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Tissue-specific HIV-1 infection: why it matters

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Abstract

The human immunodeficiency virus displays a narrow tropism for CD4⁺ mononuclear cells, and activated CD4⁺ T lymphocytes are the main target. When these cells are depleted by viral replication, bystander apoptosis and increased cell turnover mediated by immune activation, there is a progressive immunodeficiency (i.e., AIDS). Despite this specific cell tropism, HIV-infected persons demonstrate pathology in nearly every organ system. This article reviews current understanding of tissue-specific HIV-1 infection in the CNS, the genital tract, and gastrointestinal-associated lymphoid tissue.

Keywords

CNS; compartmentalization; gastrointestinal-associated lymphoid tissue; genital tract; HIV-1

HIV-1 primarily infects CD4⁺ T cells, causing depletion and eventual immunodeficiency. Despite specific cellular tropism (which also includes monocyte/macrophages, and microglial cells) pathologic involvement of nearly every organ system has been described [1,2]. Persons living with HIV demonstrate abnormalities of the hematopoietic system (e.g., anemia, leucopenia, thrombocytopenia); the nervous system (e.g., encephalitis, dementia, neuropathy); the gastrointestinal tract (e.g., enteropathy, wasting); the genitourinary system (e.g., nephropathy); and the cardiovascular system (e.g., pericardial effusions, myocarditis) [3]. Direct viral pathology does not drive the majority of these disease manifestations, and tissue-specific illness seems mostly due to the dysregulated and deficient immune system that results from CD4⁺ T-cell depletion [3]. Opportunistic infections in an immunocompromised host (e.g., TB, *Pneumocystis jirovecii* pneumonia, cryptococcal meningitis) cause much of the pathology observed in the HIV epidemic. In addition, immune system alterations during HIV infection mediate syndromes (e.g., HIV-associated myocarditis, testosterone deficiency, dementia) and likely contribute to the increased incidence of non-AIDS events observed in combined antiretroviral therapy (cART)-treated HIV-infected individuals [4,5].

Not all tissues are targets of HIV disease, but many actively participate in its pathogenesis. Some organs exist as viral compartments and reservoirs that allow HIV to persist despite cART that eliminates virus from the peripheral blood [6-8]. ‘Compartments’ are anatomic environments that restrict HIV gene flow and thus encourage viral evolution and divergence

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from virus in the peripheral blood [9-13]. On the other hand, ‘reservoirs’ are a cell type or anatomic site within which HIV or HIV-infected cells survive because of slower viral kinetics than virus present in the blood [14-16]. It is likely that these tissue compartments and reservoirs protect HIV from specific immune responses, antiretroviral therapy, and biochemical decay, and this protection ultimately provides a unique environment for host–pathogen interactions [15]. This article illustrates how compartmentalization in the CNS contributes to HIV persistence and differential evolution, reviews the aspects of the genital tract reservoir that impact HIV transmission, and proposes mechanisms by which the gastrointestinal-associated lymphoid tissue (GALT) reservoir promotes disease progression.

HIV-1 infection of the CNS

The affinity for HIV to cause disease in the CNS (i.e., brain, spinal cord and surrounding meninges) has been evident since the beginning of the epidemic [17-19]. Prior to widespread use of cART, nearly half of HIV-infected persons experienced clinically relevant symptoms of CNS disease [20]. Today many infected persons still demonstrate below-average neurocognitive performance, although the incidence of severe HIV-associated dementia has significantly declined [20,21]. These neurocognitive deficits may be explained by ongoing HIV replication within the CNS despite the use of cART, a hypothesis supported by patients with discordant viral loads in their cerebrospinal fluid (CSF) and peripheral blood [21-23]. Described instances of undetectable blood viral loads while concurrent CSF viral loads are detectable give clear evidence that the CNS can serve as an HIV reservoir. However, the CNS can also serve as a compartment, given that viral genotypes are often distinct between the CSF and peripheral blood [9-12,24].

