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Gut Innate Immunity and HIV Pathogenesis

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Abstract

Purpose of Review: In the gastro-intestinal tract, the complex network of multiple innate cell populations play critical roles not only as a first line of defense against invading pathogens and in driving adaptive immune responses, but also in maintaining intestinal homeostasis. Here, we describe the roles of various innate immune cell populations in gut immunity and detail studies investigating the impact of acute and chronic HIV infection on these cell populations.

Recent Findings: Alterations in frequencies, phenotype and/or function of innate lymphoid cells, dendritic cells, macrophages, neutrophils and innate-like T cells have been reported in people with HIV (PWH), with many of these features persisting despite anti-retroviral therapy and virological suppression.

Summary: Dysregulated gut innate immunity in PWH is a feature of gut pathogenesis. A greater understanding of the mechanisms driving impairment in the multiple different gut innate immune cell populations and the downstream consequences of an altered innate immune response on host defense and gut homeostasis in PWH is needed to develop more effective HIV treatments and cure strategies.

Keywords

HIV; innate immunity; gut

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Introduction.

Innate immunity occurs rapidly and is considered the first line of defense against invading pathogens. Innate immune responses are typically mediated through cell-dependent mechanisms (e.g. phagocytosis, cytotoxicity) and/or via secreted factors (e.g cytokines, Type I Interferons [Type I IFNs]) [1]. In the gastro-intestinal (GI) tract, innate immune cells such as innate lymphoid cells (ILCs), myeloid and plasmacytoid dendritic cells (mDCs; pDCs) and macrophages reside in the intra-epithelial layer as well as deeper into the lamina propria where they play roles not only in providing protection against invading pathogens and in driving adaptive immunity, but in maintaining immunological tolerance [1,2]. Furthermore, the human gut contains unconventional T cells with innate like properties, including mucosal-associated invariant T (MAIT) cells, γδ T cells and invariant Natural Killer T (iNKT) cells. These unusual T cells typically recognize conserved microbial molecules (e.g. lipid antigens, bacterial metabolites) presented via MHC-like molecules, but in some situations they can also be activated independently of MHC molecules [3,4]. Fundamental to the role of innate immune cells in maintaining gut homeostasis is their close interaction with the gut microbiome, a collection of commensal microbiota representing bacteria, viruses, bacteriophages and fungi. The most well studied of these, the bacterial microbiome is essential for the development and function of innate and adaptive immunity [5]. These critical innate immune cell-microbiome interactions are mediated both directly (e.g. bacteria-specific ligands binding to their specific receptors expressed by multiple innate immune cells) and indirectly via production of proteins and metabolites such as short chain fatty acids that regulate immune cell function and metabolism. Alterations in the microbial community structure, as occurs in a number of gut-associated inflammatory diseases including HIV, may therefore have a profound effect on gut innate immunity.

Frequencies of gut innate and innate-like immune cells are typically low in healthy tissue making investigations into their contributions to HIV pathogenesis difficult. However, a number of groups have met this challenge, and their investigations into the impact of HIV infection on these innate immune cells are reviewed below.

Innate Lymphoid Cells.

ILCs populate mucosal barriers including the GI tract where they play critical roles in maintaining homeostasis and in defense against pathogens [6–10]. Group 1 ILCs, which include Natural Killer (NK) cells and the more recently discovered ILC1 subset, express the transcription factors Tbet and Eomes and are primarily anti-viral with cytolytic functions and production of IFNγ. Group 2 ILCs (ILC2s) express GATA3, produce the Type 2 cytokines IL-4, IL-5 and IL-13, and are involved in immunity against helminths. Group 3 ILCs (ILC3s) express RORγt, produce IL-22, GM-CSF, IL-17 and the lymphotoxin LT-α1β2, and in addition to limiting extracellular bacterial and fungal infections, ILC3s play a role in epithelial barrier maintenance and tissue repair. A number of studies have highlighted the plasticity of ILCs whereby different subsets modify their phenotype and cytokine profiles to reflect those of their counterparts (e.g. ILC3 to ILC1 profile) in response to tissue-dependent cues including cytokines and microbiota [11].

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Group 1 ILCs.

