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# Mucosa-Associated Invariant T Cells Are Systemically Depleted in Simian Immunodeficiency Virus-Infected Rhesus Macaques

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## ABSTRACT

**Mucosa-associated invariant T (MAIT) cells contribute to host immune protection against a wide range of potential pathogens via the recognition of bacterial metabolites presented by the major histocompatibility complex class I-related molecule MR1. Although bacterial products translocate systemically in human immunodeficiency virus (HIV)-infected individuals and simian immunodeficiency virus (SIV)-infected Asian macaques, several studies have shown that MAIT cell frequencies actually decrease in peripheral blood during the course of HIV/SIV disease. However, the mechanisms underlying this proportional decline remain unclear. In this study, we characterized the phenotype, activation status, functionality, distribution, and clonotypic structure of MAIT cell populations in the peripheral blood, liver, mesenteric lymph nodes (MLNs), jejunum, and bronchoalveolar lavage (BAL) fluid of healthy and SIV-infected rhesus macaques (RMs). Low frequencies of MAIT cells were observed in the peripheral blood, MLNs, and BAL fluid of SIV-infected RMs. These numerical changes were coupled with increased proliferation and a highly public T cell receptor alpha (TCRα) repertoire in the MAIT cell compartment without redistribution to other anatomical sites. Collectively, our data show systemically decreased frequencies of MAIT cells likely attributable to enhanced turnover in SIV-infected RMs. This process may impair protective immunity against certain opportunistic infections with progression to AIDS.**

## IMPORTANCE

**The data presented in this study reveal for the first time that MAIT cells are systemically depleted in an AIDS virus infection. These findings provide a new mechanistic link with our current understanding of HIV/SIV pathogenesis and implicate MAIT cell depletion in the disease process.**

Mucosa-associated invariant T (MAIT) cells are relatively abundant in humans, comprising 1 to 10% of peripheral blood T cells (1–3) and up to 45% of liver lymphocytes (4, 5). Lower frequencies are present in the gastrointestinal (GI) tract, lung, and mesenteric lymph nodes (MLNs) (2, 6). Classically defined by the expression of a semi-invariant TRAV1-2/TRAJ33 (Vα7.2/Jα33) T cell receptor alpha (TCRα) chain (7), MAIT cells recognize microbial vitamin $B_2$ metabolites presented in association with the major histocompatibility complex class I-related molecule MR1 (8–12). These conserved features bestow widespread reactivity against an array of bacterial and fungal species (13, 14), allowing MAIT cells to act as innate-like antimicrobial guardians at mucosal sites via the secretion of proinflammatory and tissue-protective cytokines, such as interleukin 17 (IL-17), tumor necrosis factor (TNF), and gamma interferon (IFN-γ) (2, 9).

The abundance of MAIT cells in peripheral blood and mucosal tissues, combined with their broad reactivity and functional properties, suggests a key role in primary immune defense and various pathological states (2, 9). Indeed, multiple reports have described a loss of circulating MAIT cells in diseases with an inflammatory component, such as obesity and type II diabetes (15), inflammatory bowel disease (16), tuberculosis (2, 17), and human immunodeficiency virus (HIV) disease (18–20). MAIT cells also appear to be highly activated under these conditions and may be recruited to tissue sites of inflammation (15, 16, 19).

Although MAIT cells are neither directly activated nor directly infected by HIV (20), previous studies have consistently demonstrated selective depletion of this subset in the peripheral blood of HIV-infected individuals and simian immunodeficiency virus (SIV)-infected Asian macaques (18–20). However, the underlying mechanisms remain unclear. It is established that $CD4^+$ T cells are lost in the GI tract during HIV/SIV infection (21, 22). Moreover, epithelial integrity is compromised by the associated immunopathology, leading to microbial translocation and systemic immune activation (23, 24). This process could feasibly drive MAIT cell

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activation, cytokine secretion, and potential migration to sites of inflammation and/or bacterial/fungal infiltration.

In this study, we conducted a comprehensive analysis of MAIT cell populations across multiple anatomical sites in healthy and SIV-infected rhesus macaques (RMs). Our data reveal a systemic loss of MAIT cells likely attributable to increased turnover in the setting of SIV infection. These findings provide a mechanistic link with our current understanding of HIV/SIV pathogenesis and implicate MAIT cell depletion in the disease process.

## MATERIALS AND METHODS

**Animals.** The study cohort comprised 29 SIV-infected RMs (10 chronically infected with SIVsmE660, 5 chronically infected with SIVsmE543, 8 chronically infected with SIVmac239, and 6 with SIVmac239-associated simian AIDS [sAIDS]) and 25 SIV-uninfected RMs (Table 1). Peripheral blood mononuclear cells (PBMCs) were isolated by standard density gradient centrifugation. Bronchoalveolar lavage (BAL) fluid samples were filtered, centrifuged at 1,200 rpm for 8 min, and resuspended in complete medium. MLN, jejunum, and liver samples were processed into single-cell suspensions as described previously (25). All animals were housed in certified facilities and maintained in accordance with standards recommended by the American Association for the Accreditation of Laboratory Animal Care. All procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of the National Institute of Allergy and Infectious Diseases (LMM6 and LMM12).

**Viral loads.** Viral RNA levels in plasma were determined by real-time reverse transcription (RT)-PCR using the ABI Prism 7700 sequence detection system (Applied Biosystems, Carlsbad, CA, USA).

**Flow cytometry.** Multicolor flow cytometric experiments were performed using predetermined optimal concentrations of monoclonal antibodies (MAbs) with specific cross-reactivity against RM antigens (Ags). PBMCs and BAL fluid samples were processed immediately after isolation; MLN, jejunum, and liver samples were cryopreserved before analysis.

For phenotypic characterization, cells were incubated first with a phycoerythrin (PE)-conjugated active-ligand human MR1 tetramer [MR1-5-OP-RU; 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil] (8, 12) in the presence of a CCR6-specific MAb (clone G034E3; BioLegend, San Diego, CA). After subsequent staining with LIVE/DEAD fixable Aqua (Life Technologies, Grand Island, NY), the cells were incubated with MAbs against CD3 (clone SP34-2; BD Pharmingen, San Diego, CA), CD8 (clone RPA-T8; BioLegend), CD28 (clone CD28.2; Beckman Coulter, Brea, CA), CD45 (clone D058-1283; BD Horizon, San Jose, CA), CD69 (clone FN50; BD Pharmingen), and CD95 (clone DX2; BioLegend). The cells were then washed, fixed/permeabilized with Cytofix/Cytoperm buffer (BD Biosciences, San Jose, CA), and stained intracellularly with MAbs against Ki67 (clone B56; BD Pharmingen) or PLZF (clone R17-809; BD Pharmingen).

