Differentiating Immune Cell Targets in Gut-Associated Lymphoid Tissue for HIV Cure

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Abstract

The single greatest challenge to an HIV cure is the persistence of latently infected cells containing inducible, replication-competent proviral genomes, which constitute only a small fraction of total or infected cells in the body. Although resting CD4⁺ T cells in the blood are a well-known source of viral rebound, more than 90% of the body's lymphocytes reside elsewhere. Many are in gut tissue, where HIV DNA levels per million CD4⁺ T cells are considerably higher than in the blood. Despite the significant contribution of gut tissue to viral replication and persistence, little is known about the cell types that support persistence of HIV in the gut; importantly, T cells in the gut have phenotypic, functional, and survival properties that are distinct from T cells in other tissues. The mechanisms by which latency is established and maintained will likely depend on the location and cytokine milieu surrounding the latently infected cells in each compartment. Therefore, successful HIV cure strategies require identification and characterization of the exact cell types that support viral persistence, particularly in the gut. In this review, we describe the seeding of the latent HIV reservoir in the gut mucosa; highlight the evidence for compartmentalization and depletion of T cells; summarize the immunologic consequences of HIV infection within the gut milieu; propose how the damaged gut environment may promote the latent HIV reservoir; and explore several immune cell targets in the gut and their place on the path toward HIV cure.

Keywords: HIV cure, reservoir, latency, gastrointestinal tract, mucosa, T cells

Introduction

COMBINATION ANTIRETROVIRAL THERAPY (ART) has transformed HIV/AIDS from a fatal disease to a chronic manageable illness in those who can maintain lifelong ART-mediated suppression of viremia. During the course of ART, the number of HIV DNA-harboring CD4⁺ T cells gradually declines in the blood over the first 1–4 years, and then plateaus without further decline in subsequent years,^{1–3} thereby maintaining a remarkably stable pool of infected cells, including latently-infected cells that can rapidly contribute to viral rebound if treatment is interrupted.

Viral persistence in HIV-infected individuals on ART has classically been attributed to latently infected resting CD4⁺ T cells,^{4–7} which harbor integrated, replication-competent HIV and do not produce virus constitutively, but can be induced by activation to produce infectious virus. The latent proviral DNA is neither recognized by the immune system nor affected

by ART. It is unclear what mechanisms govern HIV latency *in vivo*, and prior studies have implicated mechanisms such as inhibition of HIV transcriptional initiation, inhibition of transcriptional elongation, and post-transcriptional factors.^{8,9} Traditionally, it is believed that the latently infected cells transcribe little if any HIV RNA. However, cell-associated HIV RNA can be readily detected in blood and tissues of ART-suppressed patients. For example, one recent study using highly sensitive cell-associated HIV RNA and DNA single-genome sequencing revealed that some circulating CD4⁺ T cells from ART-suppressed patients express low levels (1–62 copies/cell) of HIV RNA.¹⁰ Further study is needed to determine if these findings from total CD4⁺ T cells are recapitulated in latently infected cells. However, latently-infected cells may show varying amounts of HIV transcription, including transcription of short, incomplete, or nonpolyadenylated HIV transcripts; newly developed digital droplet PCR assays that allow measurement of total

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and elongated HIV RNAs can also support these future studies.¹¹

The mechanisms by which latency is established and maintained will likely depend on the location and cytokine milieu surrounding the latently infected cells. Therefore, it is critical to identify and characterize the exact cell types that support viral persistence in all anatomic compartments, including the gut. A recent study identified that CD32a⁺ blood CD4 T cells isolated from ART-treated HIV-1⁺ participants are highly enriched for inducible replication-competent proviruses,¹² suggesting CD32a as a potential marker for detecting latently infected cells. Given that the study only reported blood cells, further studies will be required to demonstrate the prognostic significance of CD32a in mucosal and lymphoid tissues.¹³ Importantly, HIV initiates and sustains a vicious inflammatory cycle by damaging the gastrointestinal (GI) tract, which leads to systemic exposure to gut microbial products, increased activation of T cells, and higher viral replication.^{14–16} Despite the significant contribution of gut tissue to viral replication and persistence, little is known about the cell types that support persistence of HIV in the gut.

In this review, we describe how HIV infection leads to depletion of T cells in the GI tract and discuss what is known about the HIV reservoir in the gut compared to other anatomic compartments. We give special consideration to the location and frequency of various subsets of human T cells in various lymphoid and mucosal tissues, as these will prove essential in HIV cure endeavors.

Seeding the Latent HIV Reservoir in the Mucosa

Most of our understanding of how the latent HIV reservoir is established comes from nonhuman primate models of simian immunodeficiency virus (SIV) infection and *in vitro* studies that use human cell culture systems. Upon mucosal SIV infection in rhesus macaques (RM), the viral reservoir is seeded very rapidly.¹⁷ Evidence from *in vitro* studies¹⁸ as well as HIV-infected individuals,^{19,20} indicates that the latent reservoir is also established very early in HIV infection. In agreement with these findings, initiation of ART as early as 10 days after the onset of symptoms of primary HIV-1 infection does not prevent generation of latently infected cells¹⁹; however, the size of latent reservoir can be limited by early administration of ART.^{1,21,22}

Mathematical modeling also suggests that latency is established early and is hardwired into the HIV genome to enhance lentiviral transmission across the mucosa, especially when target cells are not abundant.²³ Although the gut is rich with target cells, other factors in the mucosal milieu may contribute to rapid seeding of latently infected cells. For example, to establish a productive infection, HIV inhibits type I interferon (IFN) expression in T cells and macrophages.²⁴ HIV blocks IFN production through protease sequestering of the cytoplasmic RNA sensor retinoic acid-inducible gene I (RIG-I).²⁵ IFN resistance confers a distinct advantage to the transmitted viruses, creating a bottleneck at the mucosa and favoring selection of viruses that can replicate and spread efficiently in the face of a potent innate immune response.²⁶ *In vitro* studies also support this model, as widespread defects in IFN-I responsiveness are observed within latently HIV-infected cell lines.²⁷ Thus, latency may be established early after transmission to avoid an IFN-mediated inflammatory

response, allowing the virus to surreptitiously traffic away from the mucosa and migrate into the lymphoid tissues, where IFN resistance promotes viral replication, while creating a target-rich environment in which the virus can spread.

Direct measurements of the latent reservoir in patients on ART using limiting dilution coculture (viral outgrowth) assays show variable, but extremely slow decay rates ($t_{1/2}$ of 6–44 months) in resting CD4⁺ T cells in blood.^{28–32} In addition, latently infected CD4⁺ T cells with memory phenotypes are long-lived and undergo homeostatic proliferation and clonal expansion,^{33,34} which may add to the prolonged persistence of HIV in these cells.^{35–37} Although residual viral replication may help replenish the latent reservoir in some patients,^{29,31} even without such replenishment, the half-life of the latent reservoir is sufficiently long that these cells will persist despite lifelong ART. Lower availability/penetration of drugs in lymphoid tissues^{38,39} and peripheral tissues, such as the gut and the central nervous system, may also contribute to possible residual replication in these anatomical sites.^{39–42} Low-level persistent production of HIV may, in turn, contribute to heightened immune activation, rendering cells more permissive to infection and helping replenish reservoirs of HIV-infected cells.³¹ Phenotypic identification of latently infected cells may greatly enhance innovative strategies to selectively target these cells in infected individuals,⁴³ which would be a major milestone toward HIV cure.