Mela and colleagues were first to show depletion of NK cells (identified as $CD3^{-}CD56^{+}$) in colonic lamina propria of untreated, viremic PWH versus uninfected controls [12]. Colonic intraepithelial and lamina propria NK cells, identified by NKp46 expression, were also reduced in frequency during untreated chronic HIV infection [13]. Interestingly, rectal NK cells ($CD56^{+}CD16^{+}$) from chronically infected, viremic PWH were lower in frequency, but more activated (based on CD69 expression) versus anti-retroviral therapy (ART)-naïve PWH with plasma viral loads <2000 HIV RNA copies/ml (HIV controllers) [14]. Providing further support for a role of viral replication in driving the depletion of gut NK cells , similar frequencies of rectal NK cells relative to uninfected controls were observed in HIV controllers (rectal NK cells identified as $CD56^{+}CD16^{+}$) [15], and in the colon (identified as $CD3^{-}CD56^{+}$) [12] as well as throughout the GI tract (identified as $CD56^{+}CD94^{+}NKp44^{-}$) [16], in ART-treated PWH with viral suppression. Of note, CD94 expression was recently shown to identify a population of blood memory-like $CD56^{hi}$ NK cells with greater cytolytic activity versus $CD94^{-}$ NK cells and frequencies of which were increased in ART-naïve PWH, but not impacted in either HIV controllers or in ART-treated PWH [17]. In a recent study, Utay and colleagues highlighted that the timing of ART initiation differentially impacted frequencies of rectal NK cell subsets, identified based on CD56 and CD16 expression [18]. For example, lower frequencies of $CD56^{bright}CD16^{-}$ NK cells were observed in PWH who initiated ART during either acute or chronic infection versus untreated, chronically infected PWH; however, ART initiation during the chronic phase was associated with higher frequencies of $CD56^{bright}CD16^{dim}$ NK cells versus frequencies observed when ART was started during the acute stage. Few studies have directly investigated the impact of HIV infection on gut ILC1s. In chronically-infected and ART-treated PWH, ileum and colonic ILC1s were depleted [16,19], and ILC1s were not restored with ART, a finding linked to impaired IL-7 responses [16].

Group 2 and 3 ILCs.

ILC2s constitute only a minor fraction of total ILCs throughout the human GI tract [16] and have not been studied extensively in PWH. In one study, similar frequencies of ILC2s were observed in ART-treated PWH versus uninfected controls in the small intestine (duodenum, ileum) and in the colon [16].

In 2009, Cella and colleagues described a population of human tonsil $CD3^{-}$ cells that co-expressed NKp44 and CD56 and were capable of producing IL-22 (termed NK22 cells) [20], a population of cells now considered to be ILC3s. Using this early definition of ILC3s, frequencies of colonic $CD3^{-}$ IL-22-producing cells were increased during acute HIV infection [21], reduced in frequency in chronic infection [22] and restored to levels similar to controls during long term ART [23]. We evaluated frequencies of $NKp44^{\pm}CD56^{\pm}$ colonic cells producing IFNγ or IL-22 and observed higher frequencies of colonic $NKp44^{+}CD56^{-}$ producing IFNγ in untreated, PWH versus controls [24]. In uninfected persons, this population of ILCs typically produced IL-22, suggesting a switch to an inflammatory phenotype in PWH. Frequencies of $NKp44^{+}CD56^{-}$ $IFN\gamma^{+}$ ILC associated with colonic mDC and T cell activation and with the relative abundance of mucosa-associated *Prevotella*, a commensal bacteria found at a significantly higher relative abundance in our cohort of

PWH [25]. Our observations suggested that a critical interplay between gut ILCs, other local immune cells, and dysbiotic enteric bacteria may exist in chronic, untreated HIV infection.

In a recent study, utilizing an updated scheme to identify ILC3s based on co-expression of CD117 and CD127 and exclusion of other cells that also expressed these makers ("lineage-") [26,27], Kloverpris *et al.* observed no difference in frequencies of lineage^{-}CD127^{+}CD117^{+} ILC3s in the colon of PWH versus uninfected controls [28]. However, ART status of the PWH was not detailed in that study. In other studies, ILC3s identified as Lin^{-}CD127^{+}Rorγt^{+} using flow cytometry or evaluated by histology as CD3^{-}CD117^{+} were decreased in ART-treated PWH [17]●. Investigations of Lin^{-}CD127^{+}CD117^{+} ILC3s across the GI tract of ART-treated PWH also noted a decrease in colonic ILC3s, but intriguingly, frequencies were increased in the duodenum [16]. These compartment-specific differences were extended into functional differences, such that IL-22 production by colonic ILC3s was significantly higher in ART-treated PWH. Conversely, no HIV-associated differences were observed in frequencies of IL-22^{+} duodenum or ileum ILC3s. In a recent study, Wang and colleagues postulated that the loss of protective gut-resident ILC3s and the associated inflammatory state in the setting of HIV infection may drive the expansion of circulating memory NK cells [17]. Given that memory NK cells may have a greater ability to control HIV, studies directly investigating the role of gut ILC3s in concert with gut memory NK cells are warranted.