The pathophysiology of the CNS compartment

High replication and mutation rates of HIV can result in the development of genetically heterogeneous populations within an individual host [25]. Distinct and divergent viral populations arise within the CNS because this organ is encased in a selectively permeable physical barrier, the blood–brain barrier (BBB). The BBB is a structure of capillary endothelial cells joined by tight junctions and surrounded by a basal membrane, pericytes, and astrocyte foot processes [26]. This barrier allows the entry of nonpolar molecules via passive diffusion and active transport but limits the movement of many factors, including HIV and other pathogenic organisms, water-soluble compounds (such as antiretroviral drugs) and specific cells types (lymphocytes) [27,28]. It is likely that HIV infects the CNS during acute infection either when the BBB is disrupted by inflammatory cytokines or through entry of infected peripheral monocytes/macrophages destined to become brain residents [29-32]. Once HIV infection is established in the CNS, waning inflammation limits cell trafficking and viral gene flow between the CNS and blood, and allows the emergence of divergent viral variants [26,33].

CNS compartmentalization was first described using phylogenetic analyses of HIV *env* and *pol* sequences in brain tissue derived from autopsy samples [10-12]. The clinical and histological observations of regional brain differences in HIV-associated neuropathology led to the finding that regional differences in viral kinetics and evolution also exist, and identified the temporal lobe as a favored site of HIV replication, with evolution rates 100 times faster than other regions [24,34-36]. Viral populations distinct from the blood but clustering with the brain were also observed in samples from the spinal cord, dorsal root ganglia, cerebral spinal fluid, and meninges; confirming that the entirety of the CNS serves as a compartment [37-39]. Of these structures, the meninges contains the greatest heterogeneity of sequences, some of which cluster to the brain and others to the peripheral blood, supporting its role as a primary viral transport structure between the CNS and the

periphery [39]. The varying degrees of population heterogeneity and rates of evolution in CNS structures suggest that regional differences in local immune selection pressures, target cell availability and drug selection pressures generate HIV reservoirs within the CNS compartment [12].

The pathophysiology of the CNS reservoir

An efficient and productive HIV infection depends on the availability of activated, CCR5⁺ CD4⁺ T cells [40,41]. In response to an influx of activated lymphocytes, the CNS limits and alters the interactions between HIV and this target cell via down regulation of MHC class I and II molecules [42]. Decreasing MHC class I expression protects neurons from lysis by cytotoxic T cells (CTL), and may contribute to CTL dysfunction, ultimately modifying CTL selection pressures on HIV [43,44]. MHC class II downregulation suppresses CD4⁺ T-cell activation and proliferation and further dampens inflammation [45,46]. In addition, specific cell types within the CNS (neurons, endothelial cells, microglial cells and astrocytes) increase expression of Fas ligand, and TNF-related apoptosis-inducing ligand, which target CD4⁺ T cells for cellular death [47,48]. These CNS-protective responses minimize CNS-compartmentalized HIV exposure to activated CD4⁺ T cells, an action that alters viral kinetics and creates a viral reservoir, while also promoting the survival of virions capable of infecting other cell types such as brain-resident macrophages and microglial cells. These viral dynamics and evolutionary changes likely lead to HIV neurotropism [49].

HIV-infected monocyte-derived microglial cells, macrophages, and astrocytes in the CNS have been observed in autopsy studies, biopsy specimens, and simian immunodeficiency virus (SIV)-macaque models [50-52]. These cells have a low rate of cell turnover (surviving months to years) and an innate resistance to cytopathic effects that allow unintegrated (half-life of 30 days) and integrated forms of HIV pro virus to exist for prolonged periods [53-55]. Taken together, it is likely that these cell types act as long-lived viral reservoirs within the CNS [50-52]. Virus replication and assembly in macrophages can diverge from what is observed in CD4⁺ T cells, with unintegrated HIV DNA serving as a source of transcription factors such as Tat and altering HIV replication [28]; the involvement of membrane-bound vacuoles in virus processing [54,56]; and replication enhancement by environmental nerve growth factor [57]. Latent HIV infection of the abundant and functionally diverse astrocytes, as defined by the presence of HIV DNA within these cells, occurs rather infrequently, and the clinical relevance of this cellular reservoir is unclear since HIV infection of astrocytes is reportedly not productive *in vivo* [58-64]. It is apparent that HIV exposure alters astrocyte function, contributes to neuropathogenesis and promotes neurotropism via the induction of chemokines (MCP-1, IL-10, IL-6, nitric oxide) [65-67].

