Immunology and Cell Biology (2014), 1–12

www.nature.com/icb

ORIGINAL ARTICLE

MAIT cells are depleted early but retain functional cytokine expression in HIV infection

Caroline S Fernandez[1], Thakshila Amarasena[1], Anthony D Kelleher[2,3], Jamie Rossjohn[4,5,6], James McCluskey[1], Dale I Godfrey[1,7] and Stephen J Kent[1]

Mucosal-associated invariant T (MAIT) cells home to mucosal sites and exert antimicrobial activity against bacteria and other microorganisms. HIV infection leads to early depletion of gut T cells and translocation of bacterial products. There are reports that MAIT cells, defined by coexpression of Vα7.2 and CD161, are depleted during HIV infection and residual MAIT cells are functionally impaired. However, one study suggested that MAIT cells might remain after HIV infection but evade detection through CD161 downregulation. Thus, the impact of HIV infection on MAIT cells is unclear. We studied longitudinal blood samples from 31 HIV-infected subjects for MAIT cell numbers, phenotype and function using both standard Vα7.2/CD161 surface markers and an MR1 tetramer. We found that MAIT cells were depleted early during HIV infection, and although there was a concomitant rise in Vα7.2$^+$CD161$^-$ cells, these were MR1 tetramer negative, indicating that these are unlikely to be altered MAIT cells. Antigen-mediated activation of residual MAIT cells showed that they remained functional out to 2 years following HIV infection. Although MAIT cells are depleted in HIV infection, residual and functionally active MAIT cells persist and may still be able to assist in controlling bacterial translocation during HIV infection.
Immunology and Cell Biology advance online publication, 28 October 2014; doi:10.1038/icb.2014.91

Human Immunodeficiency Virus type 1 (HIV-1) infection is characterised by high viraemia, rapid depletion of CD4$^+$ T cells from various compartments and widespread activation of cells of the immune system.[1–3] Substantial early depletion of CD4$^+$ T cells occurs in the gut and leads to a loss of an intact gastrointestinal -tract barrier.[4,5] There is a relationship between loss of CD4$^+$ T cells in the gut and translocation of microbial products from the lumen of the gut.[2,6] Markers of microbial translocation such as lipopolysaccharide are typically elevated in the peripheral blood of HIV-infected individuals and correlate with numbers of activated T cells.[6–8] Thus, microbial translocation can drive chronic immune activation, which occurs irrespective of the stage of infection and can persist despite combination antiretroviral therapy.[9,10]

Mucosal-associated invariant T (MAIT) cells are a population of T cells capable of antimicrobial activity against bacteria and yeasts[11,12] that utilise the riboflavin metabolic pathway.[13–16] MAIT cells are found in most tissues where T cells reside, including blood, liver, gut lamina propria and mesenteric lymph nodes[17,18] and they typically comprise 1–10% of human peripheral blood T cells.[11,19,20] They have an effector memory phenotype[19,20] and express tissue-homing markers such as C–C chemokine receptor (CCR)6, CXCR6 and CCR5.[19,21,22] MAIT cells are restricted to MHC-related protein 1 (MR1).[13,18,23–25] MAIT cells are usually defined by a Vα7.2Jα33 (Vα19Jα33 in mice) semi-invariant T-cell antigen receptor (TCR) α chain, paired with a limited selection of TCR Vβ chains, typically Vβ2 or Vβ13 (Vβ6 or Vβ8 in mice).[11,26,27] A recent study has shown that human MAIT cells can also utilise other TCR Jα and Vβ genes.[28]

Human MAIT cells have previously been identified as CD4$^-$ T cells coexpressing Vα7.2 and either CD161 or IL-18Rα.[19] Phenotypically, MAIT cells are CD8$^+$ (either CD8αα or CD8αβ)[28,29] and double negative (DN) for CD4 and CD8, with low levels of CD4 expression.[20,28] An MR1 tetramer has recently been developed to identify MAIT cells based on their TCR specificity.[16,28]

Three reports have provided data showing the disappearance of circulating MAIT cells, defined by coexpression of Vα7.2 and CD161, in HIV infection[21,22,30] and one determined that MAIT cells appear to be depleted by 3 weeks after the estimated date of infection.[22] However, one of these studies provided evidence that, rather than being depleted, many MAIT cells had changed their surface phenotype by downregulating CD161, thus evading detection.[30] Furthermore, residual MAIT cells appeared to be functionally impaired, unable to produce cytokines upon activation, suggesting exhaustion.[30] *In vitro* studies have demonstrated the inability of viruses to directly activate MAIT cells, which is attributable to viruses not furnishing a MAIT cell

[1]Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Parkville, Victoria, Australia; [2]Kirby Institute, University of New South Wales, Darlinghurst, New South Wales, Australia; [3]St Vincent's Centre for Applied Medical Research, Sydney, New South Wales, Australia; [4]The Department of Biochemistry and Molecular Biology, School of Biomedical Sciences, Monash University, Clayton, Victoria, Australia; [5]Institute of Infection and Immunity, Cardiff University School of Medicine, Heath Park, Cardiff, UK; [6]Australian Research Council Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton, Victoria, Australia and [7]Australian Research Council Centre of Excellence in Advanced Molecular Imaging, University of Melbourne, Parkville, Victoria, Australia
Correspondence: Professor S Kent, Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Parkville, Victoria 3010, Australia.
E-mail skent@unimelb.edu.au
Received 31 May 2014; revised 11 September 2014; accepted 12 September 2014

ligand,[11] and that HIV does not preferentially infect or activate MAIT cells.[21] It has also been hypothesised that in HIV infection, MAIT cells home to the gut, where substantial microbial translocation from the gut lumen occurs, and undergo apoptosis through the activation of caspase-3.[21] Accordingly, although there appear to be significant changes in the MAIT cell compartment-associated with HIV infection, the extent of MAIT-cell depletion, and the responsiveness of residual MAIT cells, remains unclear.

We report on the characteristics of peripheral MAIT cells and its subsets during early HIV infection. MAIT cells were depleted during early HIV infection, and while we also observed an increase in another population of Vα7.2$^+$CD161$^-$ T cells, these did not appear to be altered MAIT cells as they failed to bind MR1 tetramer. In sharp contrast with an earlier study,[30] in our cohort of patients, peripheral MAIT cells appeared to be significantly less activated than conventional T cells during HIV infection and their function was not impaired.

RESULTS

The HIV-infected cohort

MAIT cells may have a role in controlling microbial translocation in the gut during HIV infection, but the response of MAIT cells to HIV infection remains unclear,[21,22,30] and furthermore, no studies have investigated large-cohort longitudinal samples early after primary infection.

This study of MAIT cells in HIV infection involved analyses of frozen peripheral blood mononuclear cell (PBMC) from HIV-infected subjects recruited in Australia. The cohorts were first analysed for Vα7.2$^+$CD161$^+$ (MAIT cells), Vα7.2$^+$CD161$^-$ T cells and total T cells for comparison (Supplementary Figure 1). The HIV-infected cohort comprised 97% males who were antiretroviral ART-naive, and from whom PBMC samples were collected early after HIV infection. In the Early HIV cohort, 77% of subjects were within 6 months of the estimated date of infection (mean, 4.6; median, 4; range 1–11 months). A second PBMC sample (Chronic HIV cohort) was collected from most of the subjects in the Early HIV cohort after an interval of ~21 months (median 25 months; range 18–34 months). PBMC collected from laboratory volunteers were used as control samples (healthy controls or HIV negative; 50% males).