Although as a whole, these studies suggest that HIV infection impacts gut ILC3 frequencies, future studies will need to more consistently adopt the current standard nomenclature for ILC3 identification [27] and investigate not only how HIV infection impacts frequencies, but also ILC3 function particularly in light of the their critical role in gut epithelial barrier maintenance and gut homeostasis.

Myeloid cells: mDCs and macrophages.

The mononuclear phagocyte system has historically been categorized into mDCs, monocytes, and macrophages based on phenotypical and functional characteristics; however, advancing technologies such as single cell RNAseq (scRNAseq) have highlighted that many of these phenotypic features are often overlapping [29,30]. Macrophages and mDCs are found throughout the intestinal tract where they serve as gatekeepers between the external (i.e. lumen) and internal (i.e. intra-epithelial and lamina propria) environments and mediate the delicate balance between immunogenic and tolerogenic intestinal immune responses [31–34]. In their role as potent antigen presenting cells, mDCs are instrumental in the induction of adaptive immunity against enteric microbes, including orchestrating high-affinity IgA antibodies against enteric pathogens, induction of regulatory T cells, and driving cytotoxic and T helper (Th) cell responses [33]. Our recent *in vitro* mechanistic studies demonstrated that human gut mDCs were required for commensal and pathogenic bacteria-induced IL-22 production by ILC3s [35], for IFNγ production by Group I ILCs [36] and in the commensal bacteria-induced expansion and enhanced HIV infection of lamina propria CD4 T cells [37]. These findings provide further evidence of the importance of mDC in human gut immunity via cross talk with other local innate and adaptive immune cells. Tissue macrophages consist of populations of both long-lived resident cells, primarily found in the deeper layers of the intestine, and populations of cells located close to the

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epithelial layer and in the lamina propria that are continuously replaced by circulating monocytes [31,32,34]. Of note, although classically known for their ability to phagocytose pathogens and dead cells, macrophages can also serve many similar functions to mDCs including to mediate interactions with resident enteric microbiota leading to production of cytokines, to promote regulatory T cells, or to promote IL-22 production by ILC3s.

Myeloid Dendritic Cells.

To date, few studies have investigated gut mDCs in the setting of HIV infection. In a recent study extensively interrogating the impact of untreated HIV infection on colonic mDC frequency and phenotype, we observed that, despite no significant differences in frequency, CD1c^{+} mDCs in PWH expressed an altered activation phenotype characterized by increased levels of CD40, but lower expression of CD83 [38]. Increased CD40 expression by mDC was associated with increased colonic and peripheral blood CD4 and CD8 T cell activation, constitutive production of colonic mucosa-associated inflammatory cytokines including IL-23, IL-6, TNFα and IFNγ, and with the relative abundances of dysbiotic enteric bacteria. In other studies, increased levels of indoleamine 2,3-dioxygenase 1 (IDO1) in rectosigmoid DEC205^{+} mDCs was linked to the inversion of Th17/T regulatory cell ratio in chronically infected PWH [39]. Taken together, these studies provided the initial evidence that gut mDCs may play a central role in driving gut inflammation in the setting of untreated HIV-1 infection. However, we believe it will be necessary to cultivate a greater appreciation of human gut mDC biology throughout the course of HIV disease. Given the recent observation that ART did not fully restore gene transcriptional changes in peripheral blood mDC that had occurred during untreated HIV infection [40], these future studies should include an evaluation of the function of gut mDC during viral suppression with ART to determine if a failure to restore gut mDC phenotype and function is a contributing factor to the persistence of gut dysfunction despite effective viral suppression with ART. These types of studies will be necessary to permit the design of novel treatments that target gut mDCs with the intent on reducing mDC-mediated inflammation (e.g. via anti-cytokine therapy, induction of tolerogenic mDC subsets).

Macrophages.