Inadequate levels of cART in the CNS is another mechanism responsible for maintaining a HIV reservoir. In addition to the limiting capacity of the BBB, membrane transporters such as multidrug-resistant protein, P-glycoprotein and multi-specific organic anion transporter actively pump out drug [68]. Poor drug penetration into the CNS is believed to be responsible for the persistent neurocognitive impairment evident in virologically-suppressed patients on cART and the occasional discordance observed between blood and CSF viral loads [23,69-72]. Until recently, studies evaluating the benefit of cART regimens with high CNS penetration and effectiveness were conflicting, largely due to the lack of a global standardized tool to evaluate neurocognitive impairment and to differences in characterizing the effectiveness of various antiretrovirals in the CNS [70,73]. However, recent works using the validated CNS penetration effectiveness score and a battery of neuropsychologic tests report that better neurocognitive functioning correlates with use of high CNS penetration effectiveness scoring cART regimens [74,75].

The role of the CNS in HIV persistence

The existence of viral compartments and reservoirs likely contribute to the clinical neuropathology observed in HIV-infected persons despite the use of cART that suppresses HIV to undetectable levels in the blood. In addition, an HIV sanctuary within the CNS may result in the development of drug resistance and could lead to misinterpretation of genotype resistance assessments performed in the blood, with subsequent failure of cART [76]. Perhaps the gravest consequence of the CNS as a viral sanctuary lies in its potential to be a major barrier in the quest to eradicate HIV [73]. While great strides have been made in the study of CNS-compartmentalized HIV, detailed characterization of CNS infection in early disease, the role of macrophages, microglial cells, and astrocytes in pathogenesis, and the effect of cART on symptoms, viral evolution, and viral persistence remain relatively open questions [77-80].

The genital tract compartment

Similar to the CNS, a portion of the male genital tract contains non-fenestrated capillary beds (blood–testes barrier) allowing for partial immune cell restriction and limited drug penetration [81,82]. Viral populations that diverge from the peripheral blood have been observed in seminal cells and seminal plasma, suggesting that HIV-1 compartmentalization also occurs within the male genital tract and inferring that this organ contributes to HIV persistence and evolution [83-87]. Interestingly, distinct viral populations have also been detected in female genital tracts, yet unlike in men, no strict physical barrier exists between the female genital tract and blood [88-90]. Documentation of diverse genetic viral subpopulations often serves as evidence to ‘prove’ the existence of a viral compartment associated with specific anatomy or tissue, but the methods used to evaluate divergence are inconsistent across studies [13]. Bull *et al.* utilized four different analytic methods to evaluate HIV sequences derived from single genome amplification in the blood and female genital tract, and found that compartmentalization was confirmed by three or more tests in just five of the 13 participants [91]. Reanalysis of these patients suggested that replicative bursts within a low diversity population appeared to be a more likely explanation for the differences in the viral sequences than divergent evolution due to female genital tract compartmentalization [91]. This work and a method comparison study performed by Zarate *et al.* highlight one of the major issues complicating the research of compartments and viral evolution, summarized in Box 1 [13].

The pathophysiology of the genital tract reservoir

As in the CNS, HIV infection of the male genital tract contributes to HIV evolution, drug resistance, and persistence. However, the consequence of HIV persistence in the genital tract is not restricted to the HIV-infected person, but also impacts onward transmission [92]. The risk of sexual transmission is variable (0.1–0.001% per sex act) and positively correlates with high seminal and plasma viral loads, and concurrent sexually transmitted infections (STIs) [93-99]. Retroviral particles were initially described in semen in 1984, and replication-competent HIV has been documented throughout the genital tract in cell-associated (CD4⁺ T cells, monocytes and macrophages) and cell-free forms [100]. A significant degree of diversity between cell-associated and cell-free HIV from the peripheral blood and from each other suggests that several portions of the male genital tract contribute to HIV in semen [83,92,101]. Specifically, HIV derived from the testes appears genetically distinct from HIV within the accessory glands (epididymis, prostate and seminal vesicles), perhaps because the blood–testis barrier limits cell flow from the blood [102]. Despite this physical barrier, *ex vivo* research has shown that resident testicular macrophages can be infected with HIV and are capable of producing low levels of infectious viral particles [103].

