Activation, exhaustion, and persistent decline of the antimicrobial MR1-restricted MAIT-cell population in chronic HIV-1 infection

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Mucosal-associated invariant T (MAIT) cells are an evolutionarily conserved antimicrobial MR1-restricted T-cell subset. MAIT cells are CD161+/CD8+ and numerous in blood and mucosal tissues. However, their role in HIV-1 infection is unknown. In this study, we found levels of MAIT cells to be severely reduced in circulation in patients with chronic HIV-1 infection. Residual MAIT cells were highly activated and functionally exhausted. Their decline was associated with time since diagnosis, activation levels, and the concomitant expansion of a subset of functionally impaired CD161+ CD8+ T cells. Such cells were generated in vitro by exposure of MAIT cells to Escherichia coli. Notably, whereas the function of residual MAIT cells was at least partly restored by effective antiretroviral therapy, levels of MAIT cells in peripheral blood were not restored. Interestingly, MAIT cells in rectal mucosa were relatively preserved, although some of the changes seen in blood were recapitulated in the mucosa. These findings are consistent with a model in which the MAIT-cell compartment, possibly as a result of persistent exposure to microbial material, is engaged, activated, exhausted, and progressively and persistently depleted during chronic HIV-1 infection. (Blood. 2013;121(7):1124-1135)

Introduction

HIV-1 infection is associated with a range of pathologic changes to the immune system, including systemic immune activation, CD4 T-cell loss and CD8 T-cell expansion. The state of broad and persistent immune activation develops early during infection,1,2 contributes to the rapid aging of the immune system seen during chronic progressive HIV-1 disease, and persists despite effective long-term virologic suppression by combination antiretroviral therapy (cART; reviewed in by Deeks,3 Appay et al,4 and Desai and Landay5). These pathologic processes lead to the progressive destruction of lymphoid organs and loss of CD4 helper T cells.6,7 Already during primary infection, HIV-1 depletes intestinal CD4 T cells and disrupts the structure and function of the intestinal immune system.8-13 One consequence of this is increased permeability of the intestinal epithelium with translocation of microbial products into the local tissue, the portal circulation, the liver and eventually into systemic circulation.14 This process may continue despite effective long-term cART.15,16 Disruption of immune homeostasis and barrier function at the mucosa is a considerable challenge for the host immune system because the microbial proteins, carbohydrates, and lipids form a range of antigens that will engage innate as well as adaptive immune mechanisms (reviewed by Brenchley and Douek17). Despite considerable advances in the treatment and management of HIV-1 disease, certain infections still present a significant clinical challenge particularly among HIV-infected individuals who are diagnosed at advanced stages, those who lack access to antiretroviral therapy, and those who cannot maintain adherence to therapy and clinical care.18-20 This includes an increased risk of developing bacterial pneumonia even in HIV-1–infected patients with relatively normal CD4 counts,21 indicating that impaired CD4 T-cell independent control of certain infections still exists even in the context of treated HIV-1 disease.

Mucosal-associated invariant T (MAIT) cells are a relatively recently discovered subset of unconventional, innate-like T cells that are highly abundant in mucosal tissues, liver, and peripheral blood.22-25 Human MAIT cells express an invariant T-cell receptor (TCR) carrying the Vα7.2 α-chain segment, a restricted Vβ repertoire (Vβ2 or Vβ13), and recognize antigens in complex with the evolutionarily conserved MHC-Ib–related protein (MR1).24,25 In addition to the Vα7.2 TCR segment, MAIT cells are defined by

Key Points

- Antimicrobial CD8+ MAIT cells are activated, exhausted, and progressively and persistently depleted during chronic HIV-1 infection.
- This decline in MAIT cell level and function may seriously impair the ability to mount immune responses to bacterial and fungal pathogens.

