HIV

Resident T cells stand up to HIV

Nadia R. Roan[1,2]*

HIV-specific resident memory T cells are abundant in lymphoid tissues of elite controllers and exhibit distinct functional properties.

$CD8^+$ T cells are required for effective immune control of HIV, and a variety of $CD8^+$ T cell phenotypes and functions correlate with protection from disease progression. Most of this knowledge came from studies characterizing peripheral blood of HIV-infected individuals. However, HIV infection occurs primarily in lymphoid tissues (LTs), where >98% of the body's $CD4^+$ T cells are found. A more complete view of $CD8^+$ T cell–mediated protection against HIV requires understanding the properties of these effector cells in LTs. LT T cells differ markedly from T cells in blood, one major difference being their encompassing a particular population of T cells called resident memory T cells (T_{RM}s). T_{RM}s are present not only in LTs but also in a number of other tissues, including the skin, lung, and female reproductive tract (*1*). In this issue of *Science Immunology*, Buggert *et al.* (*2*) demonstrate that HIV-specific $CD8^+$ T_{RM}s from LTs have distinct attributes and are more frequent during natural control of HIV, suggesting that T_{RM}s may play an important role in limiting HIV replication.

T_{RM}s in mice reside in tissues and do not recirculate, as demonstrated through a series of elegant parabiosis experiments (*3*, *4*). Because it is not feasible to establish tissue residence of T cells in humans, human T_{RM}s are defined according to phenotypic and functional similarities with mouse T_{RM}s. CD69 expression is commonly used as marker for identification of T_{RM}s in both mice and humans (*5*). To characterize $CD8^+$ T_{RM}s during HIV infection, the authors analyzed CD69 expression on lymph node (LN) $CD8^+$ T cells from HIV^+ and HIV^- individuals and found that expression of CD69 was higher in LNs obtained from infected individuals. Because CD69 is also a marker of early T cell activation, it was important to show that increased expression was not due to increased $CD8^+$ T cell activation. Therefore, they examined coexpression of CD69 with Ki-67 (a marker of cycling and recently activated cells) on $CD8^+$ T cells from LNs of acutely infected individuals and found that most $CD69^+$ T cells in LTs did not coexpress Ki-67. Consistent findings were observed in a simian immunodeficiency virus (SIV) infection model, in which the authors observed that CD69 expression on SIV-specific $CD8^+$ T cells in LTs did not associate with Ki-67. These findings, together with their observation that CD69-expressing $CD8^+$ T cells are more frequent in LN than in blood, suggest that CD69 expression in LTs is associated with residence in the LN.

The authors set out to better characterize $CD69^+$ T cells in LTs using mass cytometry. They confirmed no close association of CD69 expression with markers of T cell activation on $CD8^+$ T cells from a variety of LTs of uninfected individuals. Rather, CD69 tended to be coexpressed with select integrins including CD103, another marker commonly used to identify T_{RM}s (*1*), and some chemokine receptors including CXCR5, which directs cells to LN follicles. For an even deeper view, they conducted bulk RNA sequencing (RNA-seq) on sorted $CD69^-$ versus $CD69^+CD8^+$ T cells from mesenteric LN of uninfected individuals. Comparing the transcriptional signature of $CD69^+CD8^+$ T cells with established transcriptional signatures of T_{RM}s from mice revealed similarities, including decreased S1PR1 expression. Interestingly, Hobit, one of the two transcription factors associated with T_{RM} formation in mice (*6*), was not preferentially expressed in $CD69^+$ T_{RM}s, suggesting that human T cells might use different transcription factors to drive LT T_{RM} formation. Comparing the $CD69^+CD8^+$ T cell signature established in this study with that from splenic $CD69^+CD8^+$ T cells (*5*) revealed similarities, suggesting that common transcriptional signatures associated with T_{RM}s from different LTs.