Work with SIV-infected macaques revealed that infection of the testes occurs early in the disease and involves both infected macrophages and CD4⁺ T cells [104]. However, the mechanism of HIV entry and infection remains unclear. Unlike in the CNS, SIV infection of the testes in macaques is not accompanied by increased lymphocytes, markers of activation (i.e., HLA-DR), or change in cytokine expression (either proinflammatory IFN- γ , IL-1 β , TNF- α or anti-inflammatory IL-10, TGF- β) [104]. It is clear that the immune sanctuary of the testes makes HIV infection of this organ unique, but the degree of contribution to the cell-free and cell-associated HIV present in semen, and the clinical significance of that contribution remains unknown [92,104].

Acute infection of the accessory glands of the male genital tract also occurs in the SIV-macaque model [104,105]. Infected CD4⁺ T cells and macrophages are largely localized within the stroma of the accessory glands, but can be found within the secretory epithelium as well, an optimal location for release of both cell-associated and cell-free virus into the semen [92]. The prostate and seminal vesicles produce the bulk of seminal fluid and are likely to be responsible for the majority of HIV present in semen [92]. Interestingly, prostate explants are more efficiently infected by CCR5-tropic strains than CXCR4-tropic or dual-tropic strains despite lower levels of CCR5-expressing cells [106]. This finding may explain the preferential transmission of CCR5-tropic strains during sexual intercourse [107,108]. A growing body of *in vitro* and SIV-infected macaque evidence suggests that semen not only functions as a vehicle for HIV but, along with other aspects of the genital tract, may enhance transmission. The genital tract promotes onward transmission by serving as an HIV reservoir in patients on cART, and providing seminal factors that optimize target cell interactions and induce favorable immunomodulation for transmission [109-111].

The genital tract reservoir of HIV influences onward transmission

Several studies have confirmed the genital tract as a reservoir for HIV by demonstrating intermittent secretion of HIV in semen and cervicovaginal fluids despite the use of cART [112-115]. Many factors are correlated with HIV shedding in both women and men, including HIV viral load in the blood, immunologic status, genital tract inflammation, concurrent STI, pregnancy, oral contraceptive use, and cervical ectopy [116-120]. Much less is known about the predictors of viral shedding while on cART, but a publication by Henning *et al.* suggests that even in the presence of excellent cART adherence and suppressed plasma viremia, intermittent HIV shedding in women occurs, particularly with concurrent cervicovaginal inflammation [121]. Another hypothesis for continued viral shedding in the presence of cART is poor penetration of certain antiretrovirals into the genital tract, but conflicting studies necessitate more work in this area [113,115,122-124].

Complementing its role as a source of HIV, the genital tract may also be a source of other factors that promote sexual transmission. Semen is composed of a complex mixture of compounds that provide a protective and nutritive environment for spermatozoa. Assessing the impact of these components on HIV transmission has been difficult due to the intrinsic cytotoxicity of undiluted semen *in vitro* [125]. Bouhlal *et al.* noticed that HIV-1 infection of the human epithelial cell line HT-29 was enhanced when HIV was added to semen before cell culture. This effect was ablated by treatment with heat, ethylenediaminetetraacetic acid and the monoclonal antibody to complement receptor type 3, leading them to believe that complement present in semen opsonizes HIV and facilitates infection of complement receptor-expressing target cells [126,127]. Other seminal factors may promote transmission by altering the immune response to favor target cell availability and seminal virus persistence [128]. TGF- β and prostaglandin E (PGE) are present in higher quantities in the semen than elsewhere within the body and are considered to be the major immunomodulators in semen [129,130]. Inflammatory cells traffic to the female

reproductive tract following the introduction of semen, and provide an abundance of target cells for HIV, a reaction attributable to TGF- β [131,132]. Like TGF- β , PGE in semen attracts immune cells, but this molecule also inhibits their function [133]. Together, TGF- β and PGE appear to generate an optimal immune environment for HIV transmission.