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The online version of this article contains a data supplement.
the expression of CD161, the IL-18 receptor (IL-18R), and the transcription factor ZBTB16, also known as PLZF.26-28 The majority of MAIT cells are CD8+, expressing either CD8α or CD8β, with minor CD4+ or CD8/4 double-negative populations.26-29 MAIT cells recognize and respond to diverse bacterial and fungal-derived products presented by MR1 molecules expressed on antigen presenting cells (APCs), and they do this independently of the TLR pathways.27 A recent study indicated that the complementarity-determining region (CDR) 1α, CDR2α, and CDR3α of the Vα7.2 TCR are crucial for MAIT TCR recognition of a variety of enteric bacteria irrespective of the Vβ usage.30 Furthermore, a very recent study indicated that MR1–presented antigens are composed of microbial vitamin metabolites.31 After antigen recognition, MAIT cells rapidly respond by secretion of both proinflammatory and tissue-protective cytokines, such as IFNγ, TNF, IL-17, and IL-22, in an innate-like manner.22,23,27,28 MAIT cells contribute to protection against certain mycobacterial and enterobacterial infections in mice, and may play a significant role in Mycobacterium tuberculosis infection in humans.27,32-34

The role of MAIT cells in HIV-1 infection is currently unknown. In this study, we examined the levels and characteristics of MAIT cells in circulation as well as in rectal mucosa in patients with chronic HIV-1 infection. Our findings support a model whereby the MAIT-cell compartment, possibly as a result of persistent exposure to microbial material, is engaged, activated, exhausted, and progressively and persistently depleted during chronic HIV-1 infection. These findings are interpreted and discussed in the context of mechanisms of HIV immunopathogenesis and consequences for control of microbial infections in HIV-1–infected patients.

Methods

Participants

HIV–1–infected patients were from the Karolinska University Hospital Huddinge Infectious Diseases Outpatient Clinic (Stockholm, Sweden), and from the Study of the Consequences of the Protease Inhibitor Era (SCOPE), San Francisco General Hospital (SGFH), or were referred by collaborating clinicians at either the University of California, Davis (UC Davis) or the University of California, San Francisco (UCSF). Patients had no history of AIDS-defining illness in the 12 months before recruitment. Healthy HIV-uninfected individuals were recruited at the Blood Transfusion Clinic at the Karolinska University Hospital Huddinge and at the SGFH. Written informed consent was obtained from all individuals in accordance with study protocols conforming to the provisions of the Declaration of Helsinki and approved by the Regional Ethics Research Board in Stockholm and the Institutional Review Board, School of Medicine, UC Davis, and the Committee on Human Subjects Research, UCSF.

Peripheral blood and rectal biopsy tissue processing

Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood by Ficoll-Hypaque density gradient centrifugation (Piloter-Pharmacia or Axis-Shield), and either rested overnight in complete medium, or cryopreserved in liquid nitrogen. Rectal biopsy tissue was obtained at 10 to 20 cm from the anal verge by flexible sigmoidoscopy.35-37 Briefly, 20 to 25 tissue pieces (∼3 mm diameter) were collected during each procedure and placed in complete RPMI 1640 supplemented with 15% fetal calf serum (R15 medium), and immediately transported to UC Davis for processing and analysis. Rectal mononuclear cells (RMCs) were isolated from biopsy specimens after 3 washes with R15 medium and then underwent 3 rounds of digestion in 0.5 mg/mL collagenase type II (Sigma-Aldrich) at 37°C with agitation. Each digestion was followed by disruption of the tissue by passing through a syringe with a 16-gauge blunt end needle, followed by a 70-μm cell strainer. RMCs were then washed in R15 to remove collagenase and allowed to rest overnight (37°C, 5% CO₂) in R15 containing 0.5 mg/mL piperacillin-tazobactam (Zosyn; Wyeth Pharmaceuticals).

Antibodies

Anti-CD3 FITC, anti-CD3 and anti-CD69 Alexa Fluor 700, anti-CD3 and anti-CD4 Pacific Blue, anti-CD161 PE/Cy5, anti-CD38 and anti-TNF PE/Cy7, anti-CD27 and anti-HLA-DR APC-H7, anti-CD127 Alexa Fluor 647, and anti-IFNγ APCs were from BD Bioscience. Anti-CD4 ECD, and iOTest Beta Mark Kit for TCR Vβ analyses were from Beckman Coulter. Anti-Vo7.2 FITC and PE (clone 3C10), anti-CD8α Brilliant Violet 570, anti-CD57 Pacific Blue, and anti-Ki67 and anti–IL-17A Brilliant Violet 421 were from BioLegend. Anti–TIM-3 Alexa Fluor 488, anti–IL-18R PE, and anti-PLFZ APC (clone 6318100) were from R&D Systems. Anti-CD4 Qdot 705, anti-CD8 Qdot 565, and live/dead aqua fixable cell stain were from Invitrogen. Anti–Vα7.2-biotin (a kind gift from Dr Olivier Lantz, Institut Curie, Paris, France), was visualized with streptavidin Qdot 585 (Invitrogen). Anti–MR1 mAb (clone 26.5) was kindly provided by Dr Ted Hansen (School of Medicine, Washington University, St Louis, MO).

