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Tissue Issues: Mucosal T-cell Responses in HIV-1 Infection

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Abstract

Purpose of review: This review summarizes our current understanding of HIV-1-specific T-cell responses in mucosal tissues, emphasizing recent work and specifically highlighting papers published over the past 18 months.

Summary: HIV-1-specific T-cell responses have been extensively characterized; however, the vast majority of reports have focused on T-cells isolated from peripheral blood. Mucosal tissues of the genitourinary and gastrointestinal tracts serve as the primary sites of HIV-1 transmission, and provide "front line" barrier defenses against HIV-1 and other pathogens. In addition, the gastrointestinal tract remains a significant viral reservoir throughout the chronic phase of infection. Recent work on mucosal immunity has improved the standardization of tissue sampling approaches as well as provided new insights on the abundance, phenotype and distribution of HIV-1-specific T-cell populations in mucosal tissues.

Keywords

Mucosa; Genitourinary; Gastrointestinal; T-cells; Immunity

Introduction.

Mucosal tissues of the genitourinary and gastrointestinal tracts are the major sites of HIV-1 transmission, and are rich in $CD4^+$ T-cells that serve as the primary targets for infection. Numerous studies have explored the initial events occurring at these mucosal surfaces, which appear to play a major role in determining the balance between inflammation and host protective immunity. Throughout the chronic phase of infection, the gastrointestinal tract continues to serve as an important HIV-1 reservoir. This observation underscores the importance of tissue-based immune responses not only in determining the initial host-virus balance, but also in orchestrating the "long game" of defending the host throughout chronic infection. Due to the many logistical challenges associated with tissue sampling in humans, HIV-1-specific T-cell responses have primarily been studied using peripheral blood, often with the assumption that T-cell populations in blood and tissues were broadly comparable in specificity and function. However, recent work, notably including the characterization of

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tissue resident T-cell subsets in animal models and humans, has challenged this assumption. What follows is an overview of recent literature on mucosal T-cell responses, with reference to some older work in order to provide a historical perspective.

The Female Reproductive Tract.

Worldwide, most new HIV-1 infections occur as a result of sexual contact through the genital tract or the anorectal canal. HIV-1 transmission via the female reproductive tract (FRT) is less efficient than via the anorectal mucosa, as demonstrated in nonhuman primate models and supported by epidemiologic studies and mathematical modeling[1,2]. HIV-1 susceptible target cells including CCR5$^+$, CD4$^+$ T-lymphocytes are present in both the upper and lower tract[3–5]. Recently, cervical CD4$^+$ T-cells co-expressing CD69 and either α4β7 or α4β1 integrin were found to be preferential targets for HIV-1 in an *in vitro* flow cytometry-based fusion assay[6]. Earlier studies revealed CD4$^+$ Th17 cells as early targets for HIV-1 and SIVmac[7,8]. *In vivo*, viral transmission may occur through breaches in the mucosal surface; through inflamed areas associated with other sexually transmitted infections; through uptake via antigen presenting cells or by direct infection of susceptible target cells (reviewed in[9]). Several recent studies have demonstrated a major role for genital inflammation in enhancing susceptibility to HIV-1 acquisition through the FRT[10,11].

How does cell-mediated immunity respond to HIV-1 infection of the genital mucosa? HIV-1 RNA$^+$ cells are present in ectocervical tissue samples from HIV-1$^+$ women, and recent studies revealed selective infiltration and/or local expansion of CD8$^+$ T-cells in these tissues[12]. Further examination revealed that many of these cells have a “tissue-residency” phenotype, expressing CD69 alone or in combination with CD103. The number of epithelial CD103^{neg} CD8$^+$ T-cells correlated with HIV-1 viral load, and with expression of chemokines (CXCL10, CXCL9, and CCL5) in the ectocervix, suggesting a direct relationship between T_{RM} recruitment, immune activation and HIV-1 viral replication in these tissues[13]. Further work will be required to establish the antigenic specificity, clonality and effector functions of these cells.