T-Cell Subsets: Phenotypes and Compartmentalization

Memory T cells develop over decades in response to exposure to diverse antigens. By the second decade of life, memory T cells constitute up to 35% of circulating T cells.⁴⁴ This pool of memory T cells reaches a plateau by the third decade of life and remains stable throughout adulthood.^{45,46} After antigen stimulation in lymphoid organs, naive T (T_N) cells differentiate into activated effector cells that migrate to nonlymphoid tissues, where they act to clear the infection. A fraction of these cells persists as long-lived memory T cells that can be divided into three main subpopulations: central memory (T_{CM}), effector memory (T_{EM}), and tissue-resident (T_{RM}) memory T cells (Table 1). T_{CM} cells circulate between lymphatic tissues and blood, whereas T_{EM} cells survey peripheral tissues and recirculate through the lymphoid system and the blood. A more recently identified subpopulation termed “transitional memory” T (T_{TM}) cells exhibits functional and transcriptional characteristics that are intermediate to T_{CM} and T_{EM}. These T_{TM} cells can be distinguished through their additional expression of CD27 receptor.^{47,48} The long-term circulating memory pool is maintained, in part, by memory stem cells (T_{SCM}) that comprise 2%–4% of circulating memory T cells.^{49,50} T_{SCM} exhibit a gene and cell surface molecule expression profile that place them phenotypically between T_N and T_{CM}.^{51,52} T_{SCM} serve as precursors of other memory cells,⁵³ but their cellular distribution in humans is poorly understood. In contrast to all these other memory T-cell subsets, T_{RM} cells reside in peripheral and mucosal tissues. They do not reenter circulation, but remain localized and poised for rapid effector function in sites not surveyed by T_N and T_{CM} cells.^{54,55}

Most prior studies investigating various memory T-cell subsets have focused on peripheral blood samples; however, more recent analyses of tissue biopsies and organ donors have

TABLE 1. SURFACE MARKERS EXPRESSED
BY CONVENTIONAL AND NONCONVENTIONAL
T-CELL SUBSETS

Cell type	Surface marker
CD4 T cell subsets	
Naïve	CD45RA ⁺ CD45RO ⁻ CD62L ⁺ CCR7 ⁺
T _{CM}	CD45RA ⁻ CD45RO ⁺ CCR7 ⁺ CD95 ⁺
T _{EM}	CD45RA ⁻ CD45RO ⁺ CCR7 ⁻ CD62L ⁻ CD127 ⁺
T _{EMRA}	CD45RA ⁺ CCR7 ⁻ CD62L ⁻ CD27 ⁻
T _{RM}	CD45RA ⁻ CD103 ^{+/+} CD69 ⁺
T _{TM}	CD45RA ⁻ CCR7 ⁻ CD27 ⁺
T _{SCM}	CD45RA ⁺ CD45RO ⁺ CCR7 ⁺ CD28 ⁺ CD95 ⁺ CD62L ⁺
T _{H17}	CCR4 ⁺ CCR6 ⁺ CD161 ⁺
T _{H22}	CCR4 ⁺ CCR6 ⁺ CCR10 ⁺
T _{FH}	CXCR5 ⁺ ICOS ⁺ PD-1 ^{+/+}
T _{reg}	CD45RA ⁺ CD45RO ⁻ CD25 ⁺ FOXP3 ⁺ CD127 ⁻
Nonconventional T cells	
γδ T cells	CD3 ⁺ TCRγδ ⁺
MAIT cells	CD45RA ⁻ CD45RO ⁺ CD8 ⁺ CD161 ⁺ CD44 high CD27 ⁺
ILCs	
EOMES ⁺ ILC1 ⁺	NKp46 ⁺ CD56 ⁺ CD16 ⁺ CD94 ⁺ KIR ⁺
EOMES ⁻ ILC1 ⁺	NKp46 ⁺ CD56 ⁺ CD161 ⁺ CD127 ^{+/+}
ILC2 ⁺	CRTH2 ⁺ IL-33R ⁺ CD161 ⁺ ICOS ⁺ CD127 ⁺ CD117 ^{+/+}
ILC3 ⁺	CD161 ⁺ CD127 ⁺ CD117 ⁺

γδ T cells, gamma-delta T cells; ICOS, inducible T-cell costimulator; ILCs, innate lymphoid cells; MAIT, mucosal-associated invariant T.

advanced our understanding of the location and frequency of various subsets of human T cells in various lymphoid and mucosal tissues.^{46,56} Approximately half of the CD4⁺ T cells found in adult human blood have a naive phenotype. The proportions of T_{CM} and T_{EM} are similar in blood and peripheral lymph nodes, but most of the memory T cells found in various gut tissues display a T_{EM} phenotype.^{46,57} T cells are highly diverse and compartmentalized among various tissue sites,^{46,58} and the effect of memory T-cell tissue compartmentalization should be considered in the context of identifying HIV reservoirs. Since the molecular mechanisms of HIV persistence could vary based on the phenotype of HIV-infected cells and the prevailing tissue microenvironment, it is important to understand the frequency of cell subsets that harbor HIV DNA/RNA in all tissue types.

In contrast to blood, where most HIV DNA is found in central and transitional memory CD4⁺ T cells,³⁶ most HIV DNA and RNA in the gut are found in effector memory CD4⁺ T cells.⁵⁹ Although the gut contains a significant fraction of the body's memory T cells, most gut T cells also display markers of activation, suggesting that the mechanisms that maintain HIV in these cells are very different from those that maintain HIV latency in blood, where latently infected cells are primarily resting memory CD4⁺ T cells.

T-Cell Subsets: The Gut Mucosa

The GI tract, composed of a mucus layer, epithelial layer, and underlying lamina propria, constitutes a critical barrier

that serves to regulate the uptake of nutrients, water, and electrolytes, while excluding and mediating appropriate responses to a plethora of potentially harmful substances and microbes.⁶⁰ The GI tract contains the largest population of T lymphocytes, plasma cells, and macrophages in the human body, harboring up to 85% of all lymphoid tissue and over 90% of all lymphocytes.^{60,61} While both the small and large intestine are tightly connected with draining lymph nodes through the gut-associated lymphoid tissue (GALT), the small intestine is lined with organized lymphoid structures (including Peyer's patches), whereas the colon contains less structured lymphoid aggregates.^{62,63}

Most intraepithelial lymphocytes (IELs) that reside in the epithelial layer are CD8⁺ T cells. However, CD4⁺ T cells are not entirely absent from the epithelial layer, and their numbers increase toward the distal parts of the GI tract.⁶³ While TCRγδ⁺ cells are more abundant in the epithelium than in other parts of the body, they still represent a minor fraction of IELs. Unlike IELs, most T cells in the lamina propria are CD4⁺ T cells, which are present at an approximate ratio of 2:1 compared to CD8⁺ T cells.⁵⁶ Several other populations of unconventional cells, such as invariant natural killer T cells, mucosal-associated invariant T (MAIT) cells, and innate lymphoid cells, also reside in the gut.^{63,64} While naive and T_{CM} CD4⁺ T cells constitute the largest populations in the blood, the gut contains primarily T_{EM} and T_{TM} CD4⁺ T cells.⁶⁵ The lamina propria harbors a diverse pool of CD4⁺ T-cell populations, including IFNγ⁺ (T_{H1}), interleukin (IL)-4⁺ (T_{H2}), IL-17⁺ (T_{H17}), IL-22⁺ (T_{H22}), and IFNγ⁺ and IL-17⁺ (T_{H1}/T_{H17}) subsets, as well as forkhead box P3 (FoxP3)-expressing regulatory T (T_{reg}) cells producing IL-10.

T_{H17} and T_{reg} frequencies increase from the jejunum toward the colon, while frequencies of CD4⁺ T_{H1} and T_{H2} cells remain similar through the GI tract.⁶³ Within the germinal centers (GC) lies a population of CD4⁺ CXCR5⁺ PD-1⁺ lymphocytes, termed T follicular helper cells (T_{FH}), which provide help to B cells during maturation, Ig class switching, and antibody hypermutation through secretion of IL-21 and other cytokines.⁶⁶ Thus, the gut harbors a diverse array of T lymphocytes with both common and distinct phenotypic characteristics relative to T cells in other parts of the body.