In vitro infection and cell activation

Escherichia coli strain D21 was cultured overnight at 37°C in Luria broth and counted with the standard plate counting method. Bacteria were washed once in PBS and fixed in 1% paraformaldehyde for 5 minutes and then washed extensively before feeding to the PBMC culture at a multiplicity of infection (MOI) of 6 in the presence of 1.25 μg/mL anti-CD28 mAb (BD Bioscience). Cells were cultured in round-bottom 96-well plates at 10⁶ cells/well and stimulated overnight, and monensin (Golgi Stop, BD Bioscience) was added for the last 6 hours of incubation. In selected experiments, monocytes were purified from fresh buffy coats using RosetteSep monocyte enrichment cocktail (StemCell Technologies), and MAIT cells were purified from freshly isolated PBMCs using anti-Vo7.2 PE antibody followed by anti–PE-microbeads (Miltenyi Biotec) and positive selection using MACS Cell Separation (Miltenyi Biotec). The purity of both enriched monocytes and Vo7.2+ T cells were >80%. In selected experiments, MAIT cells were purified from MACS-enriched Vo7.2+ T cells by FACS-sorting (FACSARia, BD Bioscience). Monocytes were then fed PFA-fixed E coli at indicated MOIs for 3 hours, followed by the addition of Vo7.2+ T cells or MAIT cells at a 2:1 Vo7.2+ /MAIT cell:monocyte ratio. Cells were then cultured for 1 to 3 days in complete medium.

Flow cytometry

Cell-surface staining was performed using directly conjugated or biotinylated antibodies in PBS containing 2% FCS and 2mM EDTA (FACS buffer) for 20 minutes on ice. For the latter, cells were washed once in cold FACS buffer followed by 20 minutes incubation with streptavidin Qdot 585 on ice. Cells were then washed once in FACS buffer and fixed in Cytofix/Cytoperm (BD Bioscience). Intracellular staining was performed after surface staining, fixation, and 1 wash in Perm/Wash buffer (BD Bioscience) using relevant intracellular mAbs in Perm/Wash on ice for 30 minutes. Cells were washed once in Perm/Wash before data acquisition. Samples were acquired on an LSRFortessa flow cytometer (BD Bioscience) equipped with 405, 488, 561, and 639 nm lasers. Single-stained polystyrene beads (BD Bioscience) were used to determine compensation settings. Software-based compensation was performed in FlowJo Version 9 software (Tree Star).

Statistical analyses

Significant differences in independent samples were assessed using Fisher exact test for categorical variables, and t test or Mann-Whitney test for continuous variables as appropriate. Paired t test or Wilcoxon signed rank test was used to determine significance between paired samples. The Kruskal-Wallis test followed by Mann-Whitney test was used to detect differences across multiple samples. Rectal MAIT-cell frequencies were predicted from peripheral blood MAIT-cell frequency using simple linear
regression on log_{10}-transformed data to meet the normal distribution assumption. Correlations were evaluated using Spearman rank correlation. Statistical analyses were performed using Prism Version 5 software (GraphPad) and P values < .05 were considered significant.