For intracellular cytokine staining, cells were stimulated overnight at 37°C with phorbol myristate acetate (PMA) (5 ng/ml) and ionomycin (1 μM) in the presence of brefeldin A (1 μg/ml; Sigma-Aldrich, St. Louis, MO). The cells were then washed twice and stained with tetramer and surface-directed MAbs as described above (excluding CD69). After a further wash, the cells were fixed/permeabilized and stained intracellularly with MAbs against IL-17 (clone eBio64DEC17; Affymetrix eBioscience, San Diego, CA), IFN-γ (clone 4S.B3; Affymetrix eBioscience), and TNF (clone MAb11; BD Pharmingen).

At least 300,000 cells per condition were acquired using a BD LSRFortessa flow cytometer driven by FACSDiva software version 6.0 (BD Biosciences). Data were analyzed with FlowJo software version 9.4.11 (TreeStar, Ashland, OR) using a threshold of 200 collected events for each cell subset.

**Clonotype analysis.** Viable MR1 tetramer$^+$ CD8$^+$ memory CD3$^+$ T cells were sorted directly into 100 μl RNA later (Applied Biosystems) using a modified BD FACSAria flow cytometer (BD Biosciences). The median number of sorted cells was 1,550 (range, 150 to 3,000 cells) for PBMCs and 1,700 (range, 772 to 1,845 cells) for BAL fluid. Clonotypic analysis was performed as described previously (26, 27). Briefly, all expressed *TRA* gene rearrangements were amplified using an anchored template-switch RT-PCR. The products were then subcloned, sampled, sequenced, and analyzed (26, 28, 29). Rhesus macaque TRAV and TRAJ sequences were assigned according to the closest human equivalent using the international ImMunoGeneTics (IMGT) nomenclature.

**Sequence logo generation.** Sequence logos were generated in Shannon format using the Seq2Logo webserver (http://www.cbs.dtu.dk/biotools/Seq2Logo/). Each CDR3α sequence was represented per actual occurrence in the total dataset.

**Statistical analysis.** Groups were compared using the Mann-Whitney *U* test. Correlations were verified using the Spearman rank test. All analyses were performed with Prism software version 6.0f (GraphPad, La Jolla, CA).

## RESULTS

**Identification of MAIT cells in rhesus macaque peripheral blood.** The recent development of epitope-loaded human and mouse MR1 tetramers has enabled the accurate identification of antigen-specific MAIT cells directly *ex vivo* (6, 12, 30, 31). We took advantage of the fact that MR1 is highly conserved in mammals (32) to quantify and characterize a distinct population of tetramer-reactive MAIT cells in the peripheral blood and tissues of RMs. These cells were rigorously defined as MR1-5-OP-RU tetramer$^+$ CD8$^+$ memory (CD28$^{+/-}$ CD95$^+$) CD3$^+$ T lymphocytes for comparative purposes (Fig. 1A). This strategy captures the vast majority of tetramer-reactive MAIT cells in RMs. However, species-matched reagents may be required to detect low-avidity MAIT cell populations. In addition, we analyzed MAIT, non-MAIT CD8$^+$, and CD8$^-$ T cells for expression of Ki67, CD28, CD69, CCR6, PLZF, IL-17, IFN-γ, and TNF (Fig. 1B to F). MAIT cells from the peripheral blood of RMs readily produced IL-17, IFN-γ, and TNF in response to stimulation with PMA and ionomycin (Fig. 1E and F).

**Peripheral blood MAIT cell decline and activation in SIV infection.** Given multiple previous reports of peripheral blood MAIT cell loss in inflammatory disease models (2, 15–20), we assessed circulating MAIT cell frequencies in healthy and SIV-infected RMs. Lower frequencies and absolute numbers of MAIT cells were detected in the peripheral blood of SIV-infected versus SIV-uninfected RMs (Fig. 2A and B). The median frequency of tetramer-reactive MAIT cells in the peripheral blood of SIV-uninfected RMs was 1.58% (range, 0.3 to 4.8%; $n = 16$), whereas the median frequency of these cells in the peripheral blood of SIV-infected RMs was 0.43% (range, <0.1 to 1.9%; $n = 16$). These frequencies are notably lower than those detected in humans (1 to 10%) but much higher than those detected in mice (30).

Immune activation and dysregulation are common hallmarks of HIV/SIV infection. We therefore assessed markers of proliferation (Ki67) and differentiation (CD28) in the tetramer-reactive MAIT cell compartment. MAIT cells displayed a less differentiated memory phenotype (percent CD28$^+$) than non-MAIT CD8$^+$ T cells in both SIV-uninfected and SIV-infected RMs ($P < 0.0001$) (Fig. 2C). A trend was also observed toward loss of CD28 expression in SIV-infected compared with SIV-uninfected RMs ($P = 0.0670$) (Fig. 2C). Possible explanations for the decrease in circu-