Characteristics of Vα7.2$^+$ T cells in early HIV infection

To understand the response of MAIT cells and their potential to modulate cell surface CD161 during HIV infection, we first analysed T cells coexpressing Vα7.2 and CD161 in PBMC samples (Figure 1a). Live, doublet-excluded CD3$^+$ lymphocytes coexpressing Vα7.2 and CD161 were identified in the Healthy controls, Early HIV and Chronic HIV cohorts. This population was further subdivided into three populations based on CD161 staining intensity: Vα7.2$^+$CD161$^+$ (MAIT cells), Vα7.2$^+$CD161^{dim} and Vα7.2$^+$CD161$^-$ (Figure 1a). The Vα7.2$^+$CD161$^+$ MAIT-cell population was already significantly depleted by ~4 months after HIV infection (Figure 1b left panel; median, 1.1%; range, 0.3–5.5% of T cells) relative to the Healthy controls (median, 1.8%; range 0.1–4.3% of T cells; $P=0.013$). The degree of depletion was greater in the Chronic HIV cohort (median, 0.9%; range, 0.1–3.0% of T cells; $P<0.001$). Analysis of the 26 matched samples in the Early HIV and Chronic HIV cohorts revealed a significant further depletion later in HIV infection ($P=0.029$ between Early HIV and Chronic HIV cohorts by Wilcoxon-matched pairs signed-rank test). We observed a progressive decrease in Vα7.2$^+$CD161$^+$ MAIT cells during HIV infection when expressed as absolute cells numbers per microliter of blood (Figure 1c, left panel). The Vα7.2$^+$CD161^{dim} population was not different between the HIV-infected and uninfected subjects (Figures 1b and c, middle panels).

A previous report showed an increase in a Vα7.2$^+$CD161$^-$ T cell population during chronic HIV infection and suggested that these may represent MAIT cells that had downregulated CD161.[30] Although we did not observe a significant increase in these cells in early HIV infection, we also found these Vα7.2$^+$CD161$^-$ T cells were increased, as a proportion of T cells, in chronic HIV infection (Figure 1b right panel). We did not, however, observe a significant change in Vα7.2$^+$CD161$^-$ T cells during HIV infection when expressed as absolute cells per microliter (Figure 1c right panel).

MAIT cell and Vα7.2$^+$CD161$^-$ T-cell subset distribution

There is little information on the distribution of subsets of peripheral Vα7.2$^+$CD161$^+$ MAIT cells during early HIV infection. We analysed the frequencies of the CD4$^+$, CD8$^+$ and DN subsets of this population in the three cohorts studied (Figure 2a). We first assessed the proportion of Vα7.2$^+$CD161$^+$ T cells defined by the carriage of CD4, CD8 or DN for both (Figure 2b). The CD4$^+$ subset comprised the smallest fraction with a mean frequency of ~2.4% of Vα7.2$^+$CD161$^+$ T cells across all three cohorts. The CD8$^+$ cells comprised the largest fraction, with median frequencies of 75%, 65% and 65% of Vα7.2$^+$CD161$^+$ T cells in the Healthy controls, Early HIV and Chronic HIV cohorts, respectively. The DN subset comprised a smaller fraction of Vα7.2$^+$CD161$^+$ T cells, but was substantially larger than the CD4$^+$ subset, with medians of 21%, 29% and 34% in the Healthy controls, Early HIV and Chronic HIV cohorts, respectively (Figure 2b).

A study of the above subsets within the total T-cell population revealed several interesting features (Figure 2c). Depletion of the small CD4$^+$ subset of Vα7.2$^+$CD161$^+$ T cells occurred slowly during HIV infection. There was no significant depletion observed early after HIV infection ($P=0.585$), but mild depletion occurred later (Figure 2c, top panel). Narrowing the subject cohorts to only the 26 matched samples of the Early HIV and Chronic HIV cohorts revealed no significant difference when expressed as a % of T cells ($P=0.056$, Figure 2d, top panel), with a similar finding when expressed as numbers of cells μl^{-1} ($P=0.059$, Supplementary Figure 2b, top panel). A pronounced and sustained depletion of the percentage of CD8$^+$ Vα7.2$^+$CD161$^+$ T cells was observed at both the early and chronic time points of HIV infection relative to the Healthy controls (Figure 2c, centre panel), and there was no difference between the matched samples from the Early HIV and Chronic HIV cohorts ($P=0.073$; Figure 2d centre panel). A similar pattern of depletion was observed when expressed as numbers of CD8$^+$ Vα7.2$^+$CD161$^+$ T cells μl^{-1} (Supplementary Figure 2a, middle panel). However, significant depletion was observed at the chronic time point in the matched samples when absolute numbers were analysed (Supplementary Figure 2b, middle panel). In contrast, the DN subset of Vα7.2$^+$CD161$^+$ MAIT cells was not significantly depleted early ($P=0.178$), but showed significant depletion ($P=0.034$) later in HIV infection (Figure 2c, bottom panel), although there was no difference between the matched samples ($P=0.316$; Figure 2d). When expressed as numbers of DN Vα7.2$^+$CD161$^+$ T cells μl^{-1} (Supplementary Figures 2a and b, lower panels), depletion occurred in both the Early HIV and Chronic HIV cohorts relative to the Healthy controls, although there was no further depletion at the chronic time point.

Given our observation that total Vα7.2$^+$CD161$^+$ T cells were depleted, although the frequencies, but not absolute numbers, of Vα7.2$^+$CD161$^-$ T cells were increased in chronic HIV infection (Figure 1b), we first determined if the frequencies of Vα7.2$^+$ cells