Results

MAIT cells are persistently depleted in chronic HIV-1 infection

We initially determined the frequency of Vα7.2+ CD161+ MAIT cells in peripheral blood of 34 uninfected control subjects and 33 patients with chronic untreated HIV-1 infection (Figure 1A; Table 1). Levels of MAIT cells in HIV-1 patients were sharply lower compared with those of the uninfected controls (HIV+ = 1.09% ± 0.26%, HIV− = 3.92% ± 0.75%, P < .0001; Figure 1B). As previously observed,26-29 the majority of MAIT cells in blood consisted of CD8+ and CD4− CD8− (DN) subsets. We observed the presence of a CD4+ subset in all donors tested, albeit at a much smaller proportion. All MAIT-cell subsets were significantly lower in the HIV-1–infected patients (DN MAIT P = .0013; CD4 MAIT P < .0001; CD8 MAIT P = .0049; Figure 1C). In agreement with this, we did not observe significant redistribution of DN, CD4, and CD8 subsets within the total peripheral blood MAIT-cell population (supplemental Figure 1A, available on the Blood Web site; see the Supplemental Materials link at the top of the online article). The loss of MAIT cells was associated with the time since HIV-1 diagnosis, which is a surrogate marker of time since infection (r = −0.42, P = .015; Figure 1D).

We next examined whether long-term virologic control by cART could restore the frequency and numbers of peripheral blood MAIT cells in 22 patients with chronic HIV-1 infection. We measured MAIT cells just before cART initiation and again after a
Table 1. Details of HIV-1 patients and healthy controls

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<th>Stockholm cohort</th>
<th>San Francisco cohort</th>
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<td>HIV−</td>
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<td>n</td>
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<td>33</td>
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<td></td>
<td>46</td>
<td>47</td>
</tr>
<tr>
<td>Age, y‡</td>
<td>37.5 (30-51.3)</td>
<td>39 (34.5-47)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td>16 M (47.1%)</td>
<td>21 M (63.6%)</td>
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<tr>
<td>Viral loads, copies/mL</td>
<td>ND</td>
<td>78 739 ± 31 580</td>
</tr>
<tr>
<td>Time since HIV diagnosis, mo</td>
<td>NA</td>
<td>85 ± 11</td>
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ND indicates not done; NA, not applicable; and M, male.
†Comparisons were done on uninfected versus HIV-infected patients.
‡Median (IQR); mean ± SD shown for all parameters unless otherwise specified.

MAIT cells in blood from healthy donors expressed low levels of CD38, HLA-DR, and TIM-3, but these markers were significantly increased in chronically HIV-infected patients (CD38\(^{\text{HIV−}}\) < .0001; HLA-DR \(P < .0001\); TIM-3 \(P < .0001\); Figure 3A and Bi-iii). Long-term effective cART significantly decreased HLA-DR and TIM-3 expression (\(P = .011\) and .0018, respectively), but CD38 expression remained elevated (\(P = .46\); Figure 3Ci-iii). In addition, CD57 expression was increased in MAIT cells from HIV+ donors (\(P = .017\); Figure 3Biv), and this was not changed by treatment (\(P = .96\); Figure 3Civ). In contrast, the percentage of MAIT cells expressing the costimulatory molecule CD27 and CD127 (IL-7Ra) were reduced in HIV-infected individuals (CD27 \(P = .018\); CD127 \(P = .015\); Figure 3Bv-vi). CD27 expression recovered after long-term cART (\(P = .0004\), but CD127 expression remained lower (Figure 3Cv-vi).

Accumulation of CD161\(^{−}\) Vα7.2\(^{+}\) T cells

In detailed analysis of the data, we observed that the CD161\(^{−}\) Vα7.2\(^{+}\) T-cell population was significantly increased in HIV-infected patients compared with uninfected controls (\(P < .0001\); Figure 4Ai-ii). Interestingly, the total percentage of Vα7.2\(^{+}\) expressing CD3\(^{+}\) cells was not significantly different in peripheral blood between the HIV-infected patients and uninfected controls (\(P = .51\); Figure 4Aiii), suggesting that the accumulation of CD161\(^{−}\) Vα7.2\(^{+}\) T cells in chronic HIV-1 infection is not simply because of the disappearance of CD161\(^{+}\) Vα7.2\(^{+}\) MAIT cells. Furthermore, CD161\(^{−}\) Vα7.2\(^{+}\) T cells in both uninfected and infected patients were significantly lower compared with the healthy donors (Figure 4Bv-iv).
HIV-infected individuals lacked expression of Ki67, suggesting this is a nonproliferating subset (data not shown).