**TABLE 1** Study animal characteristics

| Animal | Infection status | Tissue(s) studied[a] | Disease state[b] | Plasma viremia[c] | $CD4^+$ T cell count[d] |
|---|---|---|---|---|---|
| 634 | $SIV^-$ | Jej | NA | 0 | 747 |
| DB7H | $SIV^-$ | Jej | NA | 0 | 526 |
| DBAA | $SIV^-$ | Liver | NA | 0 | Not available |
| M03 | $SIV^-$ | Liver | NA | 0 | Not available |
| DBV1 | $SIV^-$ | MLN | NA | 0 | 693 |
| DBXG | $SIV^-$ | MLN | NA | 0 | 696 |
| 485 | $SIV^-$ | MLN, Jej | NA | 0 | 269 |
| 4016 | $SIV^-$ | MLN, Jej | NA | 0 | 656 |
| DA6A | $SIV^-$ | MLN, Jej | NA | 0 | 472 |
| DCJWA | $SIV^-$ | PBMC | NA | 0 | *523* |
| DCVF | $SIV^-$ | PBMC | NA | 0 | *616* |
| DE1A | $SIV^-$ | PBMC | NA | 0 | *352* |
| DE20 | $SIV^-$ | PBMC | NA | 0 | *493* |
| DEN8 | $SIV^-$ | PBMC | NA | 0 | *707* |
| DFAi | $SIV^-$ | PBMC | NA | 0 | *879* |
| DCAV | $SIV^-$ | PBMC, BAL | NA | 0 | *632* |
| DCBC | $SIV^-$ | PBMC, BAL | NA | 0 | *382* |
| DCMV | $SIV^-$ | PBMC, BAL | NA | 0 | *711* |
| DCZ6 | $SIV^-$ | PBMC, BAL | NA | 0 | *1485* |
| DE2C | $SIV^-$ | PBMC, BAL | NA | 0 | *254* |
| DE2W | $SIV^-$ | PBMC, BAL | NA | 0 | *884* |
| 37033 | $SIV^-$ | PBMC, BAL | NA | 0 | *561* |
| 37034 | $SIV^-$ | PBMC, BAL | NA | 0 | *1453* |
| 37073 | $SIV^-$ | PBMC, BAL | NA | 0 | *298* |
| 37360 | $SIV^-$ | PBMC, BAL | NA | 0 | *258* |
| 591 | SIVsmE543 | Jej | Chronic | 2.51E5 | 186 |
| 594 | SIVsmE543 | Jej | Chronic | 8.60E3 | 292 |
| 597 | SIVsmE543 | Jej | Chronic | 1.60E3 | 484 |
| DCKG | SIVmac239 | Jej | sAIDs | 6.58E5 | 312 |
| 833 | SIVsmE543 | Liver | Chronic | 1.45E5 | 52 |
| 881 | SIVsmE543 | Liver | sAIDs (*Candida*, SV40) | 1.03E6 | 95 |
| DBPX | SIVsmE660 | Liver | Chronic | Undetected | 304 |
| PSP1010 | SIVmac239 | Liver | sAIDs | 7.80E6 | 194 |
| CE5D | SIVmac239 | MLN | sAIDs | 1.20E5 | 532 |
| CF4J | SIVmac239 | MLN | sAIDs (parainfluenza) | 2.00E5 | 241 |
| CF5T | SIVmac239 | MLN | sAIDs (*Streptococcus bovis*) | 8.00E5 | 216 |
| DB17 | SIVmac239 | MLN, Jej | sAIDs (likely pneumocystis pneumonia) | 9.20E4 | 122 |
| DB4E | SIVmac239 | MLN, Jej | Chronic | 8.10E5 | 565 |
| 851 | SIVsmE660 | PBMC | Chronic | 2.34E5 | *273* |
| 853 | SIVsmE660 | PBMC | Chronic | 7.77E4 | *742* |
| 859 | SIVsmE660 | PBMC | Chronic | 7.80E6 | *319* |
| DE1A | SIVmac239 | PBMC | Chronic | 8.23E4 | *197* |
| DE2W | SIVmac239 | PBMC | Chronic | 1.90E5 | *139* |
| CL7P | SIVmac239 | PBMC | Chronic | 7.99E3 | *342* |
| CL4C | SIVmac239 | PBMC | Chronic | 4.63E5 | *90* |
| DCJWA | SIVmac239 | PBMC | Chronic | 3.17E3 | *672* |
| 848 | SIVsmE660 | PBMC, BAL | Chronic | 1.40E6 | *140* |
| 849 | SIVsmE660 | PBMC, BAL | Chronic | 3.79E5 | *290* |
| 850 | SIVsmE660 | PBMC, BAL | Chronic | 1.43E6 | *159* |
| 856 | SIVsmE660 | PBMC, BAL | Chronic | 3.00E5 | *27* |
| 860 | SIVsmE660 | PBMC, BAL | Chronic | 9.70E5 | *88* |
| 861 | SIVsmE660 | PBMC, BAL | Chronic | 1.46E5 | *342* |
| ZG24 | SIVmac239 | PBMC, BAL | Chronic | 1.99E4 | *239* |
| ZA52 | SIVmac239 | PBMC, BAL | Chronic | 2.94E5 | *171* |

[a] Jej, jejunum; BAL, BAL fluid.
[b] Opportunistic infections at the time of sampling are shown in parentheses. NA, not applicable; SV40, simian virus 40.
[c] Number of copies of viral RNA per milliliter of plasma.
[d] Counts in italics were calculated as the number of $CD8^-$ $CD3^+$ T cells per microliter of blood; all others were calculated as the number of $CD4^+$ T cells per microliter of blood.

lating MAIT cells during HIV/SIV infection include limited proliferative capacity and increased turnover. Consistent with the latter scenario, we found that MAIT cells more frequently expressed Ki67 in the peripheral blood of SIV-infected versus SIV-uninfected RMs ($P = 0.0005$) (Fig. 2D). In the absence of SIV infection, MAIT cells less commonly expressed Ki67 in the periphery than did non-MAIT $CD8^+$ T cells ($P = 0.0008$) (Fig. 2D). In contrast, Ki67 expression frequencies were similar for both MAIT and