A review of the literature will lead to the understanding that the interplay between the genital tract and HIV is complex, and research exists that suggests the male genital tract contains aspects that both promote and hinder sexual transmission [134]. A recent article by Balandya *et al.* evaluated the effect of semen on CD4⁺ T cells *in vitro* and found that semen protected cells from infection with CXCR4 tropic strains [135]. The inhibitory effect was attributed to a decrease in the expression of CD4 and CXCR4 receptors on the surface of T cells following semen exposure. The addition of semen also protected CD4⁺ T cells from infection with CCR5-tropic strains, but to a lesser degree. Unlike with CXCR4, semen profoundly increased CCR5 expression. The conclusion was that exposure of CD4⁺ T cells to semen has an overall protective effect, but the resulting upregulation of CCR5 may contribute to the preferential R5-tropic transmission observed *in vivo* [107,108]. Taken together, these studies suggest that many factors exist both innately (the multitude of protein products in semen) and environmentally (e.g., STIs) that affect the efficiency of HIV transmission from the male genital tract.

The pathophysiology of the GALT reservoir

HIV infection is characterized by high levels of chronic immune activation, and this activation strongly correlates with disease progression and with an ‘activation/dysfunction phenotype’ of the many cells of the immune system [136-140]. Whether chronic inflammation is due to HIV replication, imbalances between Th17 and regulatory T cells, and/or microbial translocation remains unknown, but it is clear that HIV dynamics during infection are associated with perturbations in the GALT. In turn, these changes are associated with changes in immune system markers and appear to dramatically influence the course of future HIV-associated disease. GALT exists as the largest immunologic organ in the body, housing 60% of total body lymphocytes within immune-inductive (Peyer’s patches in the small intestine, and lymphoid follicles in the colon) and immune-effector (lamina propria) subcompartments [141]. The lymphoid tissue of the gut exists in a state of ‘physiologic inflammation’ due to continual exposure to antigens (gut bacteria) [142]. As has been the case in the CNS and genital tract, the GALT also serves as a reservoir for HIV and is in part responsible for driving immune activation and HIV disease progression [143-146].

The GALT reservoir fuels disease progression

Unlike the peripheral blood and other lymphoid tissue, the majority of mucosal CD4⁺ T cells display activated effector and central memory (CD45RO⁺) phenotypes with high levels of CCR5 cell surface expression [147,148]. The SIV-infected macaque model first documented the importance of GALT in the pathogenesis of HIV/SIV by revealing that severe depletion of intestinal CD4⁺ T cells occurs within days of inoculation with SIV, followed by a chronic phase of slow CD4⁺ T-cell decline. This finding has since been confirmed in humans with HIV-1 [149,150]. Further, CD4⁺ T-cell constituents in GALT are ideal HIV-1 targets, and the cascade of events that follows the elimination of these cells is thought to be critical to the pathogenesis of HIV [142,151].

CCR5-expressing CD4⁺ T cells housed in the gut lamina propria undergo the greatest degree of loss by ‘direct viral infection, activation-induced cell death, and host-derived cytotoxic cellular responses’ [93,97-101]. The decimation of GALT CD4⁺ T cells incites a proliferative response in the peripheral blood memory CD4⁺ T-cell population, presumably

a physiologic attempt to replace the lost lymphocytes; an inability to sustain this response leads to early disease progression in SIV-infected macaques [152,153]. However CD4⁺ T-cell loss in the GALT is not sufficient to lead to AIDS, as observed in nonpathogenic, nonhuman primate models, and it is possible that other events such as the loss of CD4⁺ T cells with specific functional capacities may be a key part of the mechanism [154,155].

One functional subset of CD4⁺ T cells that is particularly sensitive to HIV infection is the Th17 cell [156-158]. CD4⁺CCR5⁺ Th17 cells are characterized by the production of IL-17, and function to protect the integrity of mucosal surfaces through neutrophil recruitment, epithelial regeneration, and stimulation of defensin and mucin production [156,159]. Through the study of other diseases (inflammatory bowel disease and autoimmune Th17 deficiency) it is clear that Th17 proinflammatory functions are balanced by the anti-inflammatory functions of regulatory T cells and this balance facilitates effector CD4⁺ T-cell responses in the gut [160-163]. It is believed that the disruption of the gut mucosal barrier (presumably due to Th17 loss) results in microbial translocation, chronic immune activation and subsequent HIV disease progression [164-166]. Recent studies in nonpathogenic, nonhuman primate models, and HIV-positive elite controllers suggest that preservation of Th17 cells protects against HIV progression [154,167-169]. In further support of this hypothesis is the recent study by Chege *et al.* who evaluated blood and sigmoid biopsies from HIV-infected persons and discovered that higher Th17 frequencies correlated with reduced microbial translocation [156].

