binding of CD28 to its ligand CD80 and CD86 on APC) is provided. In the absence of the latter interaction, T cells undergo a state called ‘anergy’, in which cells can remain alive for an extended period of time but are functionally inactive [1]. Additionally, a third signal conferred by cytokines and host and bacterial metabolites is also required for induction of efficacious immune responses [2]. In an acute response, the transient overexpression of immune check point inhibitors (ICls, such as PD-1, CTLA-4, Tim-3, and LAG-3) on effector T cells puts a brake on aberrant T cell mediated responses postclearance of pathogen/infection [3,4]. However, in chronic infections [HIV, hepatitis C virus (HCV), hepatitis B virus (HBV)] and cancers, when immune cells can not eliminate the tumor or the virus, persistent antigen stimulation leads to T cell intrinsic molecular events that trigger a crippled functional state known as exhaustion [4]. Figure 1 depicts various stages of T cell dysfunction, such as exhaustion, senescence, and anergy.

Treatment of people living with HIV (PLWH) with antiretroviral therapy (ART) brings viral load to undetectable levels and promotes CD4$^+$ T cell reconstitution in a majority of patients [5]. Despite viral clearance, a small pool of cells still carries replication competent viral genomes that can be reactivated upon ART interruption. Given that CD4$^+$ T cells, abundant in the intestinal mucosa, are the primary targets during infection and are abundant in the intestinal mucosa, we hypothesized that the gut microbiota and associated metabolites play a critical role in regulating CD4$^+$T cell reconstitution[5] and HIV persistence.

**ROLE OF MICROBIOTA AND METABOLOME IN SHAPING THE IMMUNE RESPONSE**

Under normal conditions, microbes cannot pass through the intestinal epithelial barrier or are excluded from entering the systemic immune system through the coating of microbial specific secretory Immunoglobulin A antibodies. As illustrated in Fig. 2, Concurrently, innate immune cells like dendritic cells can aid in inducing tolerance against the microbiome by sampling and presenting commensal antigens to promote differentiation of resident T cells into Tregs (major producers of cytokines like Interleukin (IL)-10 and Transforming growth factor (TGF)-β) and T$_{117}$ cells (in addition to innate lymphoid cells - are major producer of cytokines such as IL-17 and IL-22) [6]. Increased IL-10 production - a STAT-3 inducing cytokine is known to reduce pro-inflammatory signals, attenuate T cell activation, impede antigen-presenting cells and B cell function [7–9] is observed in several chronic infections, including HIV, HBV, and HCV, [10]. During chronic viral infections, interfering with IL-10 signaling resulted in improved effector T cell responses, development of functional antiviral memory cells, and faster control of viral replication [11–13]. TGF-β, like IL-10, is an immunosuppressive cytokine (attenuates immune cell activation by activating an acronym from the fusion of Caenorhabditis elegans Sma genes and the Drosophila Mad, Mothers against decapentaplegic transcription factors) [14] is known to regulate the size of pathogen-specific T-cell responses and the propensity of these cells to undergo apoptosis [15]. The balance between the activity of IL-10 producing Tregs and pro-inflammatory lymphoid compartment is essential for the maintenance of gut barrier structure/function and keeping aberrant proinflammatory responses to a minimum (Fig. 2). In
addition to the direct microbe-immune interaction, the gut microbiota can produce several metabolites - such as short-chain fatty acids (SCFAs), polyamines, Adenosine triphosphate, indoles, bile acids and lipid associated metabolites, serum amyloid A (SAA) and polysaccharide A (PSA) - that bind to their specific receptors on immune cells (i.e. nucleotide-binding oligomerization domain like receptors, toll-like receptors, Aryl hydrocarbon receptor, GPR43, GPR41, P2X7, and GPCRs) and promote effector/tolerizing immune responses. Each of these families of metabolites can have a multifaceted impact on the immune response. For example, on one hand, SCFAs and tryptophan metabolites (which bind to both T cells and dendritic cells) are immunosuppressive and mediate the differentiation of TGF-β and IL10-producing Tregs [16–18]. On the contrary, these SCFAs can regulate gene expression (by inhibiting histone deacetylase) and activate GPCRs thereby promoting activation and chemotaxis of the pro-inflammatory neutrophils [19,20].