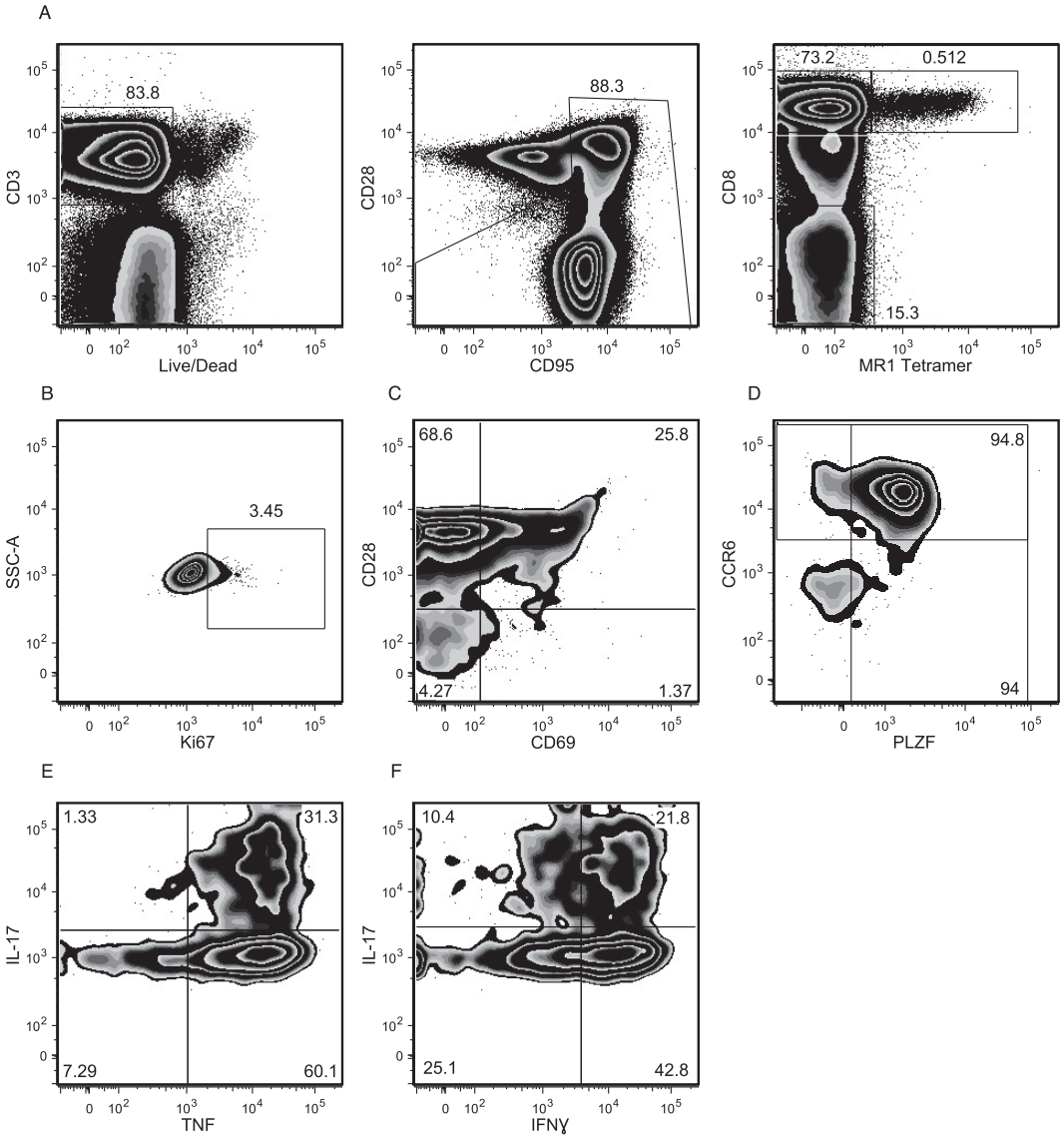


**FIG 1** Identification of MAIT cells in RMs. (A) MAIT cells were characterized as viable MR1-5-OP-RU tetramer$^+$ CD8$^+$ memory (CD28$^{+/-}$ CD95$^+$) CD3$^+$ T lymphocytes. (B to D) Representative flow cytometry plots showing expression of Ki67 ($n = 20$) (B), CD28 and CD69 ($n = 20$) (C), and CCR6 and PLZF ($n = 10$) (D) within the MAIT cell population. (E and F) Representative flow cytometry plots showing expression of IL-17 and TNF (E) and IL-17 and IFN-γ (F) by MAIT cells after overnight stimulation with PMA and ionomycin ($n = 6$).

non-MAIT CD8$^+$ T cells in the context of SIV infection (~20%) (Fig. 2D).

**Peripheral blood MAIT cell trafficking and regulation in SIV infection.** The loss of peripheral blood MAIT cells in SIV-infected RMs could potentially reflect increased trafficking to the gut mucosa in response to microbial translocation. However, we found reduced frequencies of CCR6$^+$ MAIT cells in SIV-infected versus SIV-uninfected RMs ($P = 0.0317$) (Fig. 2E). This observation is consistent with previous studies showing lower frequencies of circulating CCR6$^+$ MAIT cells in HIV-infected patients (17, 18, 20). The migration of MAIT cells to tissue sites of inflammation may therefore be impaired in the context of HIV/SIV infection.

PLZF has recently been shown to regulate CCR6 (33); it is also known to be expressed in the majority of MAIT cells (30). PLZF expression in peripheral blood MAIT and non-MAIT CD8$^+$ T cells was decreased in SIV-infected versus SIV-uninfected RMs (Fig. 2F). This finding runs contrary to a previous study of HIV-infected individuals (19) but nonetheless hints at a mechanism underlying the loss of peripheral blood MAIT cells in SIV-infected Asian macaques.

It is notable in this context that MAIT cells are not generally responsive to viral products and constitute unlikely targets for HIV/SIV infection because they rarely express the CD4 coreceptor. In line with these characteristics, no correlations were detected between MAIT cell frequencies and either plasma viral load or the number of peripheral CD4$^+$ T cells in SIV-infected RMs (Fig. 2G and H).

**Anatomical distribution of MAIT cells.** To evaluate in more detail whether tissue homing and accumulation could explain the peripheral loss of MAIT cells associated with SIV infection, we