Along with loss of Th17 cells, other HIV/SIV-related changes in the GALT could contribute to microbial translocation, including loss of myelomonocytic cells that destroy gut bacteria, apoptosis of the gut epithelium and increased epithelial permeability due to the local 'proinflammatory milieu' [169-172]. High levels of serum lipopolysaccharide, a surrogate marker for bacterial translocation, have been observed in chronically infected HIV-infected persons and in SIV-infected macaques, and appear to be tightly coupled to T-cell immune activation, CD4⁺ T-cell cycling and peripheral CD4⁺ T-cell decline [165,173,174]. However, work comparing HIV-infected persons to uninfected persons with colitis suggest that increased lipopolysaccharide alone does not cause the immune activation and peripheral CD4⁺ T-cell decline [175]. In general, it has been difficult to ascertain if microbial translocation is a participant in HIV progression or purely a symptom of dysregulated GALT [146].

HIV infection of GALT may also impact B cell function contributing to the delayed plasma antibody response observed during HIV infection [176]. Evaluation of B cells resident in the GALT of acutely HIV-infected persons demonstrates Peyer's patch follicular lysis, and a decrease in discernable B cell germinal centers. These dramatic histopathologic changes are accompanied by evidence of polyclonal B-cell activation and a shift from a predominant naive to memory phenotype in both the terminal ileum and blood, and infers that destruction of GALT during the earliest stages of HIV infection contributes to B-cell dysfunction and may result in the "high rate of decline in HIV-1-induced antibody responses and the delay in plasma antibody responses to HIV-1" observed *in vivo* [177].

The use of cART fails to completely reconstitute the gastrointestinal immune system even when started early in infection and given for prolonged periods [178]. Current hypothesized barriers to complete reconstitution include ongoing viral replication at the mucosal site (i.e., GALT as an HIV reservoir) and extensive collagen deposition within GALT [144,179]. The observation that GALT disease and chronic immune activation contribute to disease progression emphasizes the importance of understanding this site-specific infection and illustrates the value of developing new therapeutic strategies aimed at achieving complete reconstitution of the gastrointestinal immune system [146,180].

Conclusion

Studying tissue-specific infection allows an understanding of the pathogenesis behind the many organ-specific complications observed in HIV-1 disease, and can lead to the discovery of details about the pathogenesis of HIV that are critical to controlling the global HIV epidemic. Understanding the mechanisms of HIV infection in the CNS will contribute to the development of new cART strategies, and will likely be a necessary focus in the quest to eradicate HIV. Similarly, HIV infection in the genital tract and the local factors that impact sexual transmission are key to the development of novel and effective prevention strategies, including vaccine design. Lastly, understanding the events that occur with HIV infection of the GALT will lead to a broader understanding of the complex host and viral interactions that define HIV-1 pathogenesis and may open the door to innovative immunotherapy. As evident in this article and summarized in Box 2, many important questions regarding tissue-specific HIV-1 infection and its impact on HIV pathogenesis remain unanswered.

Future perspective

The impetus to understand the tissue-specific manifestations of HIV disease has begun to highlight the importance of the CNS, genital tract, and GALT in the pathogenesis of HIV. Future research efforts will fill in the details, providing novel therapeutic targets, new mechanisms of pathogenesis and definitive clinical recommendations. For the CNS, the next 5–10 years will most likely provide definitive clinical studies assessing the impact of CNS-penetrating antiretroviral regimens on neurocognitive function, quality of life and HIV persistence. As understanding of the interplay between CNS cells, local cytokines, viral interactions, and specific neurotoxicity of antiretrovirals grows, specific regimens (possibly including drugs other than antiretrovirals) will be designed to minimize CNS symptoms in persons with HIV. However, the most important advances will be made in clarifying the relevance of HIV infection in specific tissues. A complete knowledge of HIV infection of macrophages, microglial cells, and astrocytes must be obtained in order to design anti-retrovirals effective in these cells to ensure success of future efforts to eradicate HIV.