CD8$^+$ T-cells specific for HIV-1 antigens have been detected in the cervical and vaginal mucosae of women with chronic HIV-1 infection[14,15] and in the corresponding tissues of rhesus macaques chronically infected with SIVmac[16]. Efforts to track the tissue distribution of clonal HIV-1/SIV-specific T-cell populations in the reproductive tract have been relatively limited, but work in the late 1990s-early 2000s revealed that certain T-cell clones were common to blood and cervical mucosa[14,15]. Subsequent studies of cervicovaginal T-cell responses focused on their relationship to cervical inflammation and viral shedding in the context of chronic HIV-1 infection among women in South Africa[17–19]. No relationship was detected between the magnitude of cervical T-cell responses and virus shedding in genital fluids; furthermore, women whose genital secretions contained HIV-1 virus also had higher levels of proinflammatory cytokines in these secretions, suggesting a link between cervical inflammation and HIV-1 shedding[20]. Although polyfunctional HIV-1-specific CD8$^+$ T-cells could sometimes be detected in women with high CD4$^+$ T-cell counts, the presence of these cells did not prevent genital shedding of

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HIV-1[21]. Mkhize and colleagues found that cervical HIV-1-specific CD8+ T-cell responses were often maintained following successful HAART, although the corresponding responses in blood were greatly reduced; this may suggest incomplete suppression of viral replication in the lower FRT[22].

Advances in mucosal tissue sampling and preservation.

Recognizing the logistical challenges inherent in mucosal sampling, particularly in the genital tract, several recent studies have focused on optimizing minimally invasive sample collection and storage practices. Endocervical cytobrush and ectocervical biopsy sampling provide higher yields of viable leukocytes than cervicovaginal lavage[19,23]. Encouragingly, cervicovaginal leukocyte suspensions[24] and tissue biopsies[25], as well as samples from gastrointestinal mucosa, may be viably cryopreserved with minimal disruption of CD4+ and CD8+ T-cell phenotype and function. These optimized protocols may help advance further studies in this area.

HIV-1-specific T-cells in the Gastrointestinal Tract.

Experimental transmission studies in nonhuman primates, as well as epidemiologic studies of human populations, have revealed that anal intercourse is significantly more likely than vaginal intercourse to lead to HIV-1 acquisition[1,2]. The gastrointestinal (GI) tract acts as the largest lymphoid organ in the body, and houses numerous CD4+ T-cells that are highly susceptible to HIV-1; these cells are rapidly infected and depleted during acute infection[26–30]. Many reports have documented the importance of the GI tract as a reservoir for HIV-1/SIV, both before and during antiretroviral therapy[31–35]; accordingly, this mucosal tissue is of great interest for strategies to prevent or eradicate HIV-1 infection. Despite this, relatively few studies have addressed the nature and extent of antigen-specific T-cell responses in the GI tract. Early studies identified HIV-1-specific CD8+ T-cells in rectal and duodenal mucosa; these cells were able to kill HIV-1 antigen-pulsed targets in ^{51}Cr release assays [15,36]. Subsequently, epitope mapping of colorectal HIV-1-specific T-cell responses revealed significant overlap with CD8+ T-cell responses in blood[37,38]. However, closer examination of CD8+ T cells from the GI tract demonstrated that these cells contained significantly less perforin than their blood counterparts, suggesting compartment-specific differences in regulation of gene expression, and likely also in effector functions[39].

cART and Gastrointestinal HIV-1-specific T-cells.

Numerous studies, both in humans and experimentally infected nonhuman primates, have addressed the kinetics of gastrointestinal CD4+ T-cell depletion in HIV-1/SIV infection[26–30], and reconstitution of these cells following combination antiretroviral therapy (cART) [40–43]. The extent of CD4+ T-cell recovery during cART varies depending upon factors including CD4 nadir and time of cART initiation. Detailed review of this issue is beyond the scope of this article; however, the dynamics of CD4+ and CD8+ T-cell subsets are closely interrelated, and relevant to the topic of antigen-specific T-cell responses. Most reports of gastrointestinal CD4+ T-cell recovery have described CD4+ T-cell *frequency* as a percentage of all CD3+ T-cells, rather than using absolute numbers of CD4+ T-cells per unit area (or

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weight). Accordingly, these frequencies are affected by changes in the $CD8^+$ T-cell population. In a cross-sectional study, Allers and colleagues studied absolute numbers of duodenal $CD4^+$ T-cells at various stages of infection in individuals on or off cART[40]. Initiation of cART during acute, but not chronic infection was associated with preservation of gut $CD4^+$ T-cell numbers, reduced microbial translocation and decreased immune activation[40].

In a longitudinal study of cART initiation in individuals with chronic HIV-1 infection, beneficial effects of cART included partial rebound of $CD4^+$ T-cell percentages in blood, duodenal and colorectal mucosa, and decreased activation of $CD8^+$ T-cells[44]. Mucosal Gag-specific $CD8^+$ T-cell responses decreased significantly after cART initiation; this was anticipated based on prior reports of contraction of the circulating HIV-1-specific memory T-cell pool following cART[44]. In a related study of the same participant group, absolute numbers of $CD8^+$ T-cells declined in duodenum during cART, contributing significantly to the relative increase in $CD4^+$ T-cell percentages[45]. This finding is a reminder of the often-overlooked early observation that recruitment and/or expansion of $CD8^+$ T-cells occurs in the gastrointestinal lamina propria during HIV-1 infection and contributes substantially to the relative decrease in $CD4^+$ T-cell percentages[26].