We next used IL-18R as an alternative marker that, in combination with Vα7.2, also identifies the MAIT-cell population.26,27 In agreement with the results shown in Figure 1B, the IL-18R+ Vα7.2+ MAIT cells were significantly reduced in blood of HIV-infected individuals (P = .0005, Figure 4Bi-ii). We next investigated whether there was a selective loss of CD161 expression in the MAIT-cell

**Figure 2. MAIT cells are functionally impaired in chronically HIV-infected patients.** (A) The gating strategy for fixed *Escherichia coli*-activated MAIT cells in blood from HIV-1–uninfected and HIV-infected individuals. Gray dots represent unstimulated controls from the same donors. CD69, IFNγ, TNF, and IL-17 expression were determined in 10 uninfected and untreated HIV-infected individuals after an overnight stimulation with fixed *E coli* (B) and a 6 hour PMA/ionomycin stimulation (C). MAIT-cell activation and cytokine production from these HIV-infected patients were then assessed using an overnight bacterial stimulation after commencing cART (D). Box and whisker plots show median, IQR, and the 10th to 90th percentile. Tx indicates therapy.
population in HIV-infected patients. Interestingly, although the majority of IL-18R\(^+\) V\(_{α7.2}^+\) MAIT cells in uninfected control subjects coexpressed CD161, a significant proportion of IL-18R\(^+\) V\(_{α7.2}^+\) MAIT cells in HIV-infected patients had lost their CD161 expression (\(P < .0001\); Figure 4Biii). In line with this, we observed an increased frequency of CD161\(^-\) V\(_{α7.2}^+\) MAIT cells in the HIV-infected patients (\(P = .0039\); Figure 4Biv). The loss of CD161 expression within the IL-18R\(^+\) V\(_{α7.2}^+\) MAIT-cell population was significantly associated with time since HIV diagnosis (\(r = 0.52, P = .0009\); Figure 4Bv).

We next examined the TCR V\(_{β2}/V_{β13}\) usage in CD161\(^-\) V\(_{α7.2}^+\) T cells to investigate the possibility that CD161\(^-\) V\(_{α7.2}^+\) T cells in HIV-infected patients are enriched by MAIT cells that have down-regulated CD161 expression. In 13 uninfected controls, CD161\(^+\) V\(_{α7.2}^+\) MAIT cells had a preferential TCR V\(_{β2}\) and V\(_{β13}\) usage (28.1% ± 2.45%; Figure 4Ci) in agreement with a recent study.\(^{28}\) However, CD161\(^-\) V\(_{α7.2}^+\) T cells had no V\(_{β2}/V_{β13}\) bias and their V\(_{β}\) usage was significantly different from MAIT cells (14.34% ± 1.11%, \(P = .0005\); Figure 4Ci), and they also had a similar CD4/CD8 distribution compared with total CD3\(^+\) T cells (Figure 4Cii). In contrast, in 12 HIV-infected patients there was no difference in V\(_{β2}/V_{β13}\) usage between CD161\(^+\) V\(_{α7.2}^+\) MAIT cells and CD161\(^-\) V\(_{α7.2}^+\) T cells (Figure 4Ciii). In addition, the CD4/CD8 distribution in the CD161\(^-\)
**Vα7.2⁺ T-cell population in both progressor and elite controller HIV-infected patients resembled that of MAIT cells** (Figure 4Ciii).

Human MAIT cells express the transcription factor PLZF (ZBTB16). CD161⁺ Vα7.2⁺ MAIT cells from HIV-1 infected and infected individuals equally expressed PLZF (Figure 4Dii left panel). CD161⁺ Vα7.2⁺ T cells from uninfected controls had low PLZF expression, similar to Vα7.2⁻ T cells (Figure 4Dii right panel and not shown). However, CD161⁺ Vα7.2⁺ T cells from HIV-infected patients had significantly higher PLZF expression (*P* = .045; Figure 4Dii). Together, these data suggest that a CD161⁺ Vα7.2⁺ T-cell population expands, in the absence of proliferation, in patients with chronic HIV-1 infection.

**CD161⁻ Vα7.2⁺ T cells are hyper-activated and can be generated by *E. coli* stimulation**

Given the elevated levels of activation in MAIT cells in HIV-1 infected subjects, we were next interested to assess the expression of activation markers in the CD161⁻ Vα7.2⁺ T-cell population in blood. These cells were highly activated and expressed significantly higher levels of CD38, HLA-DR, and CD57 compared with MAIT cells from the same patients (*n* = 29, all *P* < .0001; Figure 5A-B). Furthermore, the CD161⁻ Vα7.2⁺ T cells showed no detectable IFNγ, TNF, or IL-17 production in response to overnight stimulation with *E. coli* (data not shown).