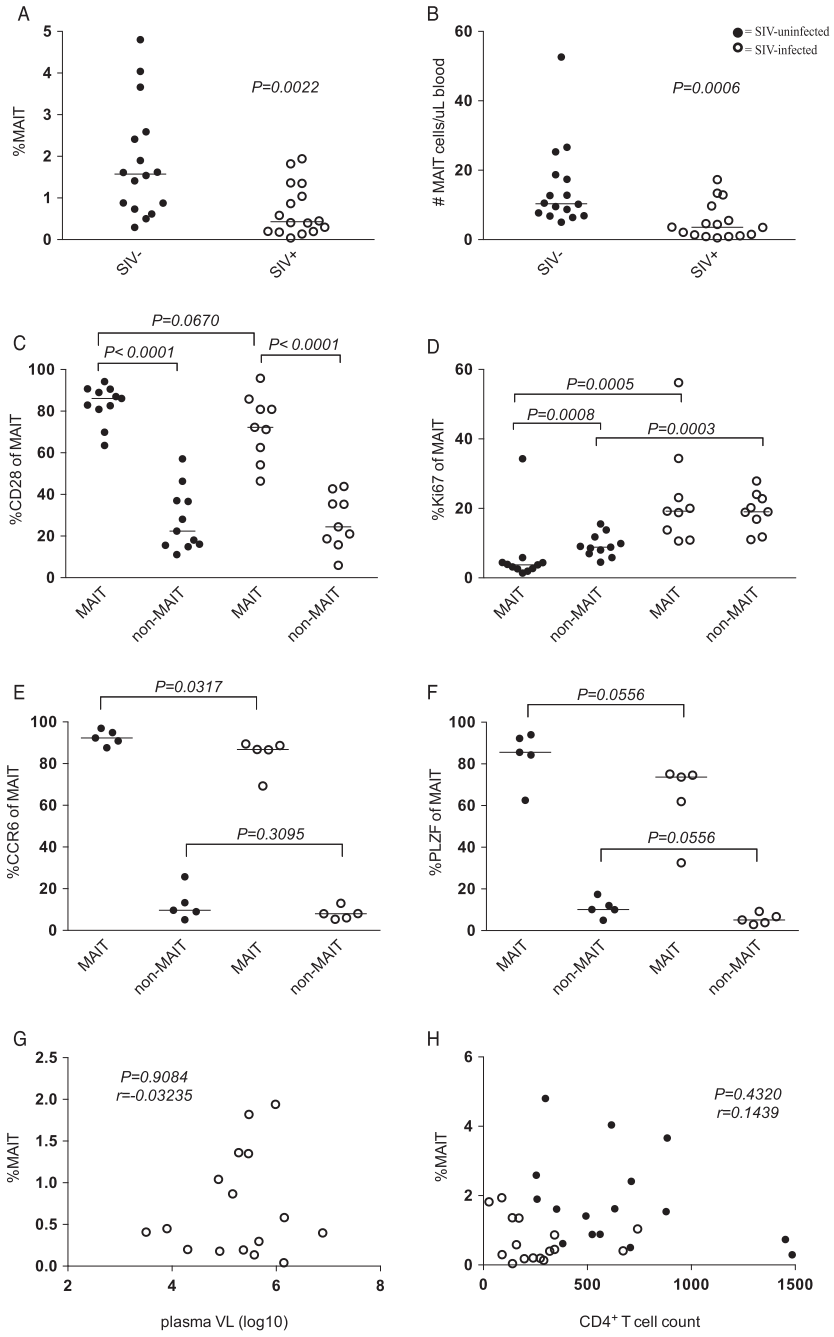


**FIG 2** Characterization of peripheral blood MAIT cells in RMs. (A) MAIT cell frequencies in peripheral blood of SIV-uninfected and SIV-infected RMs. (B) Absolute numbers of MAIT cells per microliter of peripheral blood, calculated from population percentages and lymphocyte counts. (C) Frequencies of $CD28^+$ cells in MAIT and non-MAIT (viable MR1-5-OP-RU $tetramer^-$ $CD8^+$ memory $CD3^+$ T lymphocytes) cell populations. (D) Frequencies of $Ki67^+$ cells in MAIT and non-MAIT cell populations. (E) Frequencies of $CCR6^+$ cells in MAIT and non-MAIT cell populations. (F) Frequencies of $PLZF^+$ cells in MAIT and non-MAIT cell populations. (G) Correlation between SIV plasma viral loads and MAIT cell frequencies in peripheral blood of SIV-infected RMs. (H) Correlation between $CD4^+$ T cell counts ($CD8^-$ memory $CD3^+$ lymphocytes) and MAIT cell frequencies in peripheral blood of SIV-infected RMs. (A to F) *P* values were calculated using the Mann-Whitney *U* test. The horizontal lines represent median values. SIV-uninfected, $n = 16$ total; SIV-infected, $n = 16$ total. Not all samples were available for all analyses. (G and H) Correlations were assessed using the Spearman rank test.

analyzed MAIT cell frequencies at distinct sites (BAL fluid, MLNs, jejunum, and liver) in SIV-uninfected and SIV-infected RMs. No significant differences were detected between groups in either the jejunum or liver (Fig. 3). Equivalent frequencies of MAIT cells at these sites may help to compensate for the preferential loss of IL-17- and IL-22-producing lymphocytes. In contrast, significantly lower frequencies of MAIT cells were present in the BAL fluid and MLNs of SIV-infected versus SIV-uninfected RMs ($P = 0.00117$ and $P = 0.0317$, respectively) (Fig. 3). MAIT cells were most prevalent overall in BAL fluid, with a median frequency of

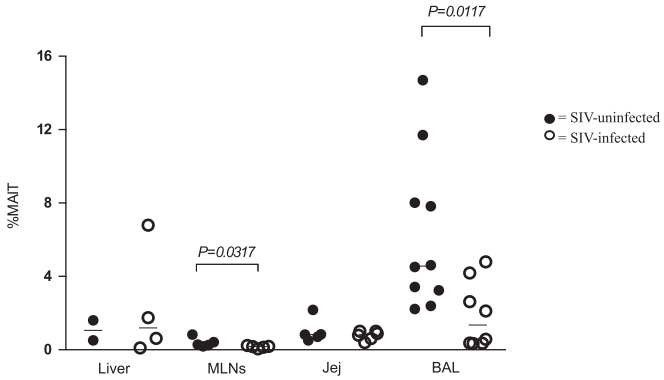


**FIG 3** Anatomical distribution of MAIT cells in RMs. MAIT cell frequencies in liver, MLNs, jejunum (Jej), and BAL fluid are shown for SIV-uninfected and SIV-infected RMs. The *P* values were calculated using the Mann-Whitney *U* test. The horizontal lines represent median values.

4.56% (range, 0.3 to 14.7%). In contrast to the high frequencies reported in human studies, however, MAIT cells comprised only 0.1 to 6.8% of the memory $CD3^+$ T cell population in the liver (Fig. 3). Notably, no significant differences in active caspase 3 expression in the peripheral blood were detected between SIV-uninfected and SIV-infected RMs (data not shown). Collectively, these data suggest that neither tissue redistribution nor apoptotic cell death contribute to the loss of circulating MAIT cells in the context of HIV/SIV infection.