Polyfunctional gastrointestinal $CD8^+$ T-cell responses and HIV-1 control.

With the development of multiparameter flow cytometry, allowing simultaneous detection of 10 analytes, more detailed analysis of T-cell function has become feasible. Analysis of colorectal T-cell responses in individuals with chronic HIV-1 infection revealed that strong, polyfunctional HIV-1 Gag-specific mucosal responses were frequently associated with low plasma viral load and well-preserved mucosal $CD4^+$ T-cells[46,47]. Elite Controllers, many of whom possessed “protective” MHC class I alleles HLA-B57 and/or B27, had particularly robust HIV-1 Gag-specific mucosal $CD8^+$ T-cell responses, co-expressing MIP-1β, TNFα, IFNγ and CD107a in response to *in vitro* stimulation[48,49]. Strikingly, in the same cohort, the frequency of HIV-1-specific polyfunctional mucosal $CD4^+$ T-cells was positively correlated with the magnitude of the mucosal $CD8^+$ T-cell response. Controllers with the strongest mucosal $CD4^+$ T-cell responses possessed class II HLA alleles DRB1*13 and/or DQB1*06, previously associated with an HIV-1 non-progression phenotype; all of these individuals also had Class I alleles associated with HIV-1 control[49]. Taken together, these findings suggest that polyfunctional mucosal T-cell responses contribute to immune control of HIV-1.

Paradoxically, colorectal $CD8^+$ T-cells exhibit low perforin expression and are weakly cytotoxic.

In contrast to their “polyfunctional” ability to produce multiple cytokines/chemokines and degranulate, colorectal $CD8^+$ T-cells from HIV-1-infected individuals rarely express *de novo* perforin in response to TCR stimulation [39,50–52]. In general, regardless of specificity, colorectal $CD8^+$ T-cells from both healthy and HIV-1-infected individuals exhibit low perforin expression and are significantly less able than blood $CD8^+$ T-cells to kill GFP-labelled target cells in redirected cytotoxicity assays[50]. In contrast to blood $CD8^+$ T-cells,

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perforin expression in colorectal CD8$^+$ T-cells is *not* elevated in Elite Controllers compared to other groups[50]. This comparatively weak expression of cytotoxic effector proteins has been associated with low expression of transcription factors T-bet and Eomesodermin, which are required for perforin-mediated cytotoxicity[50]. Importantly, these observations are consistent with independent reports demonstrating low perforin expression and weak cytolytic capacity of CD8$^+$ T-cells in human lymph node[53–55]. Taken together, these findings suggest a new paradigm in which robust cytotoxicity within the tissue microenvironment may be less beneficial for host defense than cytokine polyfunctionality.

Perforin expression is tightly regulated and maximal in tissues during acute/early infection.

Using the SIVmac model, two research groups independently documented that maximal expression of cytotoxic effector proteins in mucosal and lymphoid tissues occurs during acute/early infection, and declines rapidly thereafter[52,54]. Importantly, these findings imply loss of cytotoxic capacity during the transition from acute to chronic infection, notably within tissue sites of virus replication where the host response fails to clear persistent viral reservoirs. Studies of acute HIV-1 infection from a third group confirmed this observation, and further suggested that the early "blast" of perforin expression might actually contribute to gut epithelial damage[56]. Following this reasoning, restricted cytotoxicity might be an adaptation of tissue-resident T-cell populations to their microenvironment, with the goal of preserving barrier integrity.

It is important to note that subsequent studies of blood CD8$^+$ T-cells in acute HIV-1 infection cohorts have revealed that the cytotoxic capacity of circulating HIV-specific CD8$^+$ T-cells also changes soon after peak viremia[57]. As infection progresses to the chronic phase, perforin expression in blood is maintained in cells expressing high T-bet; however, the population of T-bet^{low} HIV-specific CD8$^+$ T-cells expands, with reduced expression of perforin during chronic infection[57]. Taken together, these studies clarify that perforin expression is tightly regulated. T-bet expression is reduced both in circulating HIV-specific CD8$^+$ T-cells during chronic infection, and more broadly, in CD8$^+$ T-cells that reside in lymphoid and mucosal tissues.

Tissue Residency and Immune Surveillance.