For the genital tract, new approaches to HIV prevention will depend on the clarification of events occurring during the sexual transmission of HIV. Antiretroviral regimens that potentially penetrate into the genital tract will be identified to minimize discordance between plasma viremia and viral levels in genital secretions. The factors that increase genital tract shedding in virologically suppressed patients on cART will be elucidated and targeted to decrease the risk of transmission in discordant couples. Most importantly, a better understanding of the contribution of the male and female genital tract environments to successful transmission events (i.e., seminal peptides, viral genetic bottlenecks, trafficking of lymphocytes, and correlates of HIV transmission at receptive mucosal sites) will lead to the development of novel non-antiretroviral interventions for prevention.

The study of HIV infection of GALT, will likely lead to a better understanding of the host immune response to HIV (specifically the role of Th17-regulatory T cells) and will identify new immunologic markers for disease progression and for initiation of cART. Trials of medications designed to decrease local gut inflammation and promote healing of GALT architecture will be attempted in concert with cART to minimize microbial translocation, and new immunologic strategies will be investigated to assess their impact on overall immune activation.

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Box 1**Methods to define viral compartmentalization**

- Tissue compartmentalization of HIV subpopulations has been documented in the CNS, genital tract, lymph nodes and breast milk [12,180-182]. The analytic methods determining the presence of compartmentalization differ across the many studies, some using simple tree clustering to claim compartmentalization and others using more rigorous statistical approaches, like the Slatkin–Maddison test. To assess the degree of agreement between common methods, Zarate *et al.* analyzed published CNS and genital tract samples from 62 patients, all of whom had at least five sequences from two compartments [13]. They evaluated six methods (three tree-based, and three distance-based) as briefly described below:
  - Slatkin-Maddison test: this tree (or phylogeny)-based analysis assesses the minimum number of migration events between the two populations based on the inferred tree. Statistical significance was determined by comparing data to the expected number of migration events that would occur in a randomly structured population.
  - Simmonds association index: this test weighs the contribution of each internal node in the phylogenetic tree based on its depth in the tree. Significance was assessed using a bootstrap sample over the structure of the inferred phylogenetic tree.
  - Correlation coefficients: correlates the distance between two sequences in a phylogenetic tree and determines if they were from the same compartment. Distance is either the number of tree branches separating the sequences or the cumulative genetic distance between sequences. The distribution of these coefficients was estimated and a $p < 0.05$ was considered significant.
  - Wright’s measure of population subdivision: compares pair-wise genetic distance between two sequences from two compartments to the mean distance between sequences from the same compartment. Significance was calculated using a population structure randomization test.
  - Nearest-neighbor statistic: measures the frequency that the nearest neighbors of each sequence were isolated from the same or different compartments.
  - Analysis of molecular variant (ANOVA) calculates associations based on the diversity of sequences between and within compartments.
- In general, tree- and distance-based methods had poor to fair agreement for determining genital tract compartmentalization. Agreement was fair to good for determining CNS compartmentalization when comparing tree-based methods to either Wrights’ measure of population subdivision or nearest-neighbor statistic. Within-class methods consistently showed moderate agreement with the tree methods, but ANOVA had poor agreement with the other distance-based methods. These discrepancies may explain the

discrepancies across published studies. Overall, this study highlights the importance of combining analytic methods for reliability and for the development of a consensus approach for reproducibility.

Box 2**Future research directions****CNS compartmentalization**

- Clarify the impact of combined antiretroviral therapy regimens with high CNS penetration effectiveness scores on neurocognitive scores, and on other factors such as mood disorders.
- Understand the role of CNS compartmentalization in HIV latency (i.e., discern if HIV-1 produced in the CNS contributes to the low-level viremia detectable in chronically suppressed patients).
- Determine the nuances of the life cycle of HIV in monocyte-derived cells with the goal of future drug design.

Genitourinary tract & transmission

- Continue to assess drug penetration in the genital tract and the effect on genital secretion and HIV viral loads.
- Differentiate between the components of semen that enhance HIV transmission and the components that protect against transmission.
- Reveal the pathway that HIV takes from semen to the CD4⁺ T cell in both the vaginal and anorectal tract to identify pharmacologic targets for prevention interventions.