**Analysis of the MAIT cell TCRα repertoire in RMs.** MAIT cells are typically characterized by the expression of a semi-invariant TRAV1-2 (Vα7.2) TCRα chain. To determine if the systemic loss of MAIT cells in SIV-infected RMs was associated with concomitant changes in the TCR repertoire, we isolated peripheral blood MAIT cells by flow cytometry and conducted a molecular analysis of all expressed *TRA* gene rearrangements in 9 SIV-uninfected and 9 SIV-infected RMs. All sequenced products expressed the *TRAV1-2* gene segment (Fig. 4A and B), thereby validating our gating strategy for MAIT cells (Fig. 1).

Next, we evaluated all TCRα sequences at the amino acid level to determine the extent of sharing among individual RMs (28). The majority of detected transcripts were public, defined on the basis of expression in more than one animal. Only 5 private TCRα sequences were identified, and 4 of them were present in the SIV-uninfected cohort (Fig. 4A and B). One of these sequences (TRA V1-2/CAVRDNNYKLSF/TRAJ20) was highly dominant in the MAIT cell repertoire (animal RHDBM6) (Fig. 4A). In contrast, the single private sequence (TRAV1-2/CAVRDGGYVLTF/TRAJ6) detected in the SIV-infected cohort was subdominant (animal RH860) (Fig. 4B).

In total, we identified 13 distinct CDR3α sequences in the peripheral MAIT cell repertoire of RMs, all of which incorporated 12 amino acids (Fig. 4C). MAIT cells are evolutionarily conserved among mammals and fairly abundant in humans. Accordingly, we found 5 public CDR3α sequences (CAVRDGDYKLSF, CAVRDS NYQLIW, CAVMDSNYQLIW, CAVSDSNYQLIW, and CASMD SNYQLIW) in RMs that were reported previously in humans (7, 10, 34) (Fig. 4C). The CAVRDSNYQLIW sequence has also been found in cattle and mice (7). Similar repertoires were present in the BAL fluid compartment, incorporating the public CDR3α sequences CAVRDSNYQLIW, CAVMDSDYKLIF, and CAVMDSN YQLIW (data not shown).

Overall, 12 of the 13 distinct CDR3α sequences were detected in SIV-uninfected RMs (Fig. 4D). In contrast, only 8 unique CDR3α sequences were found in SIV-infected RMs (Fig. 4E). Notably, the most commonly identified public TCRα chain (TRAV 1-2/CAVSDSNYQLIW/TRAJ33) in SIV-uninfected RMs (4 out of 9 animals) was not present in SIV-infected RMs. All other public sequences were distributed across both cohorts. However, there were no significant differences in absolute clonality between SIV-uninfected RMs (median, 3 clones; $n = 9$) and SIV-infected RMs (median, 2 clones; $n = 9$) ($P = 0.4397$) (data not shown).

The majority of *TRAV1-2* gene segments (67.6%) in the peripheral MAIT cell repertoire of RMs paired with the canonical *TRAJ33* gene segment (Table 2). In addition, we identified recombination events incorporating the *TRAJ20* and *TRAJ12* gene segments at lower frequencies (23.5% and 8.5%, respectively) (Table 2). These rearrangements mirror the gene preferences observed in humans (6, 10, 35). A *TRAJ6* gene segment partner was also detected in one SIV-infected RM (animal RH860) (Fig. 4B). Although not reported in a previous study (10), the *TRAJ6* gene encodes the conserved Tyr95α present in TRAJ33, TRAJ20, and TRAJ12.

In line with earlier analyses of other mammalian species, we detected Tyr95α (CDR3α position 8) in the vast majority of TCRα sequences isolated from circulating MAIT cells and BAL fluid MAIT cells in RMs (Fig. 5A and data not shown). The CDR3α loop preferentially comprised a neutral core flanked by outer hydrophobic residues in both the SIV-uninfected and SIV-infected cohorts (Fig. 5B and C). In addition, the aspartic acid residue at CDR3α position 5 was highly conserved among RMs (Fig. 5A to C). These findings align with existing structural data, which show most notably that Tyr95α forms direct contacts with the ribityl moiety of the riboflavin metabolite complexed with MR1 (6, 11, 12, 14).

## DISCUSSION

Although multiple studies have documented MAIT cell depletion in the peripheral blood of HIV-infected individuals (18–20), a clear understanding of the mechanisms that drive this phenomenon is lacking because potentially relevant tissues are difficult to access in humans. We sought to address this knowledge gap by evaluating the systemic impact of SIV infection on MAIT cell populations in RMs. As expected, MAIT cell frequencies and numbers were lower in the peripheral blood of SIV-infected versus SIV-uninfected RMs. However, these quantitative differences could not be attributed to increased trafficking to other anatomical locations. In the presence of SIV infection, MAIT cells were profoundly depleted in BAL fluid and MLNs. Moreover, no concomitant increases in MAIT cell frequencies were observed at mucosal sites. It is notable in this regard that impaired migration to the intestine and liver via reduced CCR6 expression may contribute to the depletion of circulating MAIT cells in chronic HIV/SIV infection (18).

MAIT cells provide broad-spectrum defense against bacterial and fungal species via the recognition of microbial vitamin $B_2$ metabolites bound to MR1 (36). The systemic loss of these cells described here is therefore somewhat surprising, given that microbial translocation, dysbiosis, and consequent inflammation are hallmarks of progressive HIV/SIV disease (37). Nonetheless, other IL-17-producing lymphocyte subsets are also depleted in HIV/SIV infection (38). A generic impairment of the factors that maintain