The adaptive immune system may be called upon to mount a response to antigenic challenge anywhere in the body. Mobilizing effective immune defenses therefore requires a system of lymphocyte migration that allows priming of antigen-specific cells; trafficking of antigen-experienced cells to the most relevant tissue sites of infection; and persistence of immunological memory. Nearly 20 years ago, Sallusto and Lanzavecchia proposed that memory T-cells could be subdivided into central memory cells (T_{CM}) circulating between blood and lymph nodes; and effector memory cells (T_{EM}) capable of defending "nonlymphoid" tissues including mucosal sites[58]. Originally it was thought that T_{EM} could recirculate between blood and tissues. Over the past decade, however, this paradigm has been revised by evidence establishing that some lymphocytes exist as "permanent residents" in nonlymphoid tissues[59–61]. Lymphocyte recirculation and residency have

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been analyzed using novel approaches including parabiosis, in which the circulatory systems of two laboratory animals are temporarily linked to allow circulation of non-resident cells between the animals[62,63]. Work from several groups has now established the existence of tissue resident lymphocytes in nearly all tissues studied, including the skin, gut, lung, reproductive tract, liver and others (reviewed in[64]).

The pathways leading to differentiation of tissue resident memory cells have been described in detail elsewhere[64,65]. Briefly, based upon evidence from rodent models, T_{RM} are believed to develop from killer cell lectin like receptor G1 (KLRG1)-negative effector T-cells following migration to peripheral tissues[66]. Within these tissues, exposure to locally produced cytokines, notably TGF-β, IL-15, IL-33 and TNFα, drives expression of early activation marker CD69 and intraepithelial tethering integrin $\alpha E(CD103)\beta_7$. These molecules promote tissue accumulation and retention, and have been considered hallmarks of the T_{RM} cell surface phenotype, although there are important exceptions to this generalization[62]. Although T-box transcription factors Eomesodermin and T-bet regulate $CD8^+$ T-cell development and effector functions, T_{RM} are typically T-bet^{Low} and Eomes^{Neg}[67,68].

Tissue Residents and Barrier Defense.

Unlike circulating effector memory $CD8^+$ T-cells, T_{RM} in the gastrointestinal tract appear to be maintained independently of cognate antigen for long periods of time. Situated near sites of initial pathogen exposure such as the skin or mucosal epithelium, T_{RM} can initiate rapid and robust immune defenses, notably cytokine production, which aid in mobilizing both innate and adaptive immunity. Paradigm-shifting studies in murine viral infections (notably LCMV and HSV-1) have revealed that T_{RM} can quickly detect infected cells and respond by producing cytokines (IFNγ, TNFα, and IL-2) that induce a tissue-wide antiviral state, promoting activation and/or recruitment of lymphocytes, dendritic cells and natural killer cells[69–72]. These studies provide a framework for understanding how T_{RM} cytokine polyfunctionality might provide an important advantage to the host, despite the comparatively weak cytotoxic capacity that results from low expression of T-bet and Eomes.

Re-assessing T-cell numbers and distribution in tissues.

Because of the logistical difficulties and ethical concerns inherent in obtaining fresh tissue samples from human study participants, most studies of tissue leukocytes in HIV-1 disease have relied on small tissue samples such as biopsies or surgical explants, from which leukocyte suspensions were generated by enzymatic digestion. While this approach has many advantages, it can also lead to misconceptions about the abundance and localization of tissue leukocytes *in vivo*. In a recent study, Steinert and colleagues compared two approaches for recovering memory T-cells from mouse tissues: enzymatic digestion to generate single-cell suspensions; and tissue sectioning followed by quantification of leukocyte subsets by microscopy[62]. These studies revealed that many tissue-resident T-cells do not express CD69 and/or CD103. Perhaps more surprisingly, evaluation of tissue sections by quantitative immunofluorescence microscopy revealed that the number of tissue-resident T-cells present in certain tissues was significantly greater than predicted from

single-cell suspensions, with resident T-cells greatly outnumbering recirculating cells[62]. This study provided an important cautionary lesson: over-reliance on single-cell suspensions, without complementary microscopy data, can lead to serious misconceptions about the nature and abundance of tissue leukocytes.

T_{RM} in HIV-1 infection.

To date, there have been limited studies of T_{RM} in humans; however, a core transcriptional signature for human T_{RM} has been reported and includes molecules involved in adhesion, migration and regulation that are similar to those described in rodent T_{RM}[73]. Buggert and colleagues found that HIV-1-specific $CD8^+$ T-cells within lymph nodes were predominantly T_{RM}, and elite controllers showed higher LN/blood ratios of HIV-specific $CD8^+$ T-cells compared to other HIV-1-positive participants[74]. In gene expression analyses, HIV-1-specific T_{RM} were more likely to express cytolytic proteins compared to non-T_{RM} from the same tissues[74]. This observation is intriguing given earlier results showing reduced cytotoxic capacity of $CD8^+$ T-cells from lymph node compared to those isolated from blood[53].