Frequencies of CD68^{+} or CD169^{+} macrophages were increased in duodenum of untreated PWH, normalized with ART, and related *in vitro* studies suggested that the accumulated macrophages in untreated PWH displayed impaired phagocytic ability [41]. In histological staining of colonic tissue of untreated PWH and uninfected controls, we observed higher levels of both lipoteichoic acid (LTA), a Gram-positive cell wall component, and lipopolysaccharide (LPS), a Gram-negative cell wall component in PWH [38]. Although more LTA^{+} HAM56^{+} macrophages were observed in PWH than in uninfected controls, no similar increase in frequencies of LPS^{+} macrophages was observed in that cohort, an observation in keeping with a potential defect in macrophage function and uptake of LPS and/or Gram-negative bacteria. Accumulation of colon CD14^{+} inflammatory macrophages were also noted in African AIDS patients presenting with diarrhea and/or weight loss, but without overt opportunistic infections, with frequencies positively associated with plasma LPS [42]. Thus, despite accumulation of gut macrophages during chronic HIV infection,

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their potential dysfunction may be a contributing factor to an apparent inability to limit microbial translocation.

Role of gut mDC and macrophages in cell-to-cell transmission of HIV-1.

HIV infection of target cells can occur with both cell-free viral particles and via cell-to-cell transfer of viral material [43]. In addition to membrane protrusions, cell fusion and cell engulfment processes, cell-associated HIV-1 transmission occurs through the establishment of 'infectious' or 'virological' synapses. Although a number of cellular factors have been implicated in HIV trans-infection of CD4 T cells [44], the role of CD169, also known as Siglec-1, has recently garnered attention. CD169 is induced by Type I IFNs, which consist of several cytokines that include the 12 IFNα subtypes and IFNβ [45] (discussed in more detail below). Early *in vitro* studies implicated CD169 expression by both blood-derived and tonsil mDCs and by blood-derived macrophages in mediating HIV-1 transfer to CD4 T cells [46–52]. Recent *in vitro* studies highlighted that upon virus capture, HIV-1 particles co-localize with CD169 within virus containing compartments (VCC), thus providing potential mechanisms of viral evasion [53,54] and suggesting that inhibiting CD169 uptake of HIV-1 may provide additional strategies to reduce HIV transmission and/or eradiate HIV reservoirs. *In vivo,* CD169 was required for dissemination of a murine retrovirus [55], while HIV p24 was detected within $CD169^{+}$ cervical cells of a viremic PWH [56] and $CD169^{+}$ $p27^{gag+}$ cells detected in lymph nodes of SHIV-infected macaques [46].

We recently reported that Type I IFN expression is compartmentalized during untreated, chronic HIV infection, with higher IFNβ, but lower IFNα gene expression observed in the colonic mucosa of untreated, viremic PWH versus uninfected controls [57]. Therefore, to probe potential contributions of IFNβ expression on CD169-mediated HIV trans-infection of gut CD4 T cells, we utilized an *in vitro* human intestinal cell model [58] to evaluate CD169 expression on gut mDC and macrophages in response to varying doses of exogenously added recombinant IFNβ (Fig. 1). Our initial studies suggest that IFNβ primarily drives CD169 expression on gut macrophages with minimal impact on gut mDC. Studies are underway to further investigate the underlying mechanisms governing how Type I IFNs impact CD169 expression and HIV trans-infection of gut CD4 T cells. Given that bacterial Toll-like receptor ligands such as LPS enhance CD169 expression on monocyte-derived DC [53], it will be important to determine if the threshold for IFNβ-induced CD169 by gut myeloid cells is altered in the presence of bacteria or bacterial ligands and to potentially link microbial translocation to HIV transmission and to shifting the role of Type I IFNs from protective to pathogenic [59].

Plasmacytoid Dendritic Cells.

Gut pDCs play a critical role both in anti-viral immunity and in immune regulation primarily due to being the primary producers of Type I IFNs [60,61]. Increased numbers of activated ($CD40^{+}$) colonic $CD303^{+}$ pDCs were identified in untreated PWH, and activated gut pDCs were associated with levels of colonic mDC activation, suggesting that drivers of gut DC activation may be similar for both subsets and/or that a form of cross talk occurs between these two DC subsets [38]. In duodenum of persons with HIV/AIDS, accumulating pDCs

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expressed Ki67 [62]. The authors postulated that, given that mature pDCs do not typically proliferate, the presence of Ki67^{+} CD303^{+} pDCs in colons of untreated PWH is due to the accumulation of naïve pDCs, likely of bone marrow origin. Of note, Boichuk and colleagues also noted that accumulated pDCs expressed the cytolytic molecule granzyme B and proposed that expression of this granzyme by pDC was an additional contributor to gut HIV pathogenesis [62]. In another study, higher frequencies of ileum CD303^{+}/CD123^{+} pDCs were found in untreated PWH, and pDC frequencies were associated with plasma levels of IFNα and with mucosal CD8 T cell activation. Further, pDC frequencies had not normalized after 6 months of ART [63]. In HIV-infected humanized mouse models, pDC and Type I IFNs were linked to ILC apoptosis and death and postulated as one mechanism driving depletion of gut ILC3s and ILC1s in chronic HIV infection [19,22].