A

| SIV-uninfected | | | | |
|---|---|---|---|---|
| ID | TRAV | CDR3 | TRAJ | Freq |
| RHDBM6 | 1-2 | CAVRDNNYKLSF | 20 | 71.1% |
| | 1-2 | CAVRDSNYQLIW | 33 | 20.0% |
| | 1-2 | CAVRDGDYKLSF | 20 | 8.9% |
| | | Seqs: 45 | | |
| RHDE2W | 1-2 | CAVMDSDYKLIF | 12 | 92.9% |
| | 1-2 | CAVSDSNYQLIW | 33 | 7.1% |
| | | Seqs: 28 | | |
| RHDE2C | 1-2 | CAVTDSDYKLIF | 12 | 51.4% |
| | 1-2 | CAVMDSNYQLIW | 33 | 34.3% |
| | 1-2 | CAVTDSNYQLIW | 33 | 14.3% |
| | | Seqs: 35 | | |
| RHDCZ6 | 1-2 | CAVRDSNYQLIW | 33 | 55.6% |
| | 1-2 | CASMDSNYQLIW | 33 | 28.9% |
| | 1-2 | CAVASNNYKLSF | 20 | 15.6% |
| | | Seqs: 45 | | |
| RHDCVF | 1-2 | CAVADSDYKLIF | 12 | 46.5% |
| | 1-2 | CAVMDSNYQLIW | 33 | 46.5% |
| | 1-2 | CAVRDSNYQLIW | 33 | 7.0% |
| | | Seqs: 43 | | |
| RHDE20 | 1-2 | CAVMDSNYQLIW | 33 | 39.0% |
| | 1-2 | CAVTDSDYKLIF | 12 | 39.0% |
| | 1-2 | CAVRDSNYQLIW | 33 | 12.2% |
| | 1-2 | CAVSDSNYQLIW | 33 | 4.9% |
| | 1-2 | CAVMDSNHQLIW | 33 | 2.4% |
| | 1-2 | CASMDSNYQLIW | 33 | 2.4% |
| | | Seqs: 41 | | |
| RHDCBC | 1-2 | CAVSDSNYQLIW | 33 | 100.0% |
| | | Seqs: 42 | | |
| RHDCMV | 1-2 | CAVMDSNYQLIW | 33 | 57.8% |
| | 1-2 | CAVTDSDYKLIF | 12 | 40.0% |
| | 1-2 | CAVRDSNYQLIW | 33 | 2.2% |
| | | Seqs: 45 | | |
| RHDCJWA | 1-2 | CAVSDSNYQLIW | 33 | 100.0% |
| | | Seqs: 45 | | |

B

| SIV-infected | | | | |
|---|---|---|---|---|
| ID | TRAV | CDR3 | TRAJ | Freq |
| RHCE5D | 1-2 | CAVRDSNYQLIW | 33 | 60.9% |
| | 1-2 | CAVRDGDYKLSF | 20 | 39.1% |
| | | Seqs: 46 | | |
| RH861 | 1-2 | CAVMDSNYQLIW | 33 | 66.2% |
| | 1-2 | CAVRDSNYQLIW | 33 | 33.8% |
| | | Seqs: 65 | | |
| RH860 | 1-2 | CAVMDSDYKLIF | 12 | 90.6% |
| | 1-2 | CAVRDGGYVLTF | 6 | 9.4% |
| | | Seqs: 32 | | |
| RH849 | 1-2 | CAVRDSNYQLIW | 33 | 76.2% |
| | 1-2 | CAVTDSDYKLIF | 12 | 14.3% |
| | 1-2 | CASMDSNYQLIW | 33 | 9.5% |
| | | Seqs: 42 | | |
| RH856 | 1-2 | CAVRDSNYQLIW | 33 | 94.7% |
| | 1-2 | CASMDSNYQLIW | 33 | 5.3% |
| | | Seqs: 19 | | |
| RH859 | 1-2 | CAVRDSNYQLIW | 33 | 77.4% |
| | 1-2 | CASMDSNYQLIW | 33 | 19.4% |
| | 1-2 | CAVTDSDYKLIF | 12 | 3.2% |
| | | Seqs: 31 | | |
| RH850 | 1-2 | CAVMDSNYQLIW | 33 | 50.0% |
| | 1-2 | CAVTDSDYKLIF | 12 | 46.7% |
| | 1-2 | CAVADSDYKLIF | 12 | 3.3% |
| | | Seqs: 30 | | |
| RH851 | 1-2 | CAVMDSNYQLIW | 33 | 68.3% |
| | 1-2 | CAVTDSDYKLIF | 12 | 31.7% |
| | | Seqs: 41 | | |
| RH848 | 1-2 | CAVMDSNYQLIW | 33 | 84.1% |
| | 1-2 | CAVTDSDYKLIF | 12 | 15.9% |
| | | Seqs: 44 | | |

C

| Total Animals (n=18) | | | |
|---|---|---|---|
| CDR3 | Animals with Shared Seq | # of Seqs | Freq |
| CAVMDSNYQLIW | 8 | 197 | 27.4% |
| CAVRDSNYQLIW | 10 | 167 | 23.2% |
| CAVTDSDYKLIF | 8 | 93 | 12.9% |
| CAVSDSNYQLIW | 4 | 91 | 12.7% |
| CAVMDSDYKLIF | 2 | 55 | 7.6% |
| CAVRDNNYKLSF | 1 | 32 | 4.5% |
| CASMDSNYQLIW | 5 | 25 | 3.5% |
| CAVRDGDYKLSF | 2 | 22 | 3.1% |
| CAVADSDYKLIF | 2 | 21 | 2.9% |
| CAVASNNYKLSF | 1 | 7 | 1.0% |
| CAVTDSNYQLIW | 1 | 5 | 0.7% |
| CAVRDGGYVLTF | 1 | 3 | 0.4% |
| CAVMDSNHQLIW | 1 | 1 | 0.1% |
| Total Seqs | | 719 | |

D

| SIV-uninfected (n=9) | | | |
|---|---|---|---|
| CDR3 | Animals with Shared Seq | # of Seqs | Freq in SIV- |
| CAVSDSNYQLIW | 4 | 91 | 24.7% |
| CAVMDSNYQLIW | 4 | 74 | 20.1% |
| CAVTDSDYKLIF | 3 | 52 | 14.1% |
| CAVRDSNYQLIW | 5 | 43 | 11.7% |
| CAVRDNNYKLSF | 1 | 32 | 8.7% |
| CAVMDSDYKLIF | 1 | 26 | 7.0% |
| CAVADSDYKLIF | 1 | 20 | 5.4% |
| CASMDSNYQLIW | 2 | 14 | 3.8% |
| CAVASNNYKLSF | 1 | 7 | 1.9% |
| CAVTDSNYQLIW | 1 | 5 | 1.4% |
| CAVRDGDYKLSF | 1 | 4 | 1.1% |
| CAVMDSNHQLIW | 1 | 1 | 0.3% |
| Total Seqs | | 369 | |

E

| SIV-infected (n=9) | | | |
|---|---|---|---|
| CDR3 | Animals with Shared Seq | # of Seqs | Freq in SIV+ |
| CAVRDSNYQLIW | 5 | 124 | 35.4% |
| CAVMDSNYQLIW | 4 | 123 | 35.1% |
| CAVTDSDYKLIF | 5 | 41 | 11.7% |
| CAVMDSDYKLIF | 1 | 29 | 8.3% |
| CAVRDGDYKLSF | 1 | 18 | 5.1% |
| CASMDSNYQLIW | 3 | 11 | 3.1% |
| CAVRDGGYVLTF | 1 | 3 | 0.9% |
| CAVADSDYKLIF | 1 | 1 | 0.3% |
| Total Seqs | | 350 | |