Gastrointestinal-associated lymphoid tissue & HIV-associated disease progression

- Continue to investigate the immunologic events of early infection with the goal of clarifying the relationship between HIV infection of gastrointestinal-associated lymphoid tissue (GALT) and subsequent immune activation.
- Assess if antiretroviral treatment in acute HIV infection preserves the immune system and changes the natural history of disease.
- Determine if microbial translocation is the cause of immune activation or just the result of GALT dysfunction.
- Unravel the pathophysiology underlying incomplete GALT repletion during combined antiretroviral therapy with the goal of designing non-antiretroviral therapy to promote tissue healing.

Executive summary

Tissue-specific HIV infection

- Some organ systems exist as HIV compartments (environments that restrict gene flow) and HIV reservoirs (environments that allow the survival of HIV due to different viral kinetics than in the blood).

CNS

- The blood–brain barrier limits cell trafficking and drug entry into the CNS. This results in persistence of HIV within the CNS and divergent evolution.
- HIV demonstrates considerable diversity even within the CNS, with the meninges demonstrating the most heterogeneity. It is believed that the meninges function as a primary HIV transport structure between the blood and CNS.
- Innate protective mechanisms of the CNS limit the interaction of free virions with activated CD4⁺ T cells through the downregulation of MHC I and MHC II. This may also alter viral kinetics and favor the survival of virions capable of infecting macrophages and microglial cells in the CNS.
- HIV infection of the macrophages, microglial cells, and astrocytes demonstrate altered viral kinetics compared to the CD4⁺ T lymphocyte.
- Inadequate levels of combined antiretroviral therapy (cART) in the CNS may be another mechanism responsible for the persistence and divergence of CNS-compartmentalized HIV.
- Recent work using the validated CNS penetration effectiveness score and a battery of neuropsychological testing suggests that cART regimens with high CNS penetration effectiveness scores correlate with better neurocognitive functioning.
- HIV persistence and evolution within the CNS may also contribute to the development of drug resistance in this population that would not be detectable by genotype resistance in the blood.

Genital tract

- Similar to the CNS, the male genital tract contains non-fenestrated capillary beds (blood–testes barrier) allowing for immune cell restriction and limited drug penetration.
- Distinct viral populations have also been detected in the genital tracts of women, yet unlike men, no strict physiological barrier exists in the female genital tract, which highlights a major issue complicating the research of compartments and viral evolution.
- HIV infection of the genital tract is important because of its impact on onward transmission.
- HIV is detectable in genital tract secretions even in the setting of undetectable HIV RNA in the blood, suggesting that it too is a viral reservoir.
- In women, concurrent cervicovaginal inflammation is a risk factor for HIV shedding, even in the setting of cART.

- In the male genital tract there are multiple organs that contribute to seminal HIV and provide seminal factors that may also enhance transmission.
- Complement in semen opsonizes HIV facilitating infection of complement receptor-expressing target cells *in vitro*.
- Semen may also alter the immune response to favor target cell availability and seminal virus persistence.
- Many factors exist innately (the multitude of protein products in semen) and environmentally (sexually transmitted infection) that affect the efficiency of HIV transmission.

Gastrointestinal-associated lymphoid tissue

- HIV infection is characterized by hyperimmune activation, which appears to be associated with tissue-specific infection of gastrointestinal-associated lymphoid tissue (GALT).
- A profound depletion of GALT CD4⁺ T lymphocytes occurs in early HIV-1 infection.
- A proliferative response in the peripheral blood memory CD4⁺ T-cell population follows cell loss, and an inability to sustain this response leads to early disease progression in a small population of SIV-infected macaques.
- CD4⁺CCR5⁺ Th17 cells in the GALT appear to be preferentially lost, and are believed to control the GI tract's ability to prevent bacterial intestinal invasion.
- GALT depletion is also accompanied by elevated levels of lipopolysaccharide.
- The intense inflammatory response that accompanies acute HIV infection drives and sustains CD4⁺ T-cell loss and the memory cell proliferative response, but it is unclear what drives inflammation.
- HIV infection of GALT may contribute to the dysfunction of B cells and the delayed plasma antibody response observed with HIV.
- The introduction of cART results in the recovery of peripheral CD4⁺ T cells, but reconstitution of the gastrointestinal immune system is incomplete.
- It is unknown to what degree these GALT abnormalities and subsequent GALT immune dysfunction affect the systemic immune response, but it is becoming apparent that HIV infection of GALT and the immune consequences resulting from the depletion of CD4⁺ T cells at this site are crucial components of HIV disease.