Other gut immune cells with innate or innate-like properties.

Neutrophils.

Neutrophils are polymorphonuclear leukocytes whose primary function is to kill microbes via phagocytosis, degranulation and establishment of neutrophil extracellular traps (NETosis) [64,65]. As ‘first responders’, neutrophils are also important in the establishment of the ensuing immune response and both recruit and interact with multiple other immune cells including macrophages and DCs. Few studies have directly investigated gut neutrophils in the setting of HIV infection. Neutrophil infiltration, quantified by histological staining of the enzyme myeloperoxidase (MPO), was shown to be increased in the colons of both untreated and ART-treated acute and chronically infected PWH [17,66,67]. Hensley-McBain and colleagues expanded on this work and utilized multi-color flow cytometry to confirm that colonic neutrophils accumulated in ART-treated individuals [68]●. Further, they linked increased neutrophil frequencies to prolonged neutrophil survival, a finding potentially related to features of dysbiosis (i.e. reduced *Lactobacillus*:*Prevotella* ratio).

Mucosal-associated invariant T cells.

MAIT cells are recently identified unconventional innate-like T cells that express CD161 and a semi-invariant T cell receptor (TCR; Vα7.2) and recognize microbial vitamin B metabolites presented by the highly conserved MHC Class I-like molecule MR1 [69]. The majority of MAIT cells express CD8 [70]. Activated MAIT cells are capable of killing infected cells, of inhibiting microbial growth and of secreting cytokines such as IFNγ, TNFα, IL-17 and IL-22. MAIT cells can also be activated in an MHC/TCR-independent manner via IL-12 and IL-18 and may be important in anti-viral immunity. MAIT cells, measured as the proportion of CD161^{+} cells within the CD8 T cell population, were reduced in colonic biopsies from untreated, chronically infected PWH versus uninfected controls, with restoration of MAIT cells noted in ART-treated PWH [71]. Of note, histological enumeration of colonic MAIT cells (identified using MDR1 as a substitute for CD161) also demonstrated a reduction in the proportion of MAIT cells within total CD8 T cells of chronically infected PWH; however, evaluation of frequencies per area of tissue eliminated this difference, suggesting that reduced proportions were likely a result of an overall increase in CD8 T cell frequencies [72]. Similarly, no significant loss of rectal CD161^{+}Vα7.2^{+} MAIT cells within CD8 T cells was observed in rectal tissue of chronically-

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infected PWH [70] or within $CD3^+$ $CD4^-$ T cells [73]. Of note, the low frequencies of CD4-expressing MAIT cells noted in controls was further reduced in PWH [70].

γδ T cells.

γδ T cells, defined by their expression of a unique TCR composed of one γ-chain and one δ-chain, are capable of mounting rapid cytolytic and inflammatory cytokine-mediated immune responses typically following activation via MHC Class II-independent presentation of antigens (e.g. phosphoantigens) and/or signaling through activation receptors (e.g. NKG2d) [74]. Gut γδ T cells, located in the intra-epithelial layer and underlying lamina propria, have essential roles in monitoring epithelial barrier function, scanning for indicators of cellular stress and mediating host protection against bacteria and viral infections [75]. The impact of HIV infection on gut γδ T cell frequencies may vary by location within the gut, both throughout the GI tract and between the intra-epithelial and lamina propria layers. Early studies utilizing histological analysis to specifically identify duodenal γδ T cells located in the intra-epithelial layer demonstrated an expansion of this T cell subset in individuals with late-stage HIV-1 infection and found that frequencies remained elevated despite ART [76,77]. Subsequent studies utilized multi-color flow cytometry analyses of pinch biopsies and therefore quantified γδ T cells without the ability to differentiate between those located in the intra-epithelial or lamina propria layers. In one study, percentages of rectal γδ T cells, as a fraction of total $CD3^+$ T cells, were decreased in untreated, chronically infected PWH versus controls and in PWH who initiated ART treatment either early in HIV infection or later during the chronic phase [73]. In contrast, Poles and colleagues observed an overall increase of rectal γδ T cells (similarly enumerated as a percentage of $CD3^+$ T cells) in untreated, chronically-infected PWH, and they noted an expansion of γδ T cells expressing the Vδ1 chain, but a decreased fraction of the Vδ2 γδ T cell subset [78]. In ART-treated PWH with effective viral suppression, frequencies of rectal γδ T cells and associated Vδ1 and Vδ2 subsets appeared to partially normalize [78]. Frequencies of duodenal Vδ1 γδ T cells amongst $CD3^+$ T cells were significantly lower in both acutely-infected PWH (1 of 15 were receiving ART) and chronically-infected PWH (3 of 14 were receiving ART) versus healthy controls with no differences in Vδ2 frequencies between the cohorts [79]. Interestingly, the ability of Vδ2 γδ T cells to constitutively produce IFNγ was lost during chronic infection suggesting that these cells were functionally exhausted [79]. Similar frequencies of Vδ1 γδ T cells in colon, duodenum and ileum were found between HIV controllers and uninfected controls, further suggesting a role for HIV-1 replication in alterations in γδ T cell frequencies [80].