Because MAIT cells respond to diverse microbial products, and partially down-regulate CD161 after *in vitro* *E. coli* stimulation of PBMCs (Figure 2A), we next investigated whether CD161⁻ Vα7.2⁺ T cells can be generated from MAIT cells after bacterial stimulation in vitro. CD161⁻ Vα7.2⁺ T cells expanded during a 3 days culture with relatively high levels of *E. coli* (Figure 5C), in the absence of Ki67 expression (Figure 5Cii), and overt T-cell death (as determined by Trypan-blue exclusion assay; data not shown). Furthermore, in MAIT cells purified by FACS-sorting, there was clear down-regulation of CD161 already at day 1 of culture with *E. coli* (supplemental Figure 3). Taken together, these data suggest that CD161⁻ Vα7.2⁺ T cells have a hyper-activated phenotype in chronic HIV-1 infection, and that these cells may be derived from MAIT cells as a consequence of exposure to bacterial products.
MAIT cells in rectal mucosa show similar changes as in blood but are numerically better preserved

Because of the central role of the intestinal immune system in HIV pathogenesis, we next investigated the MAIT-cell population in rectal mucosa of 9 HIV-uninfected controls and 11 chronically HIV-infected patients recruited at the SFGH (Table 1; Figure 6A). Interestingly, there was no significant loss of MAIT cells from the rectal mucosa as a percentage of total T cells in HIV-1–infected patients ($P = .65$; Figure 6B), despite a significant reduction in...
Figure 6. MAIT cells and CD161<sup>+</sup> V<sub>α7.2</sub><sup>+</sup> T cells in rectal mucosa of uninfected and HIV-infected individuals. Identification, frequency, and distribution of CD4 and CD8 subsets of rectal MAIT cells in 9 HIV-1–uninfected and 11 HIV-infected individuals (A-C). Spearman correlation between peripheral blood and rectal MAIT cells with time since HIV diagnosis (D), and a simple linear regression analysis of the relationship between frequency of blood MAIT cells and rectal MAIT cells (both are log<sub>10</sub>-transformed) in uninfected and infected individuals (E). The frequency of CD161<sup>+</sup> V<sub>α7.2</sub><sup>+</sup> T cells (F) and IL-18R<sub>α</sub> MAIT cells that did not express CD161 (G) in blood and rectal mucosa of HIV-1 infected and uninfected individuals. (H) Spearman correlation between the proportion of IL-18R<sub>α</sub> MAIT cells that did not express CD161 and time since HIV diagnosis. (I) Frequency of CD4 T cells in the rectal mucosa. Open circles represent peripheral blood and closed circles represent rectal mucosa. Box and whisker plots show median, IQR and the 10th to 90th percentile.
peripheral blood MAIT cells ($P = .033$; Figure 6B, in agreement with Figure 1B). Although rectal DN and CD8 MAIT-cell subsets were unaffected in HIV-infected patients ($P = .97$ and .59, respectively; Figure 6C), there was a significant decrease in rectal CD4 MAIT-cell frequency compared with uninfected controls ($P = .0093$; Figure 6C). Similar to the pattern in the Stockholm cohort, there was an inverse correlation between time since HIV diagnosis and levels of MAIT cells in blood ($r = −.65, P = .037$; Figure 6D, in agreement with Figure 1D), and this correlation was also present in rectal MAIT cells ($r = −.58, P = .066$; Figure 6D).