Depletion of Gut T Cells in Early HIV Infection

The dramatic depletion of CD4⁺ T cells is one of the defining hallmarks of HIV infection.⁶⁷ Studies in the pathogenic SIV model in RM suggest that during acute infection, the gut experiences a more rapid and severe depletion of CD4⁺ T cells than the peripheral blood,⁶⁸ as a result of direct infection⁶⁹ and indirectly due to apoptosis of infected and uninfected cells.⁷⁰ In acutely infected humans, a dramatic depletion of CD4⁺ T cells in the gut is observed within 4–6 weeks⁷¹ and continues through all stages of the disease.⁶⁷ In lymphoid tissues, >95% of cell death may occur by Caspase-1 induced pyroptosis of abortively infected bystander CD4⁺ T cells.⁷² In contrast, circulating CD4⁺ T cells are resistant to HIV-induced pyroptosis,⁷³ suggesting the existence of distinct mechanisms of CD4⁺ T-cell depletion in lymphoid versus circulating cells. Whether a similar pyroptotic mechanism of cell death is also responsible for rapid depletion of gut CD4⁺ T cells is yet unknown.

At the mucosal portals of entry, HIV rapidly contacts the epithelium, antigen-presenting cells, and mucosal lymphocytes

(Fig. 1). During the first hours of infection, HIV traverses the mucosal barrier into the lamina propria, where abundant CD4⁺ memory T cells reside. Several factors contribute to the increased vulnerability of gut mucosal CD4⁺ T cells and their dramatic depletion soon after HIV infection. First, the rectum is lined with dendritic cells (DCs) abundant in DC-specific ICAM-3 grabbing nonintegrin (DC-SIGN), and HIV is selectively engulfed by these DC-SIGN⁺ mononuclear cells, which are able to transfer the virus to T cells with a fourfold greater efficiency than DC-SIGN⁻ cells.⁷⁴

Secondly, compared to CD4⁺ T cells in the blood, a much higher percent of gut CD4⁺ T cells expresses markers of memory (CD45RO)⁷⁵ and the HIV coreceptor CCR5.^{76–80} Consequently, depletion of CD4⁺ T cells in the GI tract occurs predominantly in CCR5⁺CD4⁺ T cells,⁶⁷ with those exhibiting a memory phenotype being most readily infected^{81–84} and depleted,⁷⁰ while CD4⁺-naïve T cells are infected at a significantly lower frequency⁸² and are relatively spared.⁸⁴ *In vitro* data also indicate that intestinal epithelial cells may select and transfer CCR5-tropic HIV-1 by transcytosis across the tight epithelial cell monolayer,⁸⁵ which might contribute to the predominance of R5-tropic HIV during acute primary infection.^{86,87}

Third, much of the expansive surface area of the GI mucosa endures constant exposure to microbial antigens, resulting in a constant state of immunologic activation, which is characterized by high levels of HIV-1 stimulatory chemokines and proinflammatory mediators.⁸⁸

Fourth, expression of the surface receptor $\alpha 4\beta 7$ integrin, which mediates lymphocyte homing to mucosal sites, can also enhance binding to HIV Envelope protein on viral particles, leading to an increase in CD4⁺ T-cell susceptibility to infection.⁸⁹ In par with these observations, a recent study in SIV-infected monkeys found that neutralizing antibody (Ab) to $\alpha 4\beta 7$ significantly lowered detectable viral load in plasma.⁹⁰ Further evidence suggested that integrin $\alpha 4\beta 7$ can be incorporated into circulating virions, enabling the virion- $\alpha 4\beta 7$ complex to bind to mucosal addressin cell adhesion molecule-1 (MAdCAM-1)-expressing cells to enhance infection,⁹¹ which could be a potential mechanism of anti- $\alpha 4\beta 7$ Ab-mediated reduction in plasma viral load in infected monkeys.⁹⁰ The effect of the $\alpha 4\beta 7$ neutralizing Ab on gut as well as other mucosal or lymphoid tissue infection is yet unknown.

Finally, recent *in vitro* evidence suggests that HIV-mediated changes in the gut microbiome could result in lipopolysaccharide-induced upregulation of CCR5, which in turn could increase T lymphocyte susceptibility to HIV and serve to increase CCR5-tropic HIV-1 production.⁹² Together, these factors contribute to the increased susceptibility of CD4⁺ T cells to initial and ongoing infection, permitting higher levels of infection and viral replication in the gut.

Disruption of the Gut Barrier and Immunologic Sequelae During Chronic HIV Infection

HIV infection results in the marked disruption of the three major components of the GI tract: the microbial barrier, composed of commensal intestinal flora; the immunologic barrier, comprising intramucosal lymphocytes, mesenteric lymph nodes, and secreted factors responsible for mediating the host immune response; and the mechanical barrier formed by epithelial and endothelial cells connected by tight junctions.⁹³ Intestinal barrier dysfunction and subsequent immune activa-

tion coincide with HIV-mediated depletion of CD4⁺ T cells and result from the destruction of tight junctions between the single layer of epithelium that lines the vast mucosal surfaces of the GI tract, leading to enhanced microbial permeability (Fig. 1).^{16,94} The compromised structural integrity of the mucosal barrier facilitates translocation of microbial products, which significantly contribute to immune activation during chronic infection^{16,95} and may also result in delayed reconstitution of CD4⁺ T cells.⁹⁶ Microbial translocation can be further exacerbated by intestinal microbiota that degrades tryptophan through the kynurenine pathway, contributing to the dysfunction of gut mucosal CD4⁺ T_H17 and T_H22 cells.^{97,98}

Systemic immune activation arising from gut barrier dysfunction has wide-ranging, mostly deleterious consequences. Chronic immune activation is multifaceted, driven by polyclonal B-cell activation,⁹⁹ increased T-cell activation¹⁰⁰ and turnover,¹⁰¹ and dysregulation of cytokine production.¹⁰² A high turnover of CD4⁺ and CD8⁺ T cells disrupts T-cell homeostasis and contributes to lower overall half-lives of these cells.^{103,104} This turnover perpetuates HIV infection by generating more viral targets through the production of CD4⁺ T_{EM} cells from T_{CM} cells,⁸⁴ ultimately resulting in depletion and exhaustion of memory T-cell pools. Sustained immune activation may also contribute to decreased thymic function over time.^{105,106} In addition, collagen deposition and the ensuing fibrosis of lymphatic tissues create an unfavorable microenvironment for T_N cell survival and hinders immune reconstitution with ART.^{67,107,108} Moreover, decreases in digestion and absorption result from aberrant expression of genes regulating lymphocyte activation, inflammation, lipid and carbohydrate metabolism, active transport, and epithelial barrier maintenance.¹⁰⁹

The profound HIV-mediated damage to the GI tract may also contribute to a multitude of vascular, liver, renal, cardiac, and pulmonary comorbidities that are not remedied by ART.^{110,111} Even after years on ART, the absolute number of CD4⁺ T cells in the gut tissues of HIV-infected individuals is not restored to the levels observed in noninfected individuals,^{67,71,112,113} and gut CD4⁺ T-cell reconstitution lags behind that in the blood.^{71,114}

The Damaged Gut Environment Promotes Latent HIV Reservoir

Multiple studies in both untreated and ART-treated patients report that HIV levels are higher in the gut versus the blood. In untreated patients, levels of HIV p24 antigen are much higher in intestinal biopsies than in blood.¹¹⁵ Although CD4⁺ T cells constitute a much smaller percent of the total mononuclear cells in the gut, compared to peripheral blood, gut HIV DNA levels per million total cells are comparable to¹¹⁵ or greater than those in the blood¹¹⁶ of untreated patients, while HIV DNA and RNA per million CD4⁺ T cells are 13- and 10-fold higher (respectively) in the colon compared to the blood of untreated patients¹¹⁷ (Table 2).