Invariant Natural Killer T cells.

Human iNKT cells are a rare population of T cells characterized by expression of semi-invariant TCR (Vα24-Jα18 preferentially paired with Vβ11) that recognizes glycolipids presented by MHC Class I-related glycoprotein CD1d [81]. iNKT cells play important roles in immunity against a wide range of microbes including bacteria, fungi, parasites and viruses. Human iNKT cell repertoire consists of $CD4^+$, $CD8^+$, and $CD4^-CD8^-$ cells, among which $CD4^+$ iNKT cells account for approximately 50% of total iNKT cells and predominantly produce IL-4 [82]. $CD4^-$ cells mainly produce Th1 cytokines and have cytolytic activity [82,83]. In one study, rectal iNKT cells represented <0.5% of total $CD3^+$

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T cells, and lower levels were observed in chronically infected PWH due to a preferential depletion of $CD4^+$ iNKT cells. Frequencies of $CD4^+$ iNKT cells were inversely associated with plasma viremia [84]. In contrast, preservation of rectal iNKT cells were observed in ART-treated PWH compared to healthy controls, with no differences in the proportion of iNKT cells expressing CD4 [85]; production of IL-4 and IL-10 by gut iNKT cells inversely associated with systemic indicators of microbial translocation [85]. Furthermore, abundance of *Bacteroides* positively associated with iNKT frequencies and production of IL-4. Thus, iNKT cells may play an important role in local immune activation and microbial translocation and their function is potentially influenced by the enteric microbiota [85].

Type I Interferons.

Type I IFNs are a diverse family of innate cytokines that, in humans, include 12 distinct IFNα subtypes and IFNβ, all of which signal through the Type I IFN receptor (IFNR) to induce hundreds of interferon-stimulated genes (ISGs) that drive a wide range of biological activities [86]. During acute HIV-1 infection, Type I IFNs are generally considered to play a protective anti-viral role; however, during chronic infection, Type I IFNs have been associated with features of HIV-1 pathogenesis including inflammation and immune activation [59]. We recently showed compartmentalization of the Type I IFN response during untreated, chronic HIV infection [57]. IFNα subtypes were upregulated in peripheral blood mononuclear cells (PBMC), but were downregulated in colonic tissue versus controls; IFNβ gene transcripts were undetectable in PBMC, but significantly higher in colonic tissue of PWH. Furthermore, expression of canonical Type I IFN-induced viral restriction factors was elevated in colonic tissue of PWH and was associated with colon and systemic features of HIV pathogenesis. In follow up studies, *in vitro* exposure of uninfected gut CD4 T cells to dominant IFNα subtypes or IFNβ identified sets of ISGs upregulated by all Type I IFNs tested (core ISGs) as well as genes specifically induced by IFNβ (βISGs) [87]. In untreated, chronically infected PWH, core ISG expression positively correlated with IFNβ rather than IFNα transcripts, suggesting that IFNβ drove these responses. Core ISG expression positively correlated with plasma LPS, and βISGs positively correlated with plasma IL-6 levels. These findings further strengthen the link between gut Type I IFN expression and indicators of HIV pathogenesis.[88]

Several studies support a potential role of the gut microbiome in regulating Type I IFN responses. Many ISGs were upregulated in gut CD4 T cells following *in vitro* exposure with *Prevotella stercorea* [89], and Type I IFN response genes (IFNB, IFNAR1 and Mx2) in gut tissue inversely correlated with the abundance of stool *Prevotella* in ART-treated PWH [90]. In ART-treated PWH, a multi-strain probiotic supplement (Visbiome) taken twice daily over a 6 month period increased gut transcript levels of certain IFNα subtypes [91], including subtypes previously shown to exhibit the greatest anti-viral activity in human gut immune cells *in vitro* [92].