Epigenetic regulation may affect cellular identity and functions in ways not captured by transcriptional analysis, leading the authors to conduct ATAC-seq (assay for transposase-accessible chromatin with high-throughput sequencing) to compare areas of open chromatin in the sorted $CD69^-$ versus $CD69^+CD8^+$ LN T cells. The identified patterns suggested that $CD69^+$ cells exhibited features of T_{RM}s. For example, $CD69^-$ cells exhibited greater chromatin accessibility across the *S1PR1* locus, which is consistent with the ability of these cells to egress from tissues, whereas $CD69^+$ cells exhibited greater chromatin accessibility at the PRDM1 locus, which encodes Blimp1, a transcription factor that associates with Hobit to drive T_{RM} formation in mice (*6*). Notably, a previous study on human lung T_{RM}s, as defined by CD103 expression, did not find preferential expression of Blimp1 in lung T_{RM}s (*7*). Whether Blimp1 is particularly important for generation and/or maintenance of T_{RM}s in human LTs requires further study.

As a final justification that CD69 can be used as a marker of LT $CD8^+$ T_{RM}s, the authors examined CD69 expression on efferent lymph cells, which are unlikely to be T_{RM}s because they have egressed from LTs. Thoracic duct lymph (TDL) from HIV^- individuals lacked CD69 expression, which is consistent with CD69 as a marker of T_{RM}s. The authors also used histocytometry to determine in which regions of the LN $CD69^+$ cells primarily reside. Whereas $CD69^+CD8^+$ T cells resided primarily in extrafollicular regions, the smaller subset of these cells additionally expressing CD103 and PD1 was equally distributed in follicles and extrafollicular areas. These results suggest that although most $CD8^+$ T_{RM}s localize to the extrafollicular regions, the follicles are not devoid of these cells.

Having established CD69 as a marker of $CD8^+$ T_{RM}s in LTs, the authors then characterized HIV-specific $CD8^+$ T_{RM}s with tetramers. Like bulk $CD8^+$ T cells, HIV-specific $CD8^+$ T cells expressing CD69 were preferentially found in LTs over blood and TDL. These cells were also more abundant than T cells specific for cytomegalovirus (which, unlike HIV, does not primarily replicate in LTs), suggesting that active viral replication in LTs may drive

[1]Department of Urology, University of California, San Francisco, San Francisco, CA 94158, USA. [2]Gladstone Institute of Virology and Immunology, University of California, San Francisco, San Francisco, CA 94158, USA.
*Corresponding author. Email: nadia.roan@ucsf.edu

virus-specific T_{RM} formation. Furthermore, HIV-specific LT T_{RMs} were more cytolytic than their $CD69^-$ counterparts. This observation is interesting in light of previous observations made by the same group that LT HIV-specific $CD8^+$ T cells are less cytolytic than those of blood (*8*). Whether these differences in cytolytic activity result from differences in subset distribution is unclear. To further assess the properties of the HIV-specific T_{RMs}, the authors determined whether these cells express CXCR5, a receptor involved in follicle homing, which may be important for immune-mediated clearance of HIV-infected cells in LTs. Most HIV-specific $CXCR5^+CD8^+$ T cells expressed high levels of CD69, suggesting that HIV-specific T_{RMs} are capable of migrating into the follicles.

Do LT $CD8^+$ T_{RMs} help control HIV replication? To address this, the authors compared proportions of HIV-specific T_{RMs} between chronic progressors and elite controllers (ECs), the latter being individuals who exhibit natural immune-mediated control of HIV, most commonly through $CD8^+$ T cell responses. ECs exhibited higher frequencies of HIV-specific $CD8^+$ T_{RMs} than chronic progressors, suggesting that T_{RMs} are important in natural control of HIV. To further characterize these cells, the authors conducted single-cell RNA-seq. Consistent with the bulk RNA-seq experiments from HIV^- individuals, compared with their $CD69^-$ counterparts, HIV-specific T_{RMs} had lower levels of S1PR1, again suggesting tissue residence, and were enriched for transcripts that correlated with enhanced cytolytic and effector functions. These observations suggest that T_{RMs} may be poised to mount a rapid effector response against infected cells encountered in LTs. However, given that most $CD8^+$ T_{RMs} reside in extrafollicular regions, many of them may not be anatomically positioned to eliminate the major reservoir of HIV-infected $CD4^+$ T cells, which reside within the follicles. It would be of interest to determine whether the subpopulation of CXCR5-expressing HIV-specific $CD8^+$ T_{RMs} resides in the follicles and can mount a potent effector response in a manner associated with viral control.