HIV RNA, HIV DNA, and infectious virus have been detected in the gut of ART-suppressed patients, and some cross-sectional studies found little difference between ART-treated and ART-untreated patients in HIV detection frequencies or nucleic acid levels.^{118–120} A prospective study found that initiation of ART during early infection caused similar reductions in HIV RNA in blood and rectum at 6

FIG. 1. Establishment and maintenance of HIV reservoir in the gut. The healthy GI tract is composed of a mucus layer, epithelial layer, and underlying lamina propria that constitute a critical barrier to potentially harmful microbes. The GI tract contains the largest population of T lymphocytes, plasma cells, and macrophages in the human body. While naïve and T_{CM} CD4⁺ T cells constitute the largest populations in the blood, the gut contains primarily T_{EM} and T_{RM} CD4⁺ T cells and a diverse pool of other CD4⁺ T-cell populations, including T_{H17}, T_{H22}, T_{HH}, and T_{REG} cells. At the mucosal portals of entry, HIV crosses the epithelial barrier into the lamina propria, where abundant CCR5⁺CD4⁺ memory T cells reside. The rectum is lined with DCs positive for DC-SIGN (CD209), and HIV is selectively engulfed by these DC-SIGN⁺ mononuclear cells that can transfer virus to T cells. Consequently, depletion of CD4⁺ T cells in the GI tract occurs predominantly in CCR5⁺CD4⁺ T cells. The intestinal barrier dysfunction and subsequent immune activation coincide with depletion of CD4⁺ T cells. This ensues from the destruction of tight junctions between the single layer of epithelium that lines the vast mucosal surfaces of the GI tract, resulting in enhanced microbial permeability. The compromised structural integrity of the mucosal barrier facilitates the translocation of microbial products that may contribute to immune activation during chronic infection and may also result in delayed reconstitution of CD4⁺ T cells. The dramatic depletion of CD4⁺ T cells is one of the defining hallmarks of HIV infection and continues through all stages of the disease. During the course of ART and rapid reduction of HIV viremia, the number of HIV DNA-harboring CD4⁺ T cells gradually declines over the first 1–4 years, and then plateaus without further change, thereby maintaining a stable pool of latently infected cells. CD4⁺ T_{EM} cells have been shown to harbor the major proportion of HIV DNA in the gut. Other lymphocyte populations such as T_{H17}, T_{HH}, and γδ T cells are among the other potential cellular reservoirs of HIV. Although ART dramatically improves life expectancy, it does not completely restore the immune system or intestinal integrity, and, thus, health in infected individuals. ART, antiretroviral therapy; DCs, dendritic cells; DC-SIGN, DC-specific ICAM-3 grabbing nonintegrin; γδ T cells, gamma-delta T cells; GI, gastrointestinal.

TABLE 2. HIV LEVELS IN THE GUT VERSUS IN THE BLOOD

<i>Finding</i>	<i>Treatment status</i>	<i>Reference</i>
Levels of HIV p24 antigen are much higher in intestinal biopsies than in blood	Untreated	115
Gut HIV DNA levels per million total cells are comparable or greater than those in the blood	Untreated	115,116
HIV DNA and RNA per million CD4 ⁺ T cells are 13- and 10-fold higher (respectively) in the colon compared to the blood	Untreated	117
HIV DNA levels per million CD4 ⁺ T cells are higher in the gut than blood	ART	122,123
Levels of HIV DNA and unspliced HIV RNA per million CD4 ⁺ T cells are higher in four different gut sites than in blood; gut contains 1.2×10^9 infected CD4 ⁺ T cells, or up to 95% of all HIV-infected cells in the body; median HIV RNA/DNA ratio lower in the gut (except ileum) than that in the blood, suggesting that gut sites other than ileum have more “latently” infected cells	ART	124
Gut contains twice as many HIV DNA ⁺ cells as blood	ART	125

ART, antiretroviral therapy.

months, although the rectum still showed less reduction in HIV DNA.¹²¹ In ART-suppressed patients, HIV DNA levels per million CD4⁺ T cells were on average five to six times higher in the gut than blood,^{122,123} and levels of HIV DNA and unspliced HIV RNA per million CD4⁺ T cells were higher in four different gut sites than in blood.¹²⁴ One study calculated that the gut contains twice as many HIV DNA⁺ cells as blood,¹²⁵ while another estimated that the gut contains 1.2×10^9 infected CD4⁺ T cells, or up to 95% of all HIV-infected cells in the body¹²⁴ (Table 2). Based on the responses to ART intensification with one to two new antivirals, a follow-up study suggested that the ileum, but not other gut sites or blood, might be a site of ongoing replication in some patients on ART.⁴⁰ In SIV-infected, ART-suppressed monkeys, SIV envelope protein was detected in the small bowel and colon, as well as other tissues, using a whole-body antibody-targeted positron emission tomography (ImmunoPET) with ⁶⁴Cu-labeled SIV Gp120-specific antibody.¹²⁶ When multiple tissues were analyzed in ART-suppressed SIV-infected macaques, the gut had the highest levels of multiply spliced SIV RNA and the highest ratio of multiply spliced to unspliced SIV RNA.¹²⁷

Despite these differences between gut and blood, it is unclear to what degree viral variants or infected cells traffic between the gut and blood. Some studies have reported HIV sequence compartmentalization between gut and blood. In ART-treated patients, phylogenetic trees and resistance profiles of HIV-1 protease and reverse transcriptase showed differences in HIV strains between blood and rectal mucosa.¹²⁸ In chronically infected subjects not on therapy, primary *nef* quasispecies from paired plasma and sigmoid colon biopsies also showed compartmentalization.¹²⁹ In addition, phylogenetic analysis of the *Nef* protein-encoding region in untreated patients also revealed compartmentalization of viral replication in various parts of the gut.¹³⁰ However, phylogenetic analysis of HIV *env* DNA has also suggested cross-infection among resting and activated CD4⁺ T cells from blood and gut in patients on ART,^{122,131} although variants in the rectum were more heterogeneous than variants in the blood from untreated patients.¹³¹ Furthermore, when phylogenetic analysis was performed on colon, ileum, and blood samples from chronically infected individuals, no evidence of compartmentalization was found.¹³² These studies suggest that, while compartmentalization may be difficult to detect during

untreated infection, long-term ART can influence HIV sequence compartmentalization in various tissues and regions of gut, but more studies are needed to understand how exactly this occurs.

In addition to tissue-specific differences in the nature of HIV-infected cell types, the mechanisms of transcriptional control may differ between gut and blood. For example, the median ratio of HIV RNA to HIV DNA (average level of transcription per provirus) in the ileum was higher compared with the blood, suggesting that this gut site has a greater ratio of productive to latent infection. However, at all other gut sites, the median HIV RNA/DNA ratio was actually lower than that in the blood, suggesting that gut sites other than ileum have more “latently” infected cells.¹²⁴ Since most gut lymphocytes display markers of T-cell activation, “latent” infection of these cells may differ from the classic latent infection in the blood, which was originally described in resting CD4⁺ T cells. Indeed, since T-cell activation typically drives HIV transcription, it is surprising that gut cells have such low levels of HIV transcription, suggesting that they are hyporesponsive to activating stimuli, or that activation markers have different meanings in the gut and blood. Furthermore, while T-cell activation correlated positively with HIV DNA levels in the blood, it correlated negatively with HIV DNA in the gut.¹²⁴ The paradoxically low levels of transcription in the gut and the opposite correlations with T-cell activation in the gut and the blood suggest that activation has different consequences, or that HIV transcription is governed by different mechanisms, in the gut and blood.