Because MAIT cells are depleted in the blood of HIV-infected patients, we next predicted that the levels of MAIT cells in peripheral blood might be associated with the levels of rectal MAIT cells. This was indeed the case ($r^2 = 0.63, P = .0037$; Figure 6E), although this prediction did not hold up in uninfected control subjects ($r^2 = 0.25, P = .17$; Figure 6E). Levels of CD161−Va7.2+ T cells were increased in the rectal mucosa ($P = .0065$; Figure 6F), but not in blood, of these 11 HIV-infected patients ($P = .19$; Figure 6F). There was a significant increase of IL-18R+ CD161−Va7.2+ MAIT cells in peripheral blood in the SFGH HIV cohort ($P = .047$; Figure 6G), and a trend toward a similar pattern in the rectal mucosa ($P = .11$; Figure 6G). Furthermore, a significant correlation between the proportion of IL-18R+ CD161−Va7.2+ MAIT cells and the time since HIV diagnosis was present in both blood ($r = 0.81, P = .0039$), and rectal mucosa ($r = 0.74, P = .013$) in these patients (Figure 6H). As expected, the overall CD4 T-cell frequency was significantly lower in rectal mucosa of the HIV-infected patients compared with uninfected control subjects ($P = .0049$; Figure 6I). These data are consistent with a model where MAIT cells in the rectal mucosa are better preserved than MAIT cells in blood during chronic HIV-1 infection.

**Discussion**

MR1-restricted MAIT cells are believed to be an important and evolutionarily conserved component of the T-cell response against microbes. In this study, we investigated this T-cell population in HIV-1 infection and found that levels of MAIT cells are severely reduced in circulation in patients with chronic untreated HIV-1 infection. Residual MAIT cells are highly activated and functionally exhausted. The decline of the MAIT population is associated with time since diagnosis, activation levels, and the concomitant expansion of hyper-activated CD161−Va7.2+ T cells. Interestingly, this cell population can be generated in vitro by culture in the environment. Interestingly, whereas in blood there is no preferential loss of any subset of MAIT cells, CD4+ MAIT cells appear to be preferentially lost from rectal mucosa. In addition, despite the observation that MAIT-cell levels are not significantly lower in rectal mucosa in HIV-1 infected subjects than in uninfected controls, the levels of mucosal MAIT cells correlate with levels of MAIT cells in blood. In addition, MAIT-cell levels in rectal mucosa tended to correlate inversely with time since HIV diagnosis. This overall pattern is consistent with a model where MAIT cells are eventually lost at this mucosal site, but significant losses may only be seen after peripheral blood MAIT cells are severely depleted. In fact, the severe loss of CD4 T cells in rectal mucosa may lead to an underestimation of the degree of MAIT-cell loss at this site. Further studies will be required to ascertain if this is the case.

Our data indicate that MAIT-cell loss in blood is paired and linked with the appearance of a CD161−Va7.2+ population of cells. We observe that MAIT cells express elevated levels of several activation markers in HIV-1–infected patients. The loss of MAIT cells correlates with the activation levels of MAIT cells, and the CD161−Va7.2+ T-cell population express even higher levels of activation markers. Furthermore, T cells with a CD161−Va7.2+ phenotype are generated by culture of MAIT cells with E coli. These observations are consistent with a model where continuous exposure to bacterial antigens contributes to the exhaustion and depletion of MAIT cells from circulation in HIV-1–infected patients. The exhausted MAIT cells seem, however, to persist at least partly in the form of the CD161−Va7.2+ T-cell population. Interestingly, in NK cells CD161 has been shown to be down-regulated by engagement by its ligand, the lectin-like transcript 1 (LT1).39 The observation that LT1 can be induced in APCs on exposure to microbes opens a possibility for further study, as well as for therapeutic intervention. The identification of possible means to restore the function and MAIT character of these cells by immunotherapy is a high priority. Our observation that at least some of the CD161−Va7.2+ T cells retain expression of PLZF and IL-18R suggests that cytokines should be evaluated for their ability to rescue these cells. Interestingly, the loss of CD161 expression in IL-18R+ Va7.2+ MAIT cells in the rectal mucosa correlated directly with time since HIV diagnosis, suggesting that it might be untreated HIV-1 infection. The decline in MAIT cells may affect the ability to control mycobacterial as well as other opportunistic infections. It should be noted that we observed no significant recovery of MAIT-cell numbers in blood after several years of effective cART. This observation adds yet another argument for initiating cART early during HIV-1 infection. In contrast to the lack of numerical recovery, MAIT-cell function recovered at least partly on cART. Thus, treatment benefits this immune cell compartment even if the effects are modest. It is unclear how rapid the insult to the MAIT-cell compartment may be during primary HIV-1 infection, and if early treatment may rescue MAIT-cell numbers as well as their function. Future studies should address these issues.