Contribution of gut innate immunity to HIV cure strategies.

ART is highly effective at suppressing HIV viral loads in the periphery; however, it is unable to completely eradicate the virus due to the persistence of latent infection and HIV-1 viral

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reservoirs found throughout the body, including the GI tract with gut CD4 T cells reportedly representing up to 95% of all HIV-infected cells in ART-treated PWH [93–97].To date, therapeutic cure strategies involve either eliminating HIV (sterilizing cure) or providing control of HIV in the absence of ART (functional cure). The "shock and kill" approach targets latently-infected CD4 T cells by utilizing latency reversal treatments to reactivate HIV gene expression, making them susceptible to killing by cytotoxic CD8 T cell responses, a process that may be dependent on effective innate immunity [95]. Surprisingly, NK cells, pDCs and Type I IFNs, but not HIV-specific CD8 T cells were implicated in the decline in CD4 T cell-associated HIV-1 DNA following treatment with the histone deacetylase inhibitor panobinostat [98]. Blood pDC and multiple Type I IFNs inhibited establishment of *in vitro* latent infection in blood CD4 T cells; however once latency was established, IFNα demonstrated an ability to initiate HIV-1 transcription [99]. Consequently, the dysfunction that remains in gut innate immunity in ART-treated PWH may be a significant barrier to the elimination of gut HIV-1 reservoirs and development of fully-effective HIV-1 cure strategies.

In addition to harnessing the innate immune system to reduce the gut HIV-1 reservoir, HIV-1 cure strategies may also need to target innate cellular sources of the reservoir. Although debate remains, gut macrophages have been proposed as one of these potential sources [100]. Early *in vivo* studies demonstrated expression of intracellular p24 in duodenal $CD68^+$ and $CD64^+$ macrophages [101] and HIV DNA was detected in rectal $CD13^+$ myeloid cells [102]. However, Gag HIV-DNA was rarely detected in highly purified colonic myeloid cells [103]. With the recent identification of long-lived, self-renewing tissue resident gut macrophages, further studies are needed to dissect the role of this population of cells, versus myeloid cells potentially replenished by infected blood monocytes, as a potential HIV-1 reservoir. Furthermore, given that viral SIV DNA detected within small and large intestinal myeloid cells of untreated, SIV-infected macaques was associated with phagocytosis of SIV-infected T cells [104], it will be important to utilize technologies (e.g. RNAScope and single cell assays) that not only accurately define and isolate to high purity the various gut-associated myeloid subsets, but also identify bone-fide latently-infected cells, a task made more challenging in light of the low frequency of these cell populations [100].

Conclusions.

HIV-1 infection dramatically alters the gut innate immune system landscape impacting many, if not all, of the multiple innate cell populations that play vital roles in gut immunity and homeostasis. While some of these defects appear to normalize with ART and viral suppression, suggesting a role for viral replication in driving their dysfunction, many defects persist and likely contribute to the ongoing gut inflammation and epithelial barrier disruption associated with chronic immune activation in PWH. However, despite the dedication of numerous groups in overcoming the inherent difficulties in studying these typically rare immune cells in human gut tissue and understanding their contribution to HIV-associated gut pathogenesis, knowledge gaps remain. The nature of impairment needs to be expanded beyond evaluation of frequencies alone, and the impact of HIV-1 infection on innate immune function should be further explored. Moreover, although correlative analyses typically undertaken in clinical studies are informative, these should be followed up with mechanistic studies to provide possible avenues for the development of novel treatments that target

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specific innate immune cells with the ultimate goal of reducing chronic inflammation. Finally, a better understanding of the contribution of gut innate immune cells as a source of the HIV-1 reservoir as well as how their functional properties can be harnessed to provide more effective functional control of HIV are needed for the development of optimal HIV cure strategies.