It is unclear what mechanisms suppress HIV transcription in the immunologically active environment of the gut. Since most gut CD4⁺ T cells display markers of activation, it is difficult to invoke a lack of HIV transcriptional initiation due to paucity of host cell initiation factors associated with the resting state. Despite displaying a more active phenotype, likely due to exposure to gut microbial antigens, gut CD4⁺ T cells may be in a hyporesponsive or anergic state, perhaps as a means to maintain tolerance to normal flora. Future studies should investigate whether this tolerance or anergy could be mediated by epigenetic modification, negative T-cell regulators (PD-1 and CTLA-4), T_{reg} cells, or other mechanisms, and how gut CD4⁺ T cells may differ from peripheral CD4⁺ T cells in expression of cellular genes that influence HIV transcription and latency.

Immune Cell Targets in the Gut and the Path Toward HIV Cure

The gut differs from peripheral blood in the types and frequencies of HIV-infected T-cell subsets. For example, while T_{CM} and T_{TM} cells contain the largest proportion of HIV DNA in the blood,³⁶ in both ileum and rectum, effector memory $CD4^+$ T cells harbor the largest proportion of HIV DNA and RNA, and HIV DNA was also detected in leukocytes other than $CD4^+$ T cells.⁵⁹ In the following sections, we explore several major immune cell targets that could contribute to HIV persistence in the gut, and we discuss their fate and key roles in HIV pathogenesis.

Macrophages/monocytes

Myeloid cells are the first line of defense in the gut, as they present antigens and support tissue repair. The expression of CCR5 varies greatly among gut macrophages, correlating with the position within the GI tract. Macrophages within the rectum, which are more likely to be in contact with HIV during rectal viral transmission, express much higher levels of CCR5 than those in proximal regions of the GI tract.¹³³ Nonetheless, there is no consensus as to whether macrophages might serve as real reservoirs of latent, replication-competent HIV.¹³⁴ Most of the controversy stems from the fact that macrophages act as phagocytic scavengers and although HIV DNA, HIV RNA, and protein can be detected in macrophages, it might come from phagocytosis of infected $CD4^+$ T cells,^{135,136} as was documented for SIV.¹³⁷ Evidence exists both for^{138,139} and against^{79,140,141} infection of gut macrophages in ART-suppressed patients. While $CD4^+$ T cells harbor most HIV DNA and RNA in the gut, myeloid cells account for 4% of the total HIV DNA.^{142,143}

Among myeloid cells, monocytes contain HIV variants that are different than those in $CD4^+$ T cells and are genetically closely associated with variants present in long-term recipients of ART.¹⁴⁴ In line with these findings, transmitted/founder viruses have minimal tropism for macrophages, suggesting that macrophage infection occurs at a later stage after viral transmission.¹⁴⁵ However, macrophages can sustain HIV replication in the absence of T cells in humanized myeloid-only mice,¹⁴⁶ and HIV can persist in tissue macrophages even after ART treatment.¹⁴⁷ Therefore, additional research is needed to unravel the contribution of tissue-specific macrophages to the total HIV reservoir in individuals on ART.

Regulatory T cells (T_{reg})

The impact of T_{reg} cells on HIV infection and their contribution to the HIV reservoir *in vivo* are subjects of debate, and evidence suggests that T_{reg} cells may play a dual role in HIV pathogenesis.¹⁴⁸ Some studies support the beneficial impact exerted by T_{reg} cells in limiting autoimmunity, HIV replication, and $CD4^+$ T-cell depletion,^{149–152} while others argue that they contribute to disease progression and dampened HIV-specific immune responses.^{153–156} While acutely SIV-infected macaques experience a rapid depletion of T_{reg} cells in gut lymphoid tissue,¹⁵⁷ and $IL17^+$ T_{regs} derived from naive T_{reg} are selectively reduced in virologically suppressed individuals,¹⁵⁸ numerous studies concur that elevated T_{reg} frequencies are observed in both peripheral blood and the gut in ART-treated and ART-untreated HIV-infected

individuals,¹⁴⁸ particularly relative to T_H17 and T_H22 cells. Increased T_{reg} frequencies may be related to the general inflammation and tissue damage in the gut and peripheral tissues of both ART-naïve and ART-treated patients.

T_{reg} cells are susceptible to HIV infection both *in vitro*¹⁵⁹ and *in vivo*.^{157,160} Circulating T_{reg} cells express CCR5 levels similar to bulk $CD4^+$ T cells.¹⁶¹ They appear to be more susceptible to R5-tropic than X4-tropic HIV-1 infection *in vitro*, but are not preferentially infected by HIV, compared with effector T cells *in vivo*.¹⁵⁹ The burden of HIV DNA harbored by T_{reg} cells is disputed since some studies reported comparatively higher HIV DNA in T_{reg} relative to non- T_{reg} $CD4^+$ cells,^{162–164} while others observed lower levels.¹⁶⁵ Whether T_{reg} cells constitute a reservoir of HIV that harbors inducible and replication-competent virus in the gut is unknown, and more direct evidence is required.

IL-17-producing helper T cells (T_H17)

T_H17 cells are characterized by the production of IL-17A, IL-17F, and IL-22.¹⁶⁶ T_H17 can alternatively be distinguished phenotypically by their expression of CCR6,^{167,168} which regulates cell migration into various anatomic sites, including the intestinal mucosa.^{169,170} T_H17 cells play a critical role in defense and homeostasis, acting to safeguard mucosal integrity by inducing the proliferation of enterocytes, producing antibacterial defensins, and recruiting neutrophils in response to fungal or bacterial infection.¹⁶⁶

T_H17 cells harbor high levels of HIV DNA *in vivo* in ART-naïve HIV-infected individuals.¹⁷¹ In particular, peripheral CCR6 $^+$ T_H17 , along with T_H1 -/ T_H17 -polarized cells, contribute disproportionately to the pool of $CD4^+$ T cells that harbor HIV DNA, and CCR6 potentially acts as a marker for blood and gut $CD4^+$ T cells that are enriched for HIV DNA.^{172,173} Furthermore, the reported enrichment of HIV DNA and a lower ratio of cell-associated unspliced HIV RNA to DNA ratio in CXCR3 $^+$ CCR6 $^+$ cells, including T_H17 cells, are consistent with transcriptional silencing.¹⁷³ Collectively, these data allude to the potential of T_H17 to harbor latent HIV.

T_H17 cells may also undergo self-renewal or homeostatic proliferation and retain a stem cell-like molecular signature,^{174,175} a combination of attributes that could make T_H17 cells a long-lived, persistent reservoir *in vivo*.^{172,173,176,177} However, these studies did not directly assess replication competence of virus harbored by T_H17 cells. Few studies have examined the contribution of T_H17 cells to the HIV reservoir in the gut. The sigmoid HIV reservoir remains high despite long-term suppressive therapy, and HIV DNA levels in the sigmoid correlate negatively with sigmoid T_H17 frequency,¹⁷⁸ in line with previous work demonstrating a similar observation in peripheral blood.¹⁷⁹ More direct evidence is required to determine if gut T_H17 cells harbor significant levels of latent HIV, but current data suggest that T_H17 cells are an important HIV reservoir in the gut.