The gut barrier can be compromised during several inflammatory diseases like HIV, cirrhosis, and ulcerative colitis [21,22]. An inflammatory gut dysbiosis is observed to be associated with the preferential translocation of bacterial species in the *Proteobacteria phyla*. Once across the gut barrier, these species can activate innate immune cascades...
in the gut mucosa and hence indirectly propagate T cell activation/differentiation within the local mesenteric or illeal lymph nodes. Alternately, these bacterial species can migrate directly to the mesenteric lymph nodes or liver and play a critical role in shaping the migratory or effector state of the immune cascade [22,23]. Indeed, during active HIV/SIV infection, intestinal barrier injury resulting from the death of Th17 cells (primary effector CD4⁺ T cells at the site of infection) leads to the translocation of microbial/metabolites to the mucosa, liver or mesenteric lymph nodes [24–26]. The sensing of bacterial products indirectly (through the innate immune system) or directly by T cells in the context of concurrent TCR signaling (sensing of virus replication) can lead to an imbalanced Th17/Treg ratio at the site of HIV replication. This modified immune environment gradually causes a reduced abundance...
T-cell anergy, exhaustion, and senescence are the dominant dysfunctional states of T cells and are controlled by distinct active signaling processes[27–29]. While these mechanisms of T cell dysfunction share several overlapping characteristics in terms of defective proliferative activity and impaired cytotoxic activity, they differ phenotypically and functionally from effector and memory T cells. The state of exhaustion is defined as sequential loss of T cell effector function and is characterized by reduced proliferative abilities, poor metabolism, altered gene expression and epigenetic profiles, reduced effector cytokine production capacity and was associated to high expression of multiple co-inhibitory receptors.

Recent technological advances in the area of epigenetics, including scATAC seq and methyl seq, have allowed a thorough examination of the epigenetic landscape of exhausted T cells in chronic infections and cancer at the single cell level [30–33]. These studies have revealed that exhausted T cells are a unique lineage of cells that are epigenetically different from naive T cells, effector T cells and memory T cells with ~6,000 unique accessible chromatin regions (in exhaustion associated genes like Havcr2 (Tim3), Pdcd1, and BATF [30,31]). Specifically, during chronic Lymphocytic choriomeningitis virus and HIV infections, the nucleic acid sequences adjacent to the PD-1 promoter and its proximal enhancers remain fully demethylated allowing for the constitutive expression of PD-1 in exhausted T-cells [34]. Exhausted T cells display metabolic derangements including suppressed cellular respiration, reduced glucose uptake, and dysregulated mitochondrial functions [33]. PD-1 signaling in exhausted CD8+ T cells is linked with the increased activity of the transcription factor forkhead box O1 (FOXO1), which in turn sustains PD-1 expression and survival of exhausted CD8 cells[35,36].

Transcriptional studies have identified transcription factors TOX and NR4A1 as the master regulators of ICI expression as they have been observed to drive heightened frequencies of exhausted cells during chronic infection in mice and humans (HCV) [37,38]. Target genes regulated by TOX and Tcf7 promotes the generation of exhaustion precursors through the induction of Eomes and c-Myb in early chronic infection [38]. On the other hand, NR4A family members cooperate with NFAT to decrease the activity of AP-1 (bZIP) and to promote CD8+ T cell exhaustion. Indeed, the ratio of NFAT:AP-1 is observed to be the key determinant of effector T vs. exhausted T cells in acute and chronic LCMV infection. Furthermore, NFAT along with IRF-4 and BATF, can promote effector functions by repressing genes critical for induction of memory T cells (TCF-1) during chronic viral infection thus fostering exhaustion phenotype [39]. In addition to TOX and NR4A, T-bet and Eomes master transcription factors for effector and memory CD8+ T cell development [36,40–44] have also been implicated in the development of exhaustion as genetic ablation of either transcription factor impedes on the development of the exhausted T cell pool [43]. Early attempts to manipulate epigenetic pathways have shown great promise in the field of T cell exhaustion. A report by Zhang et al. [45] demonstrated that in vitro treatment of exhausted CD8+ T cells with histone deacetylase inhibitor, valproic acid, rescued T-cell exhaustion function.

Unlike T cell exhaustion, which results from continuous antigenic stimulation and induced expression of ICIs (PD-1, CTLA-4, Tim-3, LAG-3, BTLA, and TIGIT) [46], senescence is triggered by telomere shortening and/or damage signals including oxidative stress, DNA damaging agents, and mitogenic oncogenes[47]. Recent studies have shown that T cell senescence can be induced in naive/effector T cells by Tregs [10,48]. These senescent T cells do not express costimulatory molecules CD27 and CD28 [48,49] and show high expression of senescence-associated markers, including Tim-3, CD57, CD45RA, and KLRG-1 [50–53]. Similar to exhausted T cells, senescent T cells do not proliferate after TCR stimulation but unlike exhausted T-cells, they can produce high amounts of proinflammatory cytokines, such as IL-2, IL-6, IL-8, TNF-α, IFN-γ and the suppressive cytokines IL-10 and TGF-β [48,54]. In spite of these differences, senescent and exhausted CD8+ T cells share common features. For example, CD57+ senescent and exhausted CD8+ T cells express PD1, CD160, and other exhaustion markers which are associated with chronic viral infection [55]. Additionally, both subtypes do not proliferate upon antigenic stimulation. During acute infection, TGF-β restricts the size of both the effector and memory CD8+ T-cell pool, through repression of T-bet [56] and antiapoptotic gene Bcl-2 expression and by inducing the expression of pro-apoptotic genes like Bim [13]. Interestingly, elevated levels of TGF-β are observed under chronic infection with LCMV clone 13 [57]. Of note, attenuation of TGF-β signaling increased the frequency of virus specific T cells and enabled enhanced viral
control [13]. However, the molecular cascades promoting differentiation of these cells under various healthy, acute and chronic disease scenarios and their role in promoting HIV persistence remains to be elucidated.