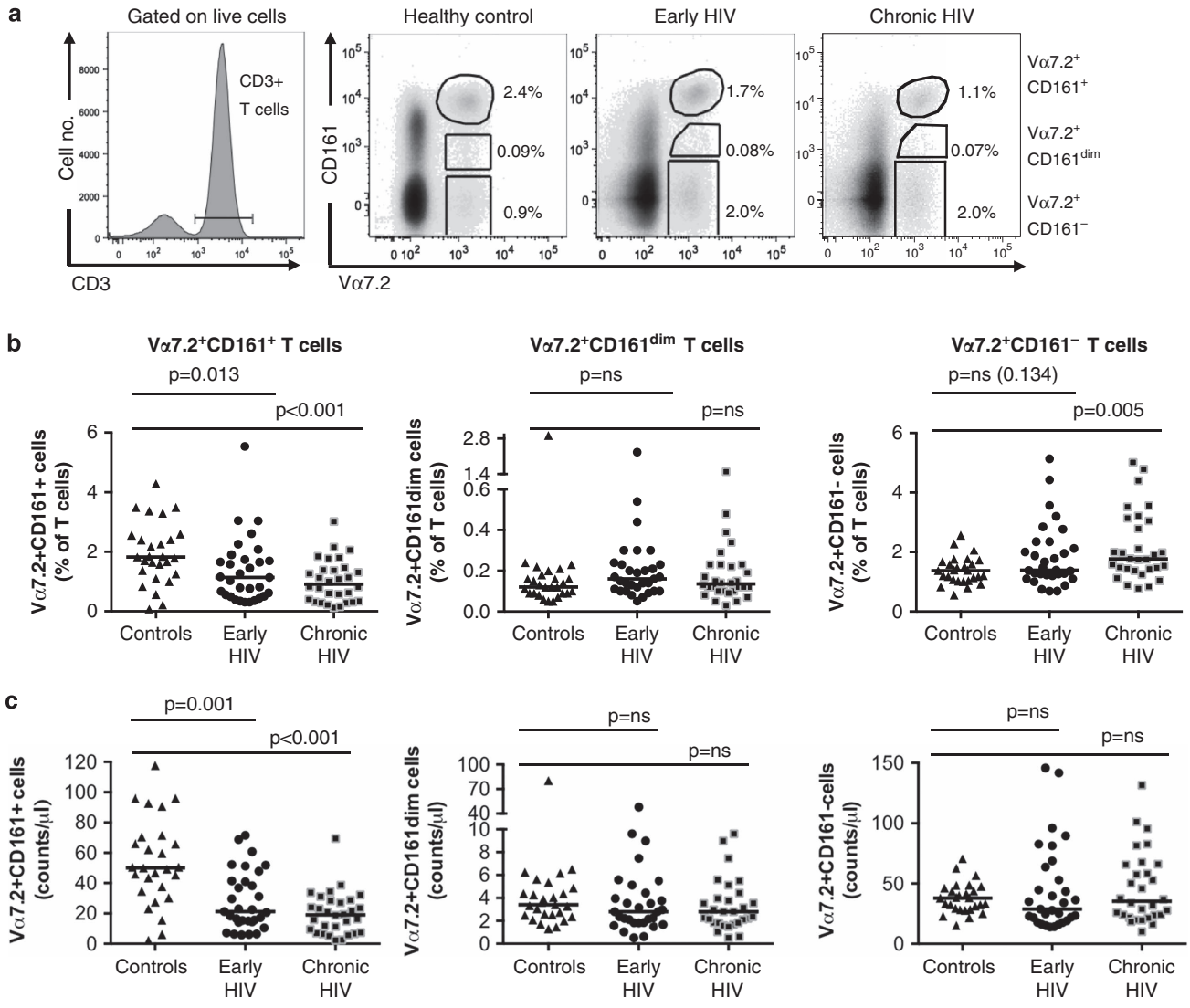

Figure 1 Characteristics of MAIT cells (Vα7.2$^+$CD161$^+$) and Vα7.2$^+$CD161$^{dim/-}$ T cells during HIV infection. (**a**) Live, double-excluded CD3$^+$ T cells were further phenotyped as Vα7.2$^+$CD161$^+$ (Vα7.2$^+$CD161^{bright}), Vα7.2$^+$CD161^{dim} or Vα7.2$^+$CD161$^-$ within a Healthy control and a matching subject from the Early and Chronic HIV cohorts. (**b**) Scatter plots of the frequencies of the above three populations within the Healthy controls (HIV negative; triangles, $n=27$), Early HIV (circles, $n=31$) or Chronic HIV (squares, $n=30$) cohorts. (**c**) Absolute numbers of the above three populations were calculated by multiplying the frequency of each population (in b. above) by the number of lymphocytes present by full blood examination (in the HIV-infected samples) or by the average lymphocyte count in blood in the controls. Bars represent medians. Kruskal–Wallis with Dunn's post test was applied to each data set.

among total T cells of the three cohorts were different (Figure 3a). The overall frequencies and ranges of total Vα7.2$^+$ T cells were not different between the three cohorts (Figure 3b). We reasoned that the apparent expansion in the frequencies of the Vα7.2$^+$CD161$^-$ population was due to depletion of the CD4$^+$ subset of this population in HIV-infected subjects, thus distorting the frequency of the pool of available T cells. Therefore, we analysed the distribution of subsets within the Vα7.2$^+$CD161$^-$ T-cell population expressed as proportions as well as absolute numbers of T cells. The CD4$^+$ subset of Vα7.2$^+$CD161$^-$ T cells showed significant depletion early in HIV infection, while significant expansion was observed in both the CD8$^+$ and DN subsets of this population (Figure 3c top panels). However, as would be expected given the effects of HIV infection on CD4+ T cells, when absolute counts were analysed, only the CD4$^+$ subset of Vα7.2$^+$CD161$^-$ T cells was significantly altered during the course of HIV infection (Figure 3c bottom panels).

MAIT cells are not as highly activated as total T cells

MAIT cells have been reported to be highly activated in chronic HIV infection.[30] We therefore studied the activation status and frequency of activated MAIT cells (Vα7.2$^+$CD161$^+$ T cells) during early HIV infection, and in comparison with total T cells and Vα7.2$^+$CD161$^-$ T cells (Figure 4). Enumeration of T cells coexpressing CD38 and HLA-DR is a better indicator of immune activation during HIV infection than single expression of either marker.[31] Initially, we examined activation of total T cells in each of the three cohorts (Figure 4a). High levels of activated T cells were observed in the Early HIV and Chronic HIV cohorts relative to Healthy controls. Both CD8$^+$ and DN subsets of total T cells were activated relative to the Healthy controls (data not shown). The frequencies of activated MAIT cells in the HIV-infected subjects were higher than in the Healthy controls, but much lower compared with activated total T cells, at a median of 0.2% for

each of the Early HIV and Chronic HIV cohorts (Figure 4b). A delineation of MAIT cell subsets expressing these activation markers demonstrated a similar degree of activation in the $CD8^+$ and DN subsets of MAIT cells (data not shown). In contrast, the $V\alpha7.2^+CD161^-$ T cells displayed high levels of activation, similar to total T cells (Figure 4c).