This study has two important implications. First, it suggests that LT T_{RMs} exist in humans, something that has been previously documented only in mice (*3*). Although it was not proven that CD69-expressing $CD8^+$ T cells from LTs are noncirculating, a difficult endeavor in humans, that these cells exhibit transcriptional and epigenetic features of T_{RMs} and are relatively absent in blood and TDL suggest that they may be bona fide T_{RMs}. Furthermore, examination of T cell receptor clonotype distribution of HIV-specific T_{RMs} from LTs of ECs revealed that these cells can exhibit clonotypic disequilibrium with their blood counterparts, arguing against the notion that these cells are free to circulate.

The second important implication is that HIV-specific T_{RMs} may be important in viral control because ECs harbor a higher frequency of these cells than chronic progressors (Fig. 1). Much remains to be answered with regard to how T_{RMs} control infection. Is control simply a consequence of LT T_{RMs} being more abundant in ECs than chronic progressors? Or are there functional differences in LT T_{RMs} between ECs and progressors? It may be that during disease progression, LT T_{RMs}, like their blood counterparts, become exhausted and dysfunctional. Of particular interest will be to determine whether the HIV-specific $CXCR5^+$ T_{RMs} from ECs exhibit distinct functional attributes that allow them to better control viral replication within the follicles, where most T follicular helper cells, a major reservoir for HIV, reside. Of equal interest will be the functional properties of other effector and memory T cells from ECs besides T_{RMs}. Because these cells have signatures distinct from T_{RMs}, do they play nonoverlapping roles in controlling HIV replication in LTs, and, if so, what exactly are these roles? Last, although HIV-specific T_{RMs} in mucosal tissues were not examined in this study, given that T cells in LN and mucosa are different (*9*) and that high levels of HIV replication occur in mucosal tissues, particularly the gut, future studies should compare the properties of mucosal T_{RMs} with their LN counterparts and investigate their respective roles in viral control. Now that we know that HIV-specific T_{RMs} have distinct attributes, gaining a better understanding of how they protect against disease progression is essential for us to harness the full potential of these powerful effector T cells that are already sitting at the primary sites of HIV replication.

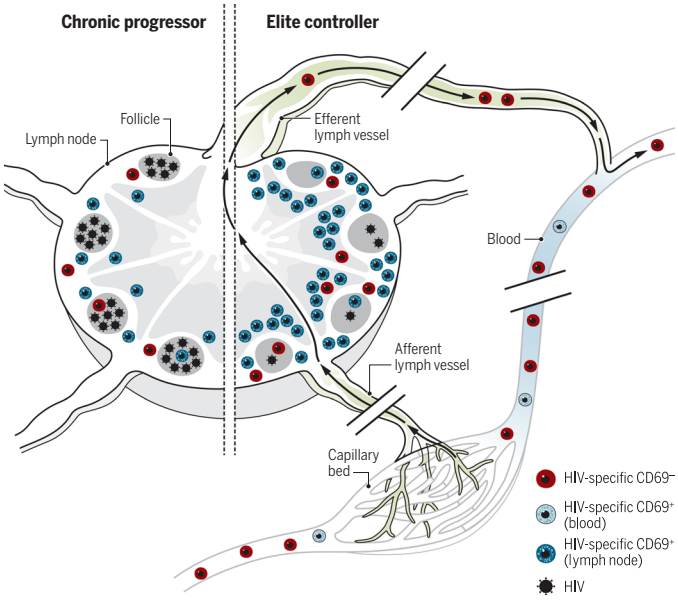

Fig. 1. Model showing the potential role of HIV-specific T_{RMs} in natural control of HIV. Circulating HIV-specific $CD8^+$ T cells move between blood and LTs via the afferent and efferent lymph. HIV-specific $CD8^+$ T cells expressing CD69 are more frequent in LTs than in blood and efferent lymph and have signatures associated with T_{RMs}. Within LT, HIV-specific T_{RMs}, as defined by CD69 expression, are more abundant and exhibit more cytolytic properties than those of their $CD69^-$ counterparts. The spatial distribution of HIV-specific T_{RMs} in the LN is not clear, but bulk $CD8^+$ T_{RMs} were found to be partially excluded from the follicles where HIV replication predominantly occurs. The study by Buggert *et al.* suggests that HIV-specific $CD8^+$ T_{RMs} are more abundant in ECs (right) than in chronic progressors (left), suggesting a role for T_{RMs} in natural control of HIV.

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