Kiniry and colleagues identified HIV-1-specific $CD8^+$ T-cells with resident memory (T_{RM}) and resident effector (rT_{EFF}) phenotypes in colorectal mucosa[75]. HIV-1-Gag-specific tissue-residents included polyfunctional cells that degranulated and produced MIP-1β, IFNγ, and in some cases TNFα in response to TCR stimulation[75]. These cells likely contribute to host defense against HIV-1, but the full extent of their contribution relative to that of non-resident populations awaits further study. In future studies, it will be important to compare the abundance and functionality of T_{RM} isolated from multiple tissue types, including gastrointestinal and reproductive mucosae as well as lymphoid tissues, in order to fully appreciate their distinct roles in host defense.

CONCLUSIONS

Mucosal tissues serve not only as the major sites of HIV-1 transmission, but also as important sites of viral replication and potential viral reservoirs during antiretroviral therapy. Although historically the vast majority of work on host immunity has focused on responses detected in peripheral blood, there is increasing awareness of the importance of mucosal tissues in determining the overall host-pathogen balance. In particular, the recent characterization of non-recirculating tissue-resident memory cells is prompting a re-examination of mucosal T-cell populations and their role in host defense. In the coming years, advances in high-definition flow cytometry and mass cytometry, imaging technologies, single-cell gene expression analysis and bioinformatics are expected to yield significant new insights regarding the nature of viral reservoirs and the characteristics of immune cells residing nearby. Ultimately such insights will be critical for designing improved strategies for HIV vaccination and reservoir eradication.

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KEY POINTS:

- Mucosal tissues serve as the major sites of HIV-1 transmission, as well as important sites of viral replication and potential viral reservoirs.
- Historically most work on host immunity has focused on responses in peripheral blood, but there is increasing awareness of the importance of mucosal tissues in determining the overall host-pathogen balance.
- The recent characterization of non-recirculating tissue-resident memory cells is prompting a re-examination of mucosal T-cell populations and their role in host defense.

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TABLE 1.

Key molecules differentially expressed by T_{RM} compared to circulating T-cells.

Category	Marker	Observations[1]	Function	References (including reviews)
Transcription Factors	**T-bet**	Downregulated in T_{RM}	Downregulation required for TGF-β responsiveness	[50, 64, 67]
	Eomes	Downregulated in T_{RM}	Downregulation required for TGF-β responsiveness	[50, 64, 67]
	KLF2	Downregulated in T_{RM}	Downregulation required for inhibition of S1PR1 and inhibition of tissue egress	[59, 64, 73]
	Hobit, Blimp1	Upregulated in mouse T_{RM}	Loss prevents T_{RM} development in mice; unclear if this holds true in humans	[64, 74]
Adhesion/Migration	**CD69**	Expressed by 50–90% of T_{RM}; varies between tissues	Downregulates S1PR1, preventing tissue egress	[59, 64, 73, 74, 75]
	CD103	Expressed mainly by oral-GI tract T_{RM}	Mucosal tethering integrin; expression varies between tissues	[59, 60, 64, 73, 74, 75]
	S1PR1	Downregulated in T_{RM}	Required for tissue egress	[59, 64, 68, 73, 74, 75]
	CD49a	Upregulated in T_{RM}	Adhesion marker	[64, 73]
	CCR7	Downregulated in T_{RM}	Required for lymph node homing; expressed on central memory cells	[59, 64, 73, 74]
	CD62L	Downregulated in T_{RM}	Required for lymph node homing; expressed on central memory cells	[59, 64, 73, 74]
Inhibitory Molecules	**PD-1**	Upregulated in T_{RM}	Inhibits T-cell activation; potentially limits inflammation-induced tissue damage	[73, 74]
	IL-10	Upregulated in T_{RM}		[73]
Activation-induced responses	**IL-2**	Expressed by higher proportions of stimulated T_{RM} vs non-T_{RM}	Stimulates NK cells and bystander memory CD8+ T-cells	[65, 71, 73]
	IFN-γ		Indirectly promotes recruitment of other immune cells (T, B); Induces broad innate antiviral responses	[65, 69, 71, 73, 74]
	Perforin	Expressed by higher proportions of T_{RM} vs non- T_{RM}, but also by higher proportions of blood vs tissue T-cells	Cytolytic capacity greater in T_{RM} vs non-T_{RM} from same tissues; greater in blood vs tissue T-cells	[50, 53, 74]

[1] as compared to expression in circulating or non-resident T-cells.