IFN- γ - and IL-17-producing helper T cells (T_H1/T_H17)

$CD4^+$ T cells producing IL-17 and IFN- γ , termed T_H1/T_H17 , are particularly susceptible to HIV infection¹⁷¹ and appear to exhibit lower levels of antiviral factors and a higher state of cellular activation,¹⁸⁰ which may contribute to their increased permissiveness to HIV infection. Despite the reduced frequency of T_H1/T_H17 cells in ART-treated HIV-infected

individuals relative to uninfected individuals, T_H1/T_H17 and T_H17 cells contribute significantly to the pool of integrated HIV DNA in peripheral blood.¹⁷² These data are in line with previous work indicating that integrated HIV persists in T_H1/T_H17 , defined by a $CXCR3^+CCR6^+$ phenotype,^{173,176} and support the *in vitro* model of latency in which the $CCR6-CCL20$ axis plays a critical role in latency establishment.¹⁸¹ Notably, the frequency of $CCR6^+$ memory T cells is higher in the colon than peripheral blood in ART-treated individuals.¹⁷² Although the inducibility and replication competence of integrated HIV DNA recovered from T_H1/T_H17 remain to be investigated, this subset likely constitutes an important reservoir for latent HIV in the gut.

IL-22-producing helper T cells (T_H22)

$CD4^+ T_H22$ cells are distinguished by their production of IL-22 and their expression of the chemokine receptors $CCR6$, $CCR4$, and $CCR10$, and the gut microbial sensor aryl hydrocarbon, which is a ligand-activated transcription factor.¹⁸² In concert with T_H17 , T_H22 promotes epithelial barrier integrity and mucosal healing through epithelial proliferation and increased production of mucus.¹⁸³ In healthy HIV-uninfected individuals, as well as in SIV-uninfected RM, the levels of T_H22 cells in the gut are comparatively lower than T_H17 cells, with the majority of IL-22-producing cells also producing IL-17.^{184,185} The imbalance of T_H22 and T_{reg} cells triggered by HIV infection in the peripheral blood potentially contributes to subsequent loss of mucosal immunity and thus systemic immune activation,⁹⁸ since the ablation of T_H22 cells is only partially rescued with suppressive ART.^{98,186} Administration of IL-21 in combination with ART has been shown to improve T_H22 reconstitution, reduce immune activation, and reduce the frequency of $CD4^+$ T cells harboring replication-competent virus in lymph nodes from SIV-infected RM.¹⁸⁷ ART treatment of SIV-infected RM also correlates with increased T_H22 cell function and decreased SIV DNA content.¹⁸⁶ However, the extent to which T_H22 cells contribute to the pool of $CD4^+$ T cells harboring replication-competent virus is undetermined. Although T_H22 cells account for a relatively small proportion of $CD4^+$ T cells in the gut and no studies have examined the levels of replication-competent HIV that they may harbor, the integral role of T_H22 cells in maintaining epithelial barrier integrity in the gut suggests that future studies should investigate the contribution of these cells to the gut HIV reservoir.

Follicular helper T cells (T_{FH})

Within GC located in lymphoid follicles of secondary lymphoid organs, critical interactions between resident B cells and T_{FH} cells promote high-affinity T-dependent antibody responses, somatic hypermutation, and antibody class switching.^{188,189} GC T_{FH} are primarily distinguished by their high expression of $Bcl-6$, a transcriptional regulator,^{190,191} and expression of $CXCR5$, a follicular homing receptor,^{192,193} as well as $CXCR4$, $CD95$, $CD154$, $BTLA$, inducible T-cell costimulator (ICOS), $CD40L$, and $PD-1$, which are neither exclusive to T_{FH} ^{188,189,194} nor uniformly expressed on all T_{FH} cells.^{192,193,195} Human T_{FH} cells express an RNA expression profile distinct from T_H1 , T_H2 , and T_H17 cells,^{190,196} and this profile can be markedly altered by HIV/SIV infection.^{197–199} This $CD4^+$ T-cell subset serves as a major reservoir for HIV.

T_{FH} cells are highly permissive to HIV and SIV^{195,197,199–202} and expand in lymphoid tissue during acute and chronic infection,^{199,203–205} although suppression of viremia by ART can attenuate T_{FH} cell expansion.^{200,203} Concomitant with this expansion is an enrichment of HIV-/SIV-infected T_{FH} cells in the lymph nodes.^{200,204,206} In contrast, SIV-infected RM experience an early and profound loss of splenic T_{FH} cells,²⁰⁷ suggesting that HIV/SIV infection has diverging effects on T_{FH} dynamics in various tissues.

T_{FH} cells support high levels of HIV infection, replication, and virus production *in vitro*²⁰⁰ and in ART-naïve patients, T_{FH} cells exhibit the highest levels of HIV DNA in lymph nodes.²⁰⁰ A newly identified population of mucosal T_{FH} cells isolated from the jejunum of RM harbors 10-fold higher levels of SIV gag DNA per cell than bulk $CD4^+$ memory cells,²⁰⁸ underscoring the importance of T_{FH} cells in the gut during HIV/SIV infection. It is unclear whether anatomic barriers facilitate HIV persistence in GC T_{FH} cells. Since the majority of $CD8^+$ cytotoxic T cells lack the chemokine receptors required for migration into B-cell follicles in lymphoid tissue,²⁰⁶ these follicles may serve as a site of “immune privilege” in which HIV-infected T_{FH} cells are not recognized and killed. However, the exclusion of $CD8^+$ T cells from follicles may not be absolute, as several reports have described populations of $CD8^+$ T cells capable of infiltrating B-cell follicles in rhesus lymph nodes²⁰⁹ and eradicating virus-infected T_{FH} cells in a mouse model of LCMV infection,^{210,211} supporting an earlier discovery of $CCR5^+CCR7^-$ cytotoxic T cells in B-cell follicles in human tonsillar tissue.²¹² The difficulty in identifying these populations may be ascribed to phenotypic and transcriptional differences that distinguish them from other $CD8^+$ T cells.²¹¹ Nonetheless, the importance of cytotoxic T cells capable of infiltrating B-cell follicles is underscored by recent work demonstrating that engineered $CD8^+$ T cells expressing human $CXCR5$ were shown to localize to B-cell follicles *in vitro*,²¹³ alluding to the possibility of a new pathway to suppress persistent viral replication in T_{FH} within these tissue sanctuaries.

In HIV-infected individuals on suppressive ART, lymph node $PD-1^+$ T_{FH} cells constitute the major cell type that can be induced to produce replication-competent virus,²⁰⁰ although the contribution of these cells to the infectious reservoir progressively decreases with time on ART.²¹⁴ $PD-1$, a marker for T-cell exhaustion and homeostatic or antigen-induced proliferation, acts as a putative marker for memory in GC T_{FH} .⁶⁶ $PD-1$ is one of three immune checkpoint molecules recently implicated in HIV persistence during ART,²¹⁵ and its expression can result in T_{FH} dysfunction after interaction with $PD-L1$ expressed on GC B cells.²¹⁶ Furthermore, a strong association exists between $PD-1$ and the immune activation marker $Ki-67$ in circulating $CD4^+$ T cells from ART-treated individuals,^{36,217} suggesting that homeostatic proliferation is a major mechanism that maintains the HIV reservoir in these cells. However, there is little agreement on expression levels of $Ki-67$ in T_{FH} cells from HIV-infected individuals,^{200,202} so it is unclear to what extent homeostatic proliferation contributes to HIV persistence in T_{FH} cells.