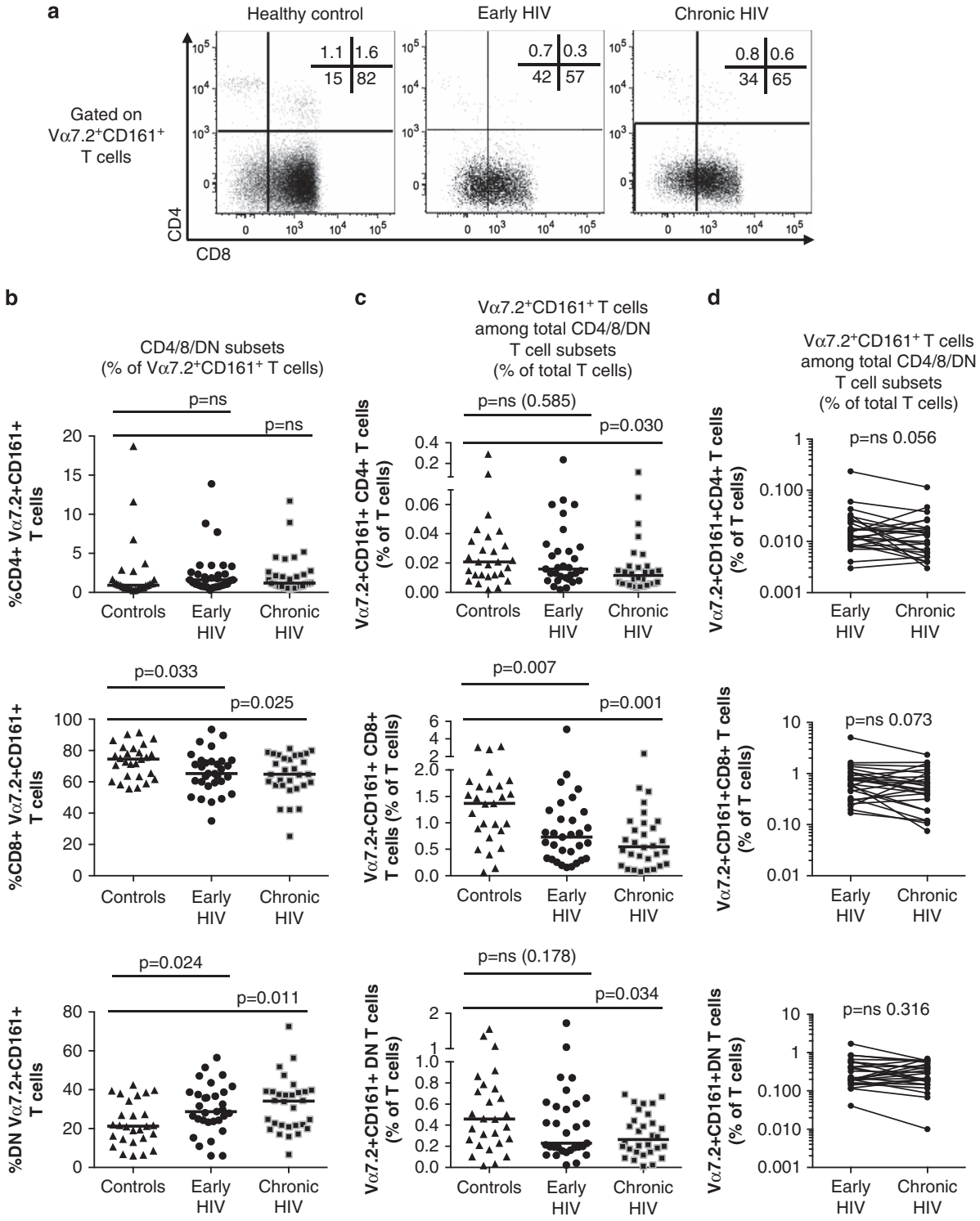

Figure 2 Early depletion of $CD8^+$ and later depletion of $CD4^-CD8^-$ (double negative or DN) MAIT cells in HIV infection. (**a**) Representative flow cytometry plots of the proportions of $CD4^+$, $CD8^+$, double positive (DP or $CD4^+CD8^+$) and double negative (DN or $CD4^-CD8^-$) subsets within $V\alpha7.2^+CD161^+$ T cells of one subject from the Healthy controls and a matching subject from each of the Early and Chronic HIV cohorts. (**b**) The proportions of $CD4^+$, $CD8^+$ or DN subsets within the $V\alpha7.2^+CD161^+$ T-cell population. (**c**) The proportion of $V\alpha7.2^+CD161^+$ cells within total $CD4^+$, $CD8^+$ or DN T cells, expressed as a percentage of total T cells. (**d**) A Wilcoxon signed-ranks test was performed between 26 matched samples of the Early HIV and Chronic HIV cohorts of each data set in (c). Bars represent medians. Kruskal–Wallis with Dunn's post test was applied to each data set, except where indicated otherwise.

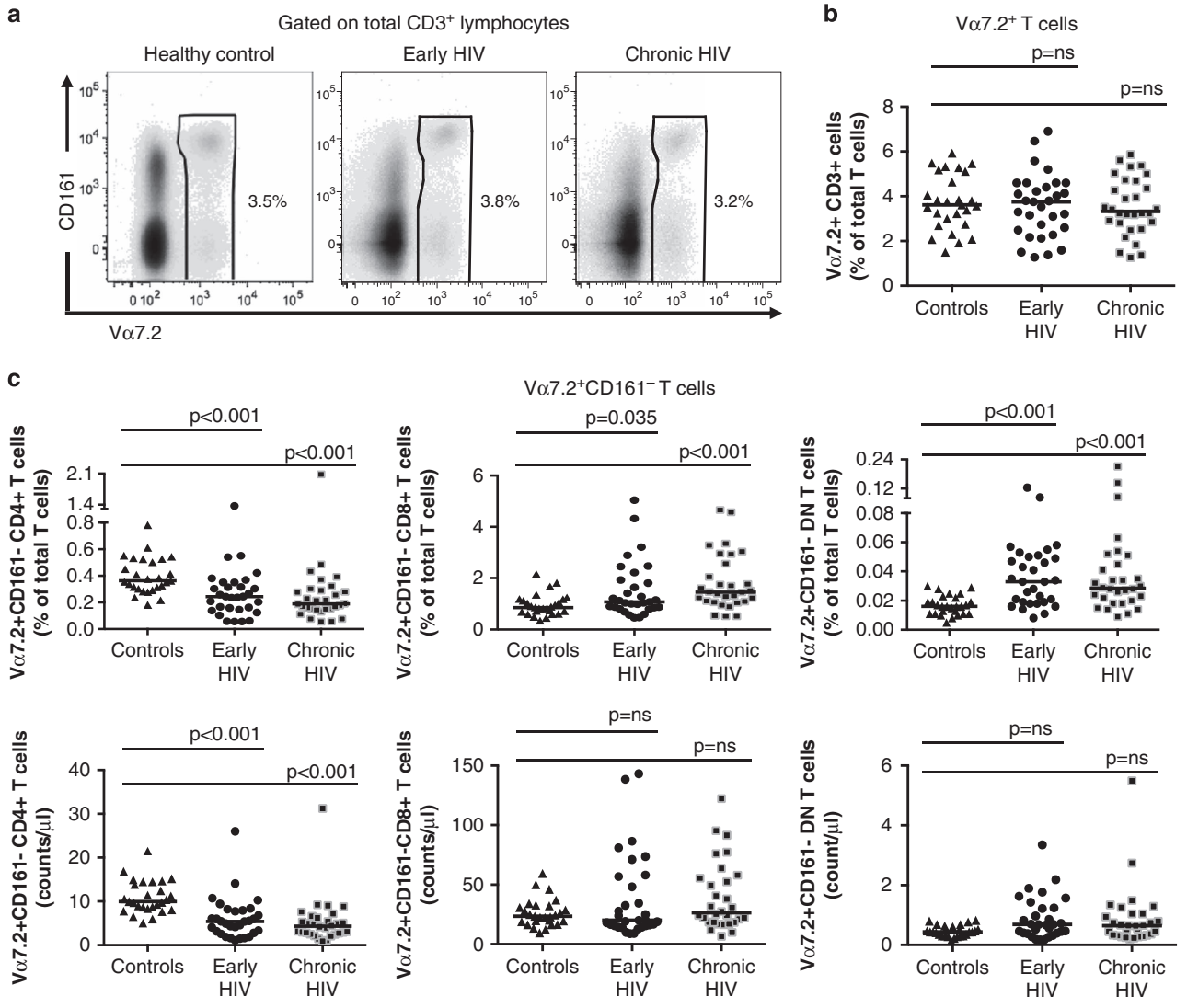

Figure 3 Expansion of Vα7.2$^+$CD161$^-$ T cells early in HIV infection. (**a**) Gating strategy to phenotype CD3$^+$ T cells expressing total Vα7.2 in a Healthy control and a matching subject from the Early and Chronic HIV cohorts. (**b**) Scatter plots of total Vα7.2$^+$ T cells in the Healthy controls, Early HIV or Chronic HIV cohorts (**c**) Scatter plots of the proportions of Vα7.2$^+$CD161$^-$ cells within total CD4$^+$, CD8$^+$ or DN T cells, expressed as a percentage of total T cells (top panels) or the absolute counts of Vα7.2$^+$CD161$^-$ T cells among each subset (bottom panels). Bars represent medians. Kruskal–Wallis with Dunn's post test was applied to each data set.

MAIT cell frequencies and HIV disease progression

MAIT cells respond to some bacteria/yeasts rather than viral products; however, they may be depleted during HIV infection.[11] We investigated whether depletion of Vα7.2$^+$CD161$^+$ MAIT cells early in infection correlated with plasma viraemia, blood CD4 T cell counts or T-cell activation (Supplementary Figure 3).