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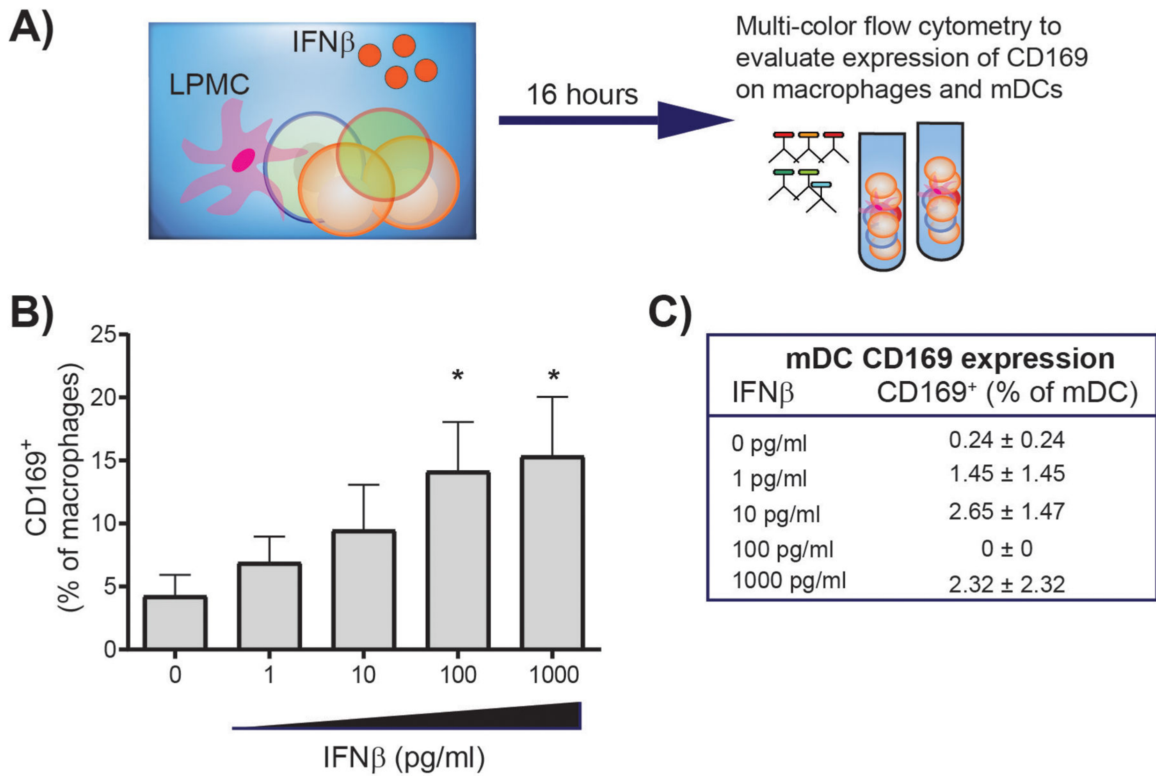

mDC CD169 expression

IFNβ	CD169+ (% of mDC)
0 pg/ml	0.24 ± 0.24
1 pg/ml	1.45 ± 1.45
10 pg/ml	2.65 ± 1.47
100 pg/ml	0 ± 0
1000 pg/ml	2.32 ± 2.32

Figure 1. *In vitro* exposure of gut macrophages to IFNβ increases CD169 expression.
Jejunum lamina propria mononuclear cells (LPMC) were cultured with increasing doses of IFNβ for 16–18hrs (N=5). Cells were collected and multi-color flow cytometry used to evaluate expression of CD169 in macrophages and mDC. Macrophages were identified as HLA-DR$^+$CD11c$^\pm$CD64$^+$ and mDC as HLA-DR$^+$CD11c$^+$CD64$^-$ within viable, CD45$^+$ CD3$^-$ CD19$^-$ cells [105,106]. A minimum of 25 events were required for analysis of CD169 expression therefore CD169 expression on mDC is in shown for 3 LPMC samples. Statistical analysis: Paired t test comparing IFNβ condition to no IFNβ; *$P<0.05$. LPMC were isolated from patients undergoing elective abdominal surgery and were designated discarded tissue from macroscopically normal sites. Samples from patients with a history of Inflammatory Bowel Disease, HIV-1 infection, treatment with immunosuppressive drugs, or recent chemotherapy (within 8 weeks) were excluded from the study. LPMC were isolated from tissue samples in a two step-procedure to remove epithelial cells followed by collagenase-digestion to release LPMC as previously described [37,58,107]. All patients undergoing surgery signed a release form to allow unrestricted use of discarded tissue for research purposes. Protected patient information was de-identified to the laboratory investigators. Research associated with the use of LPMC was reviewed by the Colorado Multiple Institutional Review Board (COMIRB) at UC-AMC and deemed Not Human Subject Research.