Despite perturbation of T_{FH} cell counts in tissues, such as the lymph nodes and spleen, during HIV infection, recent evidence suggests that there are no differences in gut GC T_{FH} , B cells, or IgA^+ B cells between virologically suppressed HIV-infected individuals and uninfected controls.²¹⁸ Nonetheless, peripheral (p T_{FH}) cells are highly susceptible

to HIV infection, particularly if they express PD-1, and virus reactivation can be observed following *ex vivo* stimulation.²¹⁹ Although this observation has yet to be recapitulated in T_{FH} cells from the gut, it suggests that T_{FH} cells contribute to HIV latency *in vivo*. Further evidence supporting the contribution of gut T_{FH} cells to the HIV reservoir comes from humanized mouse models of HIV, which suggest that T_{FH} cells rapidly accumulate in gut mucosal tissues and are most permissive to HIV infection.²²⁰ Finally, the ileum is particularly enriched with lymphoid follicles^{221,222} and exhibits higher average levels of HIV transcription relative to blood and other gut sites,¹²⁴ suggesting the ileum may be a site for HIV persistence in T_{FH} cells. Together, these data underscore the importance of this $CD4^+$ T-cell subset as a potential HIV reservoir in the gut.

Transitional memory T cells (T_{TM})

Peripheral T_{TM} , along with T_{CM} , are major cellular reservoirs for HIV DNA in circulating $CD4^+$ T cells.³⁶ Relative to T_N , T_{SCM} , and T_{CM} , T_{TM} cells express higher levels of CCR5, which may contribute to their susceptibility to HIV infection.²²³ Homeostatic proliferation driven by IL-7-induced mitosis of infected T_{TM} may help maintain the size, genetic diversity, and persistence of the HIV reservoir in T_{TM} .³⁶ In individuals with low $CD4^+$ T-cell counts, T_{TM} account for a greater proportion of the HIV DNA, with additional contribution from T_{CM} that differentiate into T_{TM} cells.³⁶ The finding that T_{TM} likely harbor high levels of total or integrated proviral DNA³⁶ is echoed by other studies in treatment-naïve acutely infected individuals,²²⁴ ART-treated individuals,^{21,172,225–227} and long-term nonprogressors.²²⁸ Although one study questioned how much the resting T_{TM} compartment contributes to the pool of cells harboring infectious proviruses,²²⁹ another showed inducible, replication-competent HIV in T_{TM} of posttreatment HIV controllers and implicated this population as the main contributor to the HIV-1 reservoir.²² In addition, HIV is more robustly reactivated in the T_{TM}/T_{EM} subsets than T_{CM} by a retinoid, all-trans retinoic acid,¹⁷² and NF- κ B inducers (e.g., PMA + ionomycin, anti-CD3 + anti-CD28 antibodies, and prostratin) preferentially reactivate HIV provirus from latently infected T_{TM} *in vitro*.²³⁰ Moreover, PD-1 is highly expressed in T_{TM} during both acute and chronic HIV-1 infection and does not normalize to levels seen in uninfected individuals, despite prolonged ART treatment.²³¹ Increased PD-1 expression also correlates with inducible virus,²¹⁵ providing further evidence implicating T_{TM} as an important HIV reservoir.

Little is known about the contribution of T_{TM} to the HIV reservoir in the gut. Despite high levels of T-cell activation in the gut even during suppressive ART,¹¹³ a paradoxically low level of HIV transcription is observed.¹²⁴ T_{TM} account for a larger proportion of $CD4^+$ T cells in the ileum and rectum relative to peripheral blood,⁵⁹ and HIV DNA levels per $CD4^+$ T cell are higher in both gut sites than in blood.¹²⁴ Furthermore, rectal T_{TM} display lower average levels of HIV transcription per infected cell than peripheral blood T_{TM} ,⁵⁹ alluding to existence of transcriptionally silent HIV harbored by gut T_{TM} . With these data, T_{TM} likely play a considerable role in HIV persistence in the gut, although further research is required to evaluate the contribution of T_{TM} as a cellular reservoir for latent HIV in the gut.

Tissue-resident memory T cells (T_{RM})

Most of the memory T cells found in mucosal tissues do not recirculate to the blood; instead, they are retained locally in specific tissue sites. Referred to as tissue-resident memory T (T_{RM}) cells,^{232,233} they can respond rapidly upon reexposure to cognate antigens.^{54,65} Thus, T_{RM} cells are an effective first line of defense against invading pathogens in mucosal tissues.^{233,234} In general, less is known about $CD4^+$ T_{RM} cells and their functions than about $CD8^+$ T_{RM} cells.⁶⁵ However, $CD4^+$ T_{RM} cells have been identified in lung, skin, and mucosal surfaces, where they seem to direct protective responses and coordinate the recruitment of immune cells.⁶⁵

Phenotypically, $CD4^+$ T_{RM} cells are distinguished from circulating T_{EM} populations based on their surface expression of CD69.^{65,235} In humans, putative T_{RM} cells in mucosal, lymphoid, and peripheral tissue sites are also identified by expression of CD69.⁵⁶ More than 80% of the $CD4^+$ T cells in colon, skin, and tonsil are $CD69^+$, whereas this population is mostly absent in blood.^{56,57,236} The lectin CD69 has traditionally been thought of as an early marker of T-cell activation, since it is transiently upregulated early after T-cell receptor stimulation or in response to proinflammatory cytokines, including type I IFNs (IFN- α and IFN- β) and tumor necrosis factor. CD69 contributes to T-cell retention in tissues by regulating sphingosine-1-phosphate receptor 1, which controls the egress of lymphocytes from certain tissues. Interestingly, in both ileum and rectum, the largest proportion of HIV DNA and RNA is present in $CD4^+$ T_{EM} cells,⁵⁹ and since T_{RM} cells in the gut have phenotypes similar to T_{EM} cells,⁵⁶ the previously studied gut T_{EM} reservoir may also encompass T_{RM} cells.

T_{RM} cells respond to inflammatory and tissue-specific environmental factors, which also determine their resident memory phenotype. For example, high expression of transforming growth factor-beta within the epithelium induces the expression of CD103 integrin on a subset of T_{RM} cells.^{237–239} CD103 allows T cells to interact with epithelial E-cadherin and to be retained in the epithelium.²⁴⁰ Compared to $CD8^+$ T_{RM} cells, a very small fraction of $CD4^+$ T_{RM} cells are positive for CD103.^{56,241} High frequencies of $CD103^+$ $CD4^+$ T_{RM} cells in the colon, lung, and liver also express CCR5,⁵⁶ indicating that these cells could be susceptible to HIV infection. While the exact contribution of T_{RM} cells to the HIV reservoir in the gut or other tissues remains unknown, adipose tissue in ART-treated individuals contains HIV-1 DNA, and most of the $CD4^+$ T cells in adipose tissue are $CD69^+$, suggesting that they belong to the T_{RM} population. Coculturing of adipocytes from uninfected donors enhanced HIV infection of memory $CD4^+$ T cells in a contact-independent manner, indicating that adipocytes promote HIV infection in memory $CD4^+$ T cells by secretion of unknown soluble factors.²⁴² In another study, $CD4^+$ T cells with T_{RM} phenotype ($CD45RO^+$ $CD69^+$) in adipose tissues were found to harbor HIV DNA, and these cells produced HIV RNA upon *in vitro* reactivation with allogenic preactivated $CD4^+$ T cells.²⁴³ Furthermore, $CD4^+$ $CD69^+$ T cells collected from female cervix were preferential targets for infection with a CCR5-tropic pseudovirus in tissue culture.²⁴⁴ Therefore, T_{RM} cells in the gut may also serve as a pool of latently infected cells.^{33,245}