Total MAIT cell frequencies in the Early HIV cohort tended to correlate inversely with early plasma viral load (Supplementary Figure 3a; $r=-0.305$, $P=0.095$). When the same correlations were done with the CD8$^+$ or DN subsets of MAIT cells, we found no significant correlation with either subset (Supplementary Figures 3b and 4c). Early MAIT cell frequencies were not predictive of chronic plasma viral load (Supplementary Figure 3d; $r=-0.104$, $P=0.612$). Analyses of MAIT cell frequencies of the Early HIV cohort and early CD4$^+$ T-cell counts or early activation levels of total T cells demonstrated no significant relationship (Supplementary Figures 3e and f). Similarly, early MAIT cell frequencies were not predictive of chronic CD4$^+$ T-cell counts or chronic T-cell activation levels (Supplementary Figures 3g and h). This concurs with previous studies where no correlation between HIV viraemia and peripheral MAIT cell levels was observed.[21,22,30]

MR1-tetramers do not bind to Vα7.2$^+$CD161$^-$ T cells

The depletion of Vα7.2$^+$CD161$^+$ T cells and expansion in the frequency of Vα7.2$^+$CD161$^-$ T cells raised the possibility that CD161 was simply being downregulated on MAIT cells as proposed in a recent study.[30] During the course of our studies, an MR1-tetramer reagent[16] became available to identify MAIT cells based upon their TCR specificity. This reagent was used to directly investigate whether the Vα7.2$^+$CD161$^-$ T cells were MAIT cells. We stained a subset of 10 frozen PBMC samples from the Chronic HIV cohort and the Healthy controls simultaneously with Vα7.2, CD161 and the MR1-tetramer loaded with the MAIT cell antigen 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (MR1:5-OP-RU) or the control MR1 tetramer

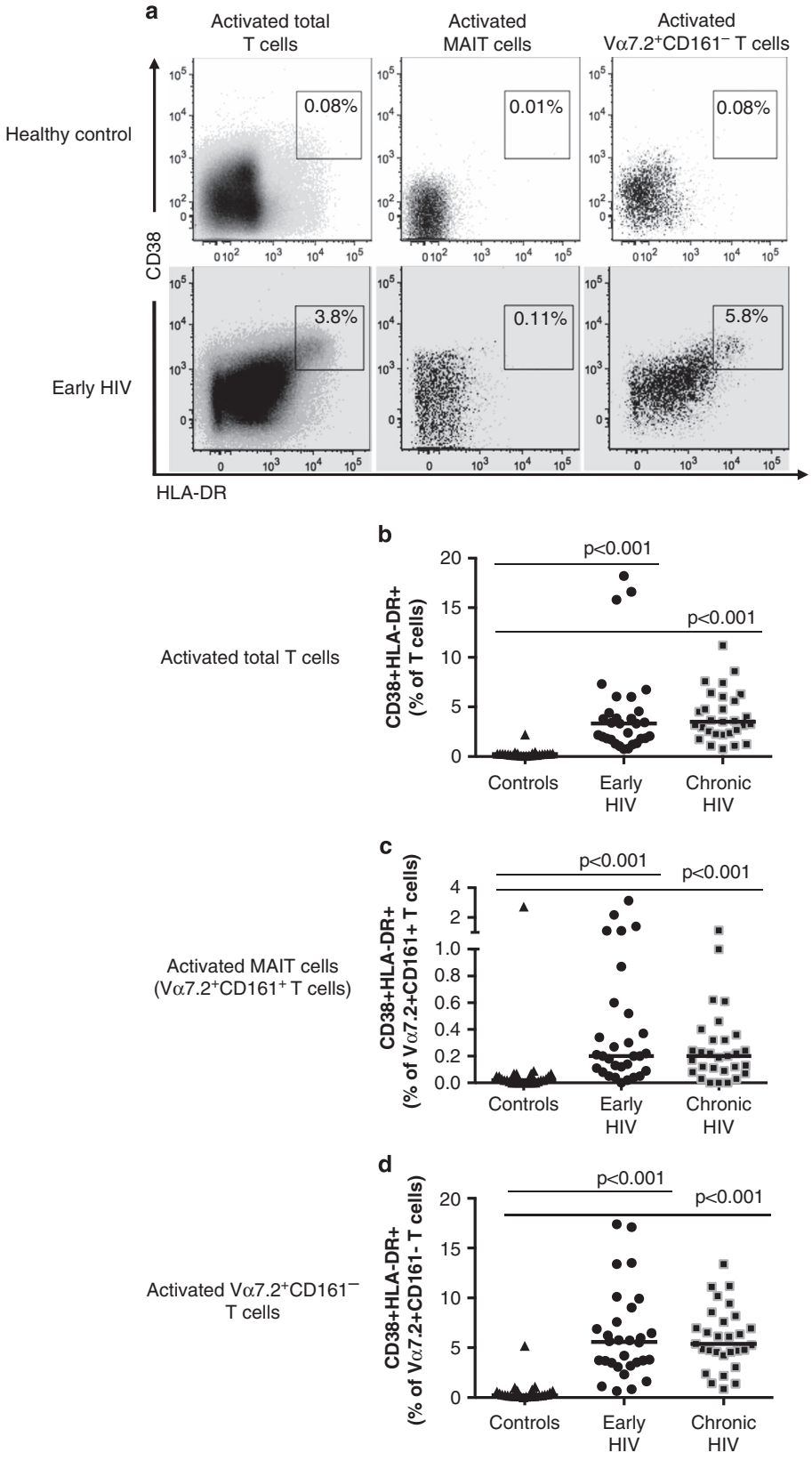

Figure 4 MAIT cells are activated in HIV infection, but less activated compared with conventional T cells. (**a**) Representative density plots from a healthy control and an Early HIV subject. Coexpression of the activation markers CD38 and HLA-DR on total T cells (**b**), total MAIT cells (**c**), and Vα7.2+CD161− T cells (**d**). Bars represent medians. Kruskal–Wallis with Dunn's post test was applied to each data set.

loaded with the MAIT non-activating antigen 6-FP (MR1:6-FP; Figure 5a). The MR1:5-OP-RU tetramer for MAIT cells was highly specific for CD161$^+$ T cells and did not bind CD161$^-$ T cells in either HIV$^+$ subjects (Figure 5b; median, ~0.083% of T cells) or Healthy controls (not shown; median, 0.052% of T cells). An MR1tet$^+$ low staining population that is not evident in the MR1:6-FPtet plot was found to be negative for Vα7.2, indicating this population is not MAIT cells (Figure 5c). An average of over 95% of Vα7.2$^+$CD161$^+$ T cells were MR1:5-OP-RU tetramer$^+$ in both Healthy controls and the Chronic HIV cohorts (Figure 5d). MR1:5-OP-RU tetramer$^+$