**FIG 4** TCRα repertoire of MAIT cells in RMs. (A and B) *TRAV* gene usage, CDR3α amino acid sequence, *TRAJ* gene usage, and frequency (percent) of TCRα chains isolated from MAIT cells in the peripheral blood of SIV-uninfected (A) and SIV-infected (B) RMs. The colors highlight public sequences shared among animals. (C to E) CDR3α amino acid sequences, sharing among individual animals, total count, and frequency (percent) of TCRα chains isolated from MAIT cells in the peripheral blood of all ($n$ = 18) (C), SIV-uninfected ($n$ = 9) (D), and SIV-infected ($n$ = 9) (E) RMs. The colors highlight public sequences and correspond with those in panels A and B.

IL-17 production may therefore contribute to the global loss of MAIT cells in this context. In line with this possibility, our data show that peripheral blood MAIT cells in SIV-infected RMs express relatively low levels of PLZF, which is known to play a key role in Th17 differentiation (33).

Our results further suggest that MAIT cell depletion in SIV-infected RMs does not occur as a consequence of apoptotic death via caspase 3. However, exhaustion due to ongoing microbial stimulation could feasibly account for the loss of MAIT cells in chronic HIV/SIV infection. Consistent with this possibility, we

**TABLE 2** *TRAJ* gene usage of peripheral blood MAIT cells in RMs

| | All RMs | | SIV-uninfected RMs | | SIV-infected RMs | |
|---|---|---|---|---|---|---|
| *TRAJ* | No. of sequences | Frequency (%) | No. of sequences | Frequency (%) | No. of sequences | Frequency (%) |
| 33 | 486 | 67.6 | 228 | 61.8 | 258 | 73.7 |
| 12 | 169 | 23.5 | 98 | 26.6 | 71 | 20.3 |
| 20 | 61 | 8.5 | 43 | 11.7 | 18 | 5.1 |
| 6 | 3 | 0.4 | | | 3 | 0.9 |

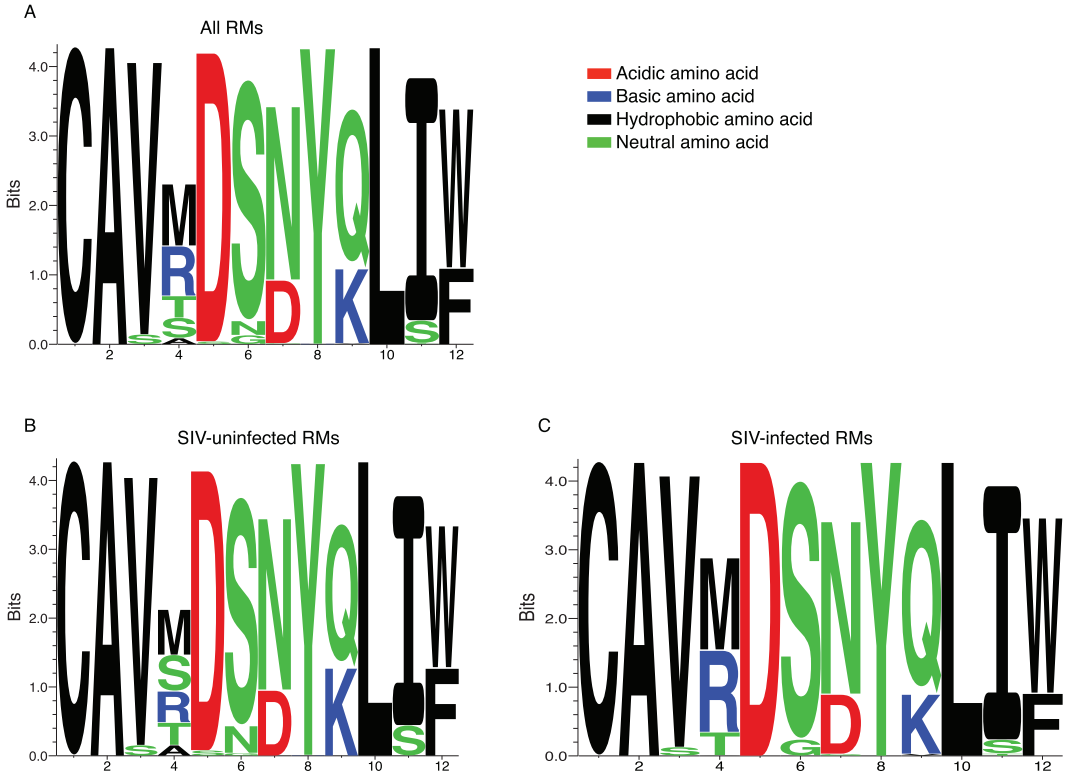


**FIG 5** CDR3α amino acid preferences of MAIT cells in RMs. Shown is amino acid enrichment (bit size) at each position in the CDR3α repertoire of MAIT cells isolated from the peripheral blood of all ($n = 18$) (A), SIV-uninfected ($n = 9$) (B), and SIV-infected ($n = 9$) (C) RMs. The bit size corresponds with the amino acid frequency at each position.

detected increased frequencies of Ki67$^+$ MAIT cells in SIV-infected RMs, suggesting greater proliferation and turnover relative to MAIT cells in SIV-uninfected RMs. Moreover, analysis of peripheral MAIT cell TCRα sequences revealed a nonsignificant trend toward more restricted and highly public repertoires in SIV-infected than in SIV-uninfected RMs. It is established that commonly shared TCRα sequences among MAIT cells can be generated efficiently via the process of convergent recombination, suggesting that they may be more easily replenished from the naive pool in the event of peripheral exhaustion and subsequent clonal deletion (39). This scenario likely explains the limited number of private TCRα sequences detected in SIV-infected RMs.

Collectively, these data are compatible with the hypothesis that microbial translocation drives a systemic loss of MAIT cells in HIV/SIV infection. This enhanced mechanistic understanding may facilitate the development of novel therapies to boost antimicrobial immunity in individuals with AIDS.

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