Gamma-delta T cells

HIV-1 may reside in nonconventional T cells, such as gamma-delta T cells ($\gamma\delta$ T cells). The $\gamma\delta$ T cells are distinct

from $\alpha\beta$ T cells,²⁴⁶ account for 0.5%–5% of adult murine or human CD3⁺ cells,²⁴⁷ are mainly CD4⁺ CD8⁺,^{248,249} and are more abundant in mucosal tissues than in peripheral blood or lymph nodes.²⁴⁷ There are two major subsets of $\gamma\delta$ T cells in humans, distinguished by the expression of V δ 1 or V δ 2 genes.^{250,251} V δ 1 T cells respond to antigens from pathogenic bacteria,^{252,253} whereas stress-related chemical compounds activate V δ 2 cells.^{246,250,254} The $\gamma\delta$ T cells exert protective functions against a number of viral infections, including HIV,²⁵⁵ although infection with HIV-1 persistently dysregulates subsets of circulating and mucosal $\gamma\delta$ T cells.²⁵⁶ In healthy individuals, V δ 1 cells are less abundant than V δ 2 cells in blood,²⁴⁷ whereas in mucosal sites such as the GI tract and endocervical and vaginal tissues, V δ 1 T cells predominate.²⁵⁷

In mucosal tissues, the V δ 1 T cells are also the most severely diminished subset in chronically HIV-infected patients.^{258–260} During ART, even in patients with CD4⁺ T-cell reconstitution, $\gamma\delta$ T-cell frequency and function are not restored to normal levels.²⁶¹ A fraction of both peripheral and mucosal $\gamma\delta$ T-cell populations expresses CD4, CCR5, and CXCR4.^{258,262–264} Peripheral V δ 2 T cells express higher levels of CCR5 and can be infected with HIV-1 *in vitro*.^{265,266} Interestingly, peripheral V δ 2 cells from ART-treated patients harbor HIV-1 DNA in quantities that exceed those of resting CD4⁺ T cells, and the majority of these cells contain replication-competent virus.²⁶⁷ However, the exact contribution of $\gamma\delta$ T cells to the gut HIV reservoir remains unknown.

Other nonconventional T cells, such as NKT and MAIT cells, are also severely diminished in numbers or frequencies in HIV-infected humans.^{268–270} However, no evidence suggests that these nonconventional T cells serve as HIV reservoirs.

Although the following cell types are not typically found in the gut, they may still contribute to the gut reservoir upon activation, differentiation, and subsequent migration to the gut.

Stem cell memory T cells (T_{SCM})

T_{SCM} cells are permissive to HIV-1 infection with both R5- and X4-tropic viruses,^{223,271,272} which can yield latently infected T_{SCM} at a similar propensity to other CD4⁺ T-cell subsets.²⁷¹ There is no consensus regarding the contribution of T_{SCM} cells to the latent HIV reservoir in the gut. To date, the overwhelming majority of research has focused on T_{SCM} in peripheral blood. In this regard, one study demonstrated that T_{SCM} represent a very stable reservoir *in vivo*, and that a high frequency of HIV-1 provirus was detected in purified T_{SCM} from virologically suppressed, HIV-infected individuals.²⁷³ This study reported that the per-cell levels of HIV-1 DNA were highest in the CD4⁺ T_{SCM} cell subset (compared to T_N , T_{CM} , T_{EM} , and T_{TD}) irrespective of treatment.²⁷³ In contrast, another study observed that T_{SCM} harbor the lowest level of integrated HIV-1 DNA in individuals on suppressive ART.²⁷⁴ These disparities may reflect differences in the study participants or the methods used to sort T_{SCM} and measure viral DNA. Nonetheless, these studies agree that the contribution of CD4⁺ T_{SCM} to the pool of infected cells may increase over time, concomitant with the decline of other T cell subsets *in vivo*.^{273,274}

Although T_{SCM} may represent an exceptionally stable and durable component of the HIV latent reservoir in peripheral CD4⁺ T cells,^{53,274} T_{SCM} cells in NHP models are virtually

absent from mucosal surfaces, including the jejunum and rectum.⁵³ Furthermore, both CD4⁺ and CD8⁺ T_{SCM} are more prevalent in the peripheral blood than in GALT from both healthy and untreated HIV-infected individuals.²⁷⁵ Additional studies are needed to investigate the contribution of T_{SCM} cells to the gut HIV reservoir, but this contribution might be limited due to the low frequency of T_{SCM} cells in the gut.

Central memory T cells (T_{CM})

T_{CM} cells are long-lived cells that constitute a major cellular reservoir for HIV in peripheral blood.^{36,229,276} The CD4⁺ T_{CM} subset harbors a high frequency of viral DNA in the blood, exhibiting ~10-fold greater levels of viral copies than terminally differentiated memory cells.⁸² Blood T_{CM} cells are also enriched for cells infected with replication-competent virus.²²⁹ Numerous studies in mice and humans demonstrate that T_{FH} cells develop into memory cells and are part of the T_{CM} compartment.⁶⁶ Recent findings suggest that p T_{FH} , defined by their expression of CXCR5, constitute a large proportion of circulating T_{CM} cells and are highly susceptible to HIV infection.²¹⁹ In ART-suppressed individuals, p T_{FH} cells harbor inducible virus capable of reactivation upon *ex vivo* stimulation.²¹⁹ Importantly, 20% of human T_{CM} CD4⁺ T cells are estimated to express CXCR5, suggesting that T_{FH} make up a large proportion of the memory pool.⁶⁶ However, memory T_{FH} cells predominantly reside in the spleen, lymph nodes, and bone marrow.⁶⁶ Relative to peripheral blood, the proportion of T_{CM} cells as a percentage of all CD4⁺ T cells is very low in the GI tract, where cells with a more differentiated phenotype, such as T_{TM} , T_{EM} , and T_{RM} , predominate.^{59,124} Thus, T_{CM} are unlikely to contribute significantly to the reservoir of latent HIV in the gut.

Conclusions and Future Perspectives

On the path toward HIV cure, further studies are needed to understand the contribution of gut tissue and gut cell subsets to the HIV latent reservoir. Since mechanisms of HIV persistence may vary significantly depending on the cell type and tissue microenvironment, therapeutic strategies for HIV eradication will require identification of all cellular subsets and mechanisms that contribute to viral persistence in ART-treated patients. Although studies on circulating CD4⁺ T cells have provided valuable knowledge about the mechanisms of cell death and viral persistence that lead to immunodeficiency, tissue CD4⁺ T cells have very different phenotypic and functional properties, and hence may involve significantly different mechanisms of HIV persistence. Moreover, the proportion of the body's CD4⁺ T cells in mucosal tissues is much higher than in the blood, which adds to the tremendous value of studying rare subsets of CD4⁺ T cells in various tissue compartments. In addition, virus production in the gut leads to breakdown of the mucosal barrier, microbial translocation, and local and systemic immune activation, which may further increase viral replication or reactivation from latency and contribute to the organ damage observed even in ART-treated patients. Arresting this vicious cycle of immune activation requires restoration of the gut mucosal barrier, which will require new therapies that can eliminate residual virus production from ongoing replication or latent reservoirs. Thus, understanding how the gut environment

contributes to HIV persistence may be critical both for new therapies aimed at reducing HIV-associated immune activation and for strategies aimed at functional or complete HIV cure.

Although recent advances in cytometry and other molecular techniques have allowed deep phenotyping and molecular characterization of various immune cells from blood and mucosal tissues, technical challenges limit the ability to quantify viral DNA/RNA or replication-competent virus from rare subsets of CD4⁺ T cells from hard-to-obtain mucosal tissues. In addition, current enzymatic digestion protocols allow recovery of only a small fraction of immune cells from tissue biopsies. A comparison of cell numbers by immunohistochemistry and flow cytometry on human recto-sigmoid biopsies suggested that only ~20% of T cells can be recovered by enzymatic digestion procedures for downstream analyses.⁶² Therefore, improvements in methodologies for maximum cell recovery and viral detection would be helpful to determine rare cell types that contribute to HIV persistence.

The observation that proviral sequences do not significantly change in the HIV-1 reservoir during suppressive therapy²⁷⁷ raises hope that phenotypic identification of true latently infected cells in patients on long-term ART may greatly enhance innovation of strategies to selectively target these cells, which would be a major milestone toward HIV cure.

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