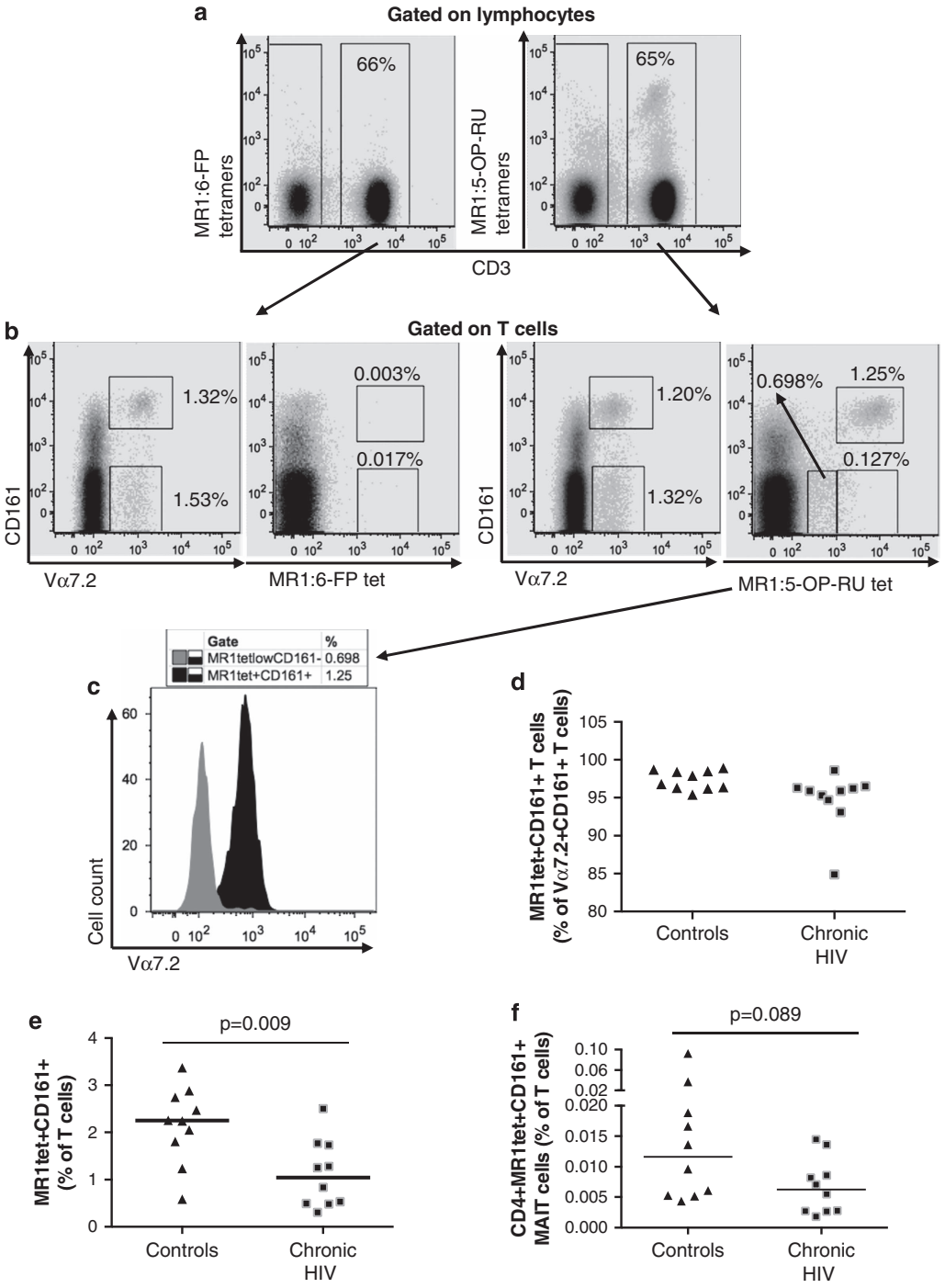

Figure 5 Vα7.2$^+$CD161$^-$ T cells do not bind MR1 tetramers. (**a**) Representative density plots of lymphocytes from one subject in the Chronic HIV cohort positive for CD3 and MR1 tetramers loaded with 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (MR1:5-OP-RU) or 6-formyl pterin (MR1:6-FP). (**b**) Expression of T cells positive for MR1:6-FP tetramer or MR1:5-OP-RU tetramer, Vα7.2 and CD161 was determined. (**c**) There is negligible background staining of T cells with MR1:6-FP tetramer as evidenced by Vα7.2 binding. (**d**) The majority of Vα7.2$^+$CD161$^+$ T cells are MAIT (MR1:5-OP-RUtet$^+$CD161$^+$) cells. (**e**) Depletion of MR1:5-OP-RUtet$^+$ MAIT cells in the Chronic HIV cohort. (**f**) Nonsignificant depletion of CD4$^+$ MAIT cells in the Chronic HIV cohort relative to controls. A Mann–Whitney test was used in (**e**) and (**f**).

MAIT cells of the HIV$^+$ subjects are significantly depleted compared with those of healthy controls ($P=0.009$; Figure 5e).

We found that only ~30% of CD4$^+$Vα7.2$^+$CD161$^+$ T cells were positive for the MR1:5-OP-RU tetramers (medians of 31% and 28%; range, 10–78% and 4–66%, respectively, of the Healthy controls and Chronic HIV cohorts). In agreement with a previous report,[28] only ~1% of all MR1:5-OP-RU tetramer binding MAIT cells were CD4$^+$ (range, 0.3–2.8% in Healthy controls and 0.2–1.7% in Chronic HIV). Interestingly, analysis of the frequencies of the CD4$^+$ MR1:5-OP-RU tetramer$^+$ MAIT cell subset within total T cells revealed no significant depletion in the Chronic HIV cohort relative to the Healthy controls ($P=0.089$ by Mann–Whitney Rank Sum U test; Figure 5f). This suggests that the depletion of CD4$^+$Vα7.2$^+$CD161$^+$ T cells observed in the Chronic HIV cohort (Figure 2c top panel) is driven mainly by non-MAIT cells.

MAIT cells have reduced CCR6 expression in HIV infection

A characteristic of MAIT cells is their homing to the gut via chemokine receptors such as CCR6.[19,21] We therefore analysed MR1:5-OP-RU tetramer$^+$ MAIT cells for their expression of CCR6. CCR6 expression level was reduced on MAIT cells in PBMC samples from subjects with chronic HIV infection compared with the healthy controls (Figures 6a and b). This reduction in CCR6 expression on MAIT cells was reflected both in the proportion of MAIT cells expressing CCR6 and the mean fluorescent intensity of CCR6 expression (Figure 6b). In contrast, the Vα7.2$^+$CD161$^-$ non-MAIT