MAIT-cell levels in rectal mucosa appear to be better preserved compared with those in blood. One possible reason for this could be recruitment of MAIT cells to intestinal mucosa from blood. Interestingly, MAIT-cell levels are reduced in the blood, but enriched in the lungs, of individuals with active M tuberculosis infection.27,32 Already in early HIV-1 infection the mucosal immune system and mucosal barrier functions are compromised.8-13 MAIT-cell recruitment is a plausible way for the immune system to try to compensate for the lack of CD4 helper T cells to defend barrier integrity in the HIV-1 infected mucosa. As MAIT cells are primarily CD8+ they may be more resilient in this environment. Interestingly, whereas in blood there is no preferential loss of any subset of MAIT cells, CD4+ MAIT cells appear to be preferentially lost from rectal mucosa. In addition, despite the observation that MAIT-cell levels are not significantly lower in rectal mucosa in HIV-1 infected subjects than in uninfected controls, the levels of mucosal MAIT cells correlate with levels of MAIT cells in blood. In addition, MAIT-cell levels in rectal mucosa tended to correlate inversely with time since HIV diagnosis. This overall pattern is consistent with a model where MAIT cells are eventually lost at this mucosal site, but significant losses may only be seen after peripheral blood MAIT cells are severely depleted. In fact, the severe loss of CD4 T cells in rectal mucosa may lead to an underestimation of the degree of MAIT-cell loss at this site. Further studies will be required to ascertain if this is the case.

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possible to restore the rectal MAIT population by immunotherapeutic intervention.

MAIT cells respond to E coli stimulation by high-level production of IFNγ and TNF, and they also express some IL-17. However, the residual MAIT-cell population in chronically HIV-1–infected patients show severely suppressed production of IFNγ and TNF, and IL-17 is undetectable. In particular the loss of IL-17 production is striking and may have important implications given the role of this cytokine in defense against extracellular pathogens.41 The mechanisms of this unresponsiveness is at present unclear, but the cells appear to largely retain the ability to respond to PMA/ionomycin, suggesting that the defect may reside in the triggering and signal transduction pathway. The functionality of the residual MAIT-cell population recovers partly on effective cART, supporting this notion. We observe alterations in surface expression of several activation-associated receptors in MAIT cells in HIV-1–infected patients, and most of these do not revert when patients start cART. However, the levels of the inhibitory TIM-3 receptor decrease and the costimulatory CD27 increase after long-term effective cART. Future studies should evaluate the role that these receptors may play in MAIT-cell dysfunction in HIV-1 infection. Another possible avenue to explore in this regard is the functionality of APCs in HIV-1–infected subjects with regard to MR1-mediated antigen presentation and expression of costimulatory ligands. Numerous immune evasion pathways have been described for HIV-1 including down-modulation of phagocytosis,42-44 and the interference with MHC class I and CD1d antigen presentation.45,46 In particular, the parallel with the CD1d-restricted invariant natural killer T cells is interesting as this population shares many similarities with MAIT cells and displays a similar functional impairment in HIV-1–infected patients.47

In summary, we found that the MAIT-cell compartment is severely compromised in chronic HIV-1 infection. Given the size of this T-cell subset and their role in recognition of bacterial and fungal pathogens, it is reasonable to believe that this observation may have clinical significance. The relative preservation of MAIT cells in rectal mucosa is interesting and may suggest preferential homing to the intestinal mucosal barrier. Future studies should aim to address this possibility, to examine the role of MAIT cells in the control of opportunistic pathogens, to investigate the mechanisms of MAIT-cell exhaustion, as well as the ability of early cART to rescue the MAIT-cell compartment.

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Authorship

Contribution: E.L. and J.K.S. conceived and designed the experiments, and wrote the paper; E.L. and A.G. performed the experiments and analyzed data; M.F.Q., M.M., M.S., A.S., S.G.D., J.N.M., and P.W.H. identified patients, acquired and processed critical samples; B.L.S. provided critical material, supervised work at UC Davis, and wrote the paper; M.M., A.S., J.A., M.F.Q., and P.W.H. contributed to paper preparation; and JKS supervised the work.

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References


Activation, exhaustion, and persistent decline of the antimicrobial MR1-restricted MAIT-cell population in chronic HIV-1 infection

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