HIV PERSISTENCE: T CELL DYSFUNCTION RESULTING FROM ABBERRATIONS IN PLASMA METABOLOME AND MICROBIOME COMPOSITION

It is well established that within 3 months of initiation of ART, viral loads plummet and a resulting increase in CD4+ T cell counts is observed. However, gut barrier dysfunction and associated pro-inflammatory responses continue to persist up to 12-months after the start of therapy. Indeed, in recent work from our group (Nganou-Makamdop, Talla, Sharma et al. Cell, in press), we observed that CD4+ T cell reconstitution during the first year on ART was driven by heightened pro-inflammatory innate immune responses and higher expression of Th17. These responses were complemented by higher levels of IL-17A, IL-1β, and IL-6 in the plasma. A closer assessment of the plasma microbial burden (i.e. PathSeq to quantify circulating microbial reads – a direct readout of recent immune encounters with the microbiota) during this inflammatory phase revealed that low microbial diversity observed during early ART was characterized by increased relative abundance of nucleotide reads from the Proteobacterial phyla (i.e. lower Firmicute/Bacteroidetes phyla abundance) and synergized with a glycolytic metabolic milieu (higher lactate and pyruvate). In contrast, by the second year post-ART (when more gut barrier improvement is expected) a restoration of microbial diversity, dampening of innate inflammatory cascades (lower IL-1β, IL-6) and increase in homeostatic milieu was observed. Unlike gut recovery during early ART, immune non-responders (PLWH who maintain low CD4+ T cell counts – less than 350 counts/mm3 blood – despite prolonged ART) present with long-lasting gut barrier dysfunction. Recently, [58**] we observed that the lack of CD4+ T cell reconstitution in a subset of these subjects (i.e. ‘senescent’ INRs - exemplified by a TGF-β induced senescent peripheral blood gene expression profile) was associated with higher HIV persistence and was driven by an abundance of butyrate/bile acid rich plasma metabolome and microbial reads from the Firmicute phyla. This observation prompted us to review immune mechanisms regulated by the microbiome that impact gut dysbiosis and HIV persistence post-ART (Fig. 2).

Transient viral antigen stimulation leads to activation of AP1 and NFAT dependent effector T cells that promote a robust antiviral response. However, under chronic antigen stimulation, NFAT promotes exhaustion by inducing expression of ICIs like PD1, TIM3, TIGIT, CTLA4 and lowered secretion of IL-2, TNF-α, IFN-γ, and Granzyme B in effector T cells [59]. The resulting increased frequencies of exhausted CD8+ T cells renders the immune system incapable of eliminating HIV infected CD4+ T cells, thereby promoting the persistence of HIV and poor recovery of CD4+ T cells despite prolonged combination antiretroviral therapy (cART) therapy. In addition to exhaustion, our recent data (described above) shows that systemic senescence in non-responders to cART therapy is driven by TGF-β signaling and likely results from an increase in β-hydroxybutyrate dependent induction of TGF-β producing Treg differentiation (validated in vitro). We further show that in vitro latency establishment was more profound when latency establishment media was supplemented with TGF-β. Although the direct role of individual metabolites in promoting exhaustion and senescence have not been studied, their intrinsic property of promoting immune regulation either through binding on receptors of immune cells or serving as HDAC inhibitors makes them ideal candidates for key regulators of these processes. In one recent study, authors established that butyrate and propionate can suppress antigen specific CD8+ T cell activation by limiting IL12 production from APC [60] while another recent report demonstrated that acetate promotes histone acetylation, chromatin accessibility and anticancer effector function in T cells [61]. These data indicate that, T cell dysfunction (and associated HIV persistence) can result from an ‘excessive/exhaustive’ pro-inflammatory response or an anti-inflammatory TGF-β induced ‘senescent’ respond. Identifying subjects that are delineated along these lines and targeting T cell senescence or exhaustion may help design therapeutic intervention that can help purge the HIV reservoir and reduce HIV persistence.

CONCLUSION and PERSPECTIVES

In cART, one of the main obstacles post successful treatment remains poor recovery of CD4+ T cell counts and HIV persistence. Growing evidence demonstrates that microbiota associated metabolites regulate the gene expression profiles of immune cells and are linked to various inflammatory disorders. Continuous encounter of microbes and metabolites in intestinal area due to breach of intestinal barrier leads to activation of innate and adaptive immune responses. Considering the significant contribution of various metabolites to immune programming and modulation, future detailed studies should be conducted for
understanding their roles in regulating T cell senescence and exhaustion in the context of HIV infection and persistence.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

■■ of outstanding interest


This review very well covers the the impact of gut microbiome on immunity and also been highlighted as:

Papers of particular interest, published within the annual period of review, have

■■ of outstanding interest