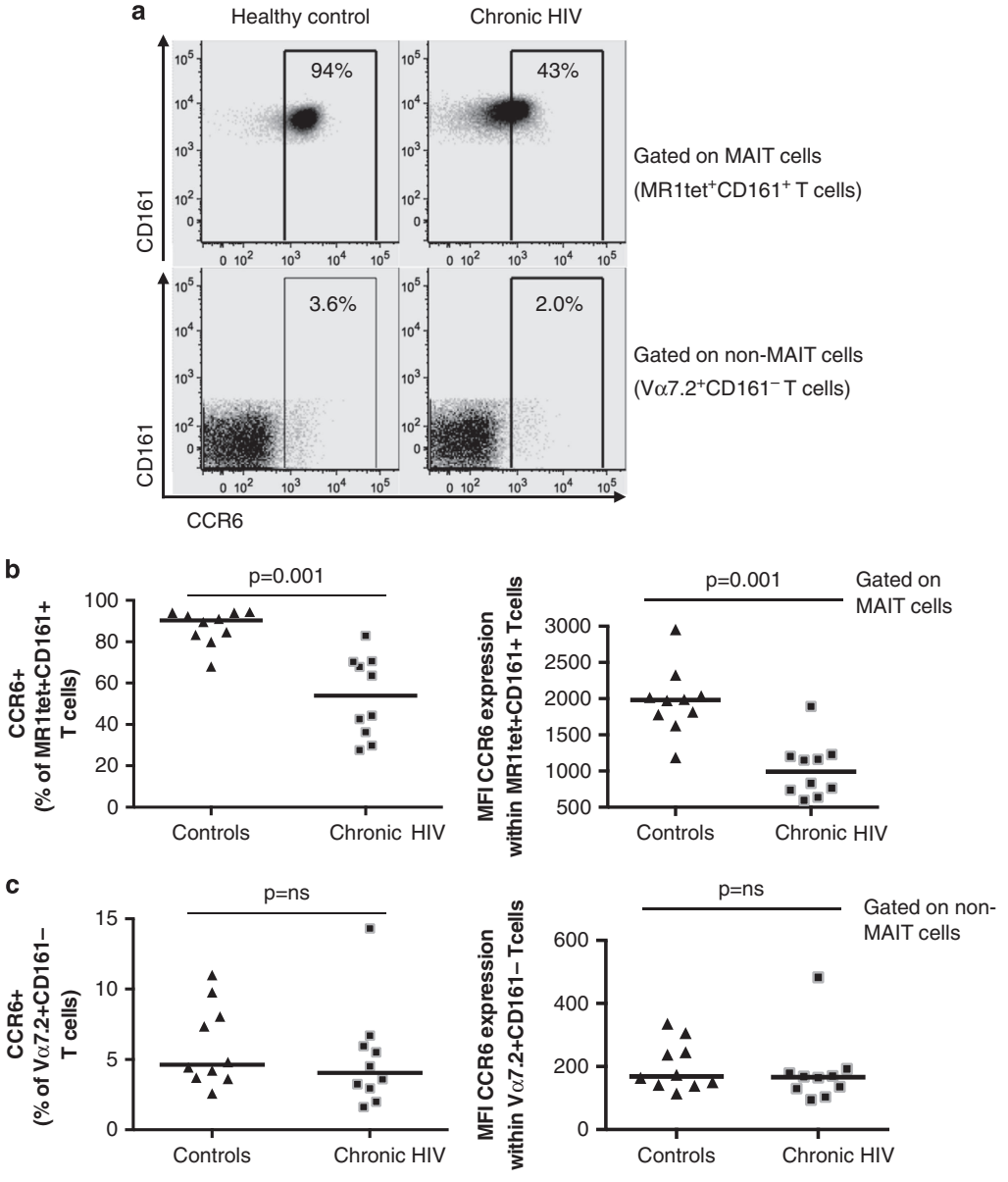

Figure 6 MAIT cells are deficient in CCR6 expression in chronic HIV infection. (**a**) Representative density plots of CCR6 expression within MR1:5-OP-RU tet$^+$CD161$^+$ MAIT cells and Vα7.2$^+$CD161$^-$ non-MAIT cells in a Healthy control and a Chronic HIV subject. (**b**) CCR6 expression among MR1:5-OP-RUtet$^+$ CD161$^+$ MAIT cells in Healthy controls and Chronic HIV subjects, expressed as percentages or mean fluorescent intensities (MFI). (**c**) CCR6 expression among Vα7.2$^+$CD161$^-$ non-MAIT cells in Healthy controls and Chronic HIV subjects, expressed as percentages or MFI. Bars represent medians. A Mann–Whitney test was applied to each data set.

population were nearly all CCR6$^-$ and were unchanged in the chronic HIV setting (Figures 6a and c).

MAIT cells from HIV-infected subjects have normal function

MAIT cell function against *Escherichia coli* has been reported to be impaired in subjects with chronic HIV infection compared with healthy controls.[30] The availability of the MAIT cell ligand, synthetic rRL-6-CH_2OH (or 6-HM for simplicity) as well as the MAIT non-activating ligand 6-formyl pterin (6-FP)[28,32] allowed us to study functional responses to these synthetic ligands. Activation-induced TCR downregulation has previously been described for innate lymphocytes such as NKT cells.[33–35] To circumvent the apparent loss of MAIT cells due to TCR downregulation, we gated on total lymphocytes positive for MR1:5-OP-RU tetramer and expressing high levels of CD161 (Figure 7a). MAIT cells were then further phenotyped for intracellular expression of IFNγ or TNF (Figure 7b). Expression of

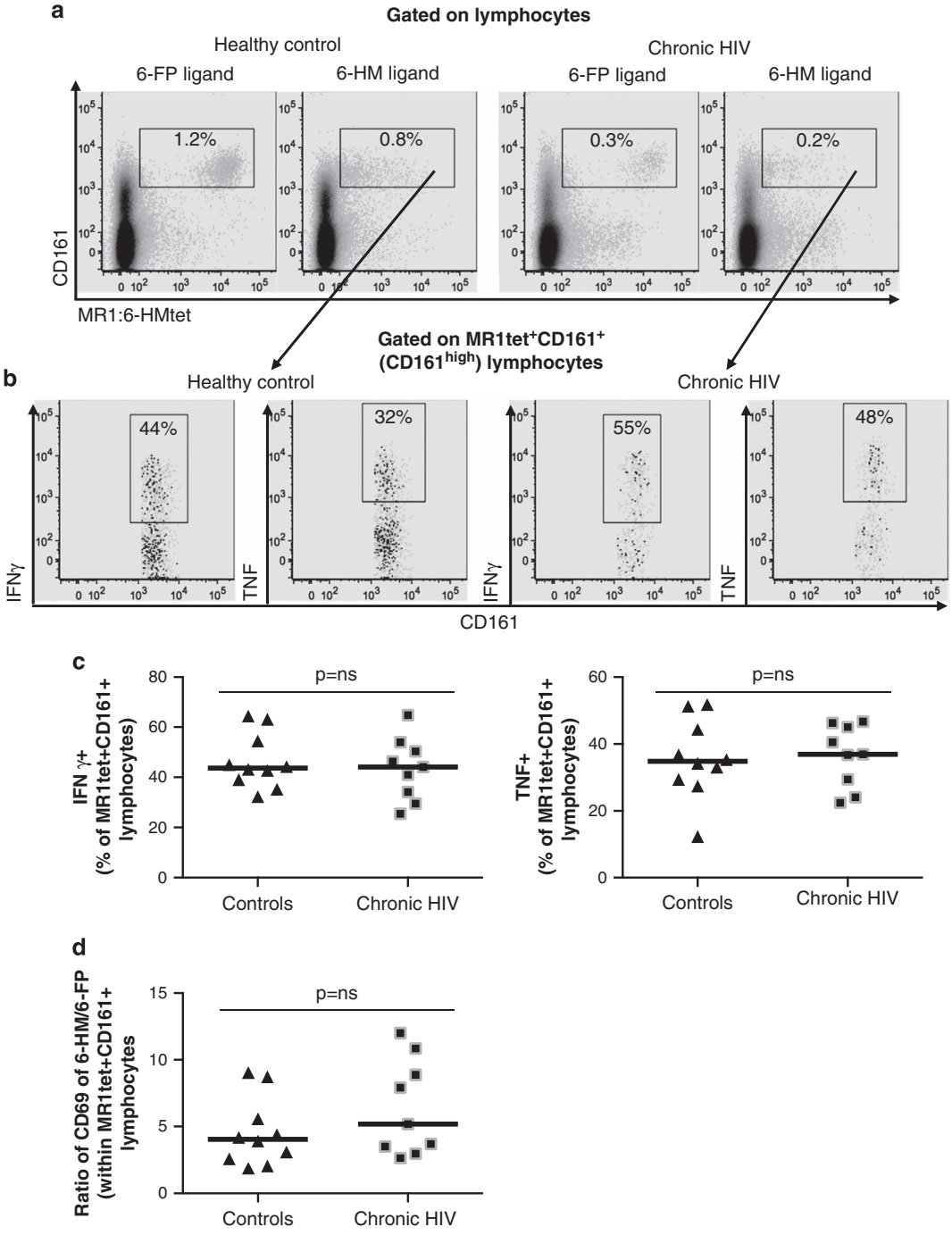

Figure 7 MAIT cells are fully functional in chronic HIV infection. (**a**) MAIT cells were identified as lymphocytes double positive for MR1:5-OP-RU tetramer and CD161. (**b**) Gating of 6-HM-activated MAIT cells expressing IFNγ or TNF. (**c**) Intracellular IFNγ or TNF expression from MAIT cells in the Healthy control or Chronic HIV cohort. Values are background subtracted from 6-FP-stimulated samples. (**d**) Ratio of CD69 expression in 6-HM/6-FP stimulated samples among the MR1:5-OP-RUtet$^+$CD161$^{+(high)}$ lymphocytes. Bars represent medians. A Mann–Whitney analysis was applied to each data set.

cytokines from MAIT cells of HIV chronically infected subjects was no different to that of healthy controls (Figure 7c). Further, HIV-infected subjects upregulated the activation marker CD69 to a similar degree as healthy controls (Figure 7d). Although CD69 upregulation in the two cohorts was comparable, we observed that cytokine expressing MAIT cells (MR1:5-OP-RUtet$^+$ lymphocytes) of the healthy controls downregulated CD161 (CD161^{dim}) more readily (Supplementary Figures 4a and b top and bottom panels). MR1:5-OP-RUtet $^+$CD161$^-$ lymphocytes expressed negligible amounts of either cytokine (data not shown).

As MAIT cells are CD3$^+$, the conventional method of identifying MAIT cells is to gate on CD3$^+$ lymphocytes followed by further gating on MR1:5-OP-RUtet$^+$ CD161$^+$ T cells (Supplementary Figure 4c). We found that such a gating strategy resulted in significantly lower IFNγ or TNF expression ($P=0.011$ and $P=0.041$, respectively) in the Chronic HIV cohort compared with the Healthy controls cohort (Supplementary Figure 4d(i)). However, analyses of MAIT cell numbers revealed no significant difference in cytokine expression (Supplementary Figure 4d(ii)). When cytokine expression from MR1:5-OP-RUtet$^+$CD161$^+$ lymphocytes (Figure 7b) was back-gated onto total lymphocytes we observed that the CD3$^-$ lymphocyte population expressed the greatest amount of cytokines, consistent with activation-induced CD3 downregulation of the MAIT cells (Supplementary Figure 4e).

DISCUSSION

MAIT cells are innate T cells that utilise a semi-invariant T-cell receptor to recognise riboflavin metabolites derived from many different bacteria and yeasts.[15,36] The response of MAIT cells to viral infections such as HIV is currently unclear.[21,22,30] We found blood MAIT cells were depleted early after infection, and this impacted on both the CD8$^+$ and DN MAIT cell subsets. We observed a concomitant expansion in the frequency, but not the absolute numbers, of a Vα7.2$^+$CD161$^-$ T-cell population that was previously proposed to represent MAIT cells that may have downregulated CD161.[30] In addition, using a MAIT-cell-specific tetramer, we found that this Vα7.2$^+$CD161$^-$ population did not contain MAIT cells. Further, in contrast with a previous report,[30] we report that MAIT cells in chronic HIV-infected patients only moderately upregulated activation markers compared with other T cells and were fully capable of producing cytokines.

The massive depletion of CD4$^+$ T lymphocytes in the gut and the subsequent disruption of the gastrointestinal mucosal barrier enables microbial pathogens and their products to enter the blood of HIV/SIV-infected subjects.[6–8] MAIT cells express gut-homing markers and are thought to migrate to the gut during HIV infection, resulting in their apparent loss from the periphery during HIV infection.[21] MAIT cells upregulate activation markers such as CD69 and CD25, and expand in response to bacterial infection *in vitro*.[11,21] It was therefore unexpected that our data showed lower levels of activated peripheral MAIT cells compared with other T cells, considering the microbial translocation occurring in HIV infection.[6–8] Future studies could correlate markers of microbial translociation with MAIT-cell activation and loss. Highly activated MAIT cells may migrate to mucosal sites and undergo apoptosis, although we also found a loss of the tissue-homing marker CCR6 in chronic HIV infection. A previous study found MAIT cells in the rectal mucosal biopsies of chronically HIV-infected subjects[30] and that frequencies of CD8$^+$ and DN Vα7.2$^+$ CD161$^+$ T cells, were similar between HIV-infected and -uninfected subjects, suggesting either a *de novo* preservation of mucosal MAIT cells or an influx of these cells from peripheral blood. In support of the latter notion, MAIT cells appear to migrate from the blood of subjects with bacterial infections, such as tuberculosis, and accumulate at the sites of infections.[11,12] A recent report shows MAIT cells are reduced in the colon of subjects with chronic HIV infection, which were restored after antiretroviral therapy.[37] Thus, the mechanism of MAIT-cell depletion from peripheral blood in HIV infection is currently unclear. An advantage of our study over earlier reports is that we studied longitudinal samples from a median of ~4 months (early) to ~2 years (chronic) after seroconversion, whereas previous studies were cross-sectional in nature.

We speculate that the early loss of gut integrity, translocation of microbial products into the bloodstream during early HIV infection results in increased activation induced cell death of MAIT cells in the periphery and that this progressively worsens over time. Thus, the level of depletion in our cohort of chronic HIV individuals at ~2 years was not as severe as that reported in the study from Leeansyah *et al.*[30] at ~6–8 years post infection . This, combined with increased migration of MAIT cells from the blood to the gut, would result in depletion of peripheral blood MAIT cells as observed in this and previous studies.[21,22,30]

An important difference between our study and the previous report[30] is that our subjects were followed without antiretroviral therapy for a median of over 2 years and we did not find functional impairment of MAIT cells in response to activation. We utilised a specific ligand in conjunction with identifying MAIT cells using the brightly staining MR1 tetramer reagent. Leeansyah *et al.*[30] demonstrated significant MAIT-cell functional impairment in therapy-naive subjects infected with HIV for ~6–8 years using a whole-bacterial stimulation assay, identifying MAIT cells as Vα7.2$^+$CD161$^+$ T cells.[30] This may suggest that MAIT cells become functionally impaired later in HIV infection (that is, between 2 and 6–8 years). However, we found that downregulation of surface CD3/TCR markers on MAIT cells upon stimulation *in vitro* can make it difficult to accurately identify MAIT cells in these assays.

In conclusion, we found that peripheral blood MAIT cells were depleted early in HIV infection, but that remaining cells retain functional cytokine expression in response to activation. Furthermore, the Vα7.2$^+$ CD161$^-$ T cells that accumulate following infection do not appear to be MAIT cells as defined by MR1-tetramer staining. The depletion of antimicrobial MAIT cells at an early stage following HIV infection has important implications for the immunopathogenesis of this disease.

METHODS

Study subjects

The HIV-infected PBMC samples analysed were collected from antiretroviral therapy-naive subjects in the Core01/Phaedra cohort recruited from Australia. Briefly, PBMC samples were collected from 37 HIV-infected subjects, donors of whom 77% were within 6 months of seroconversion (mean, 4.6; median, 4; range, 1–11 months; Early HIV cohort). The time from infection was interpolated as either midpoint between a negative and positive enzyme-linked immunosorbent assay, or 3 month prior to a positive detuned enzyme-linked immunosorbent assay. A second PBMC sample was collected from most of the same donors after an interval of ~ 21 months (median 25; range, 18–34 months; Chronic HIV cohort). Some PBMC samples from each cohort were non-viable following thawing and a small number of samples were excluded from each cohort prior to data analyses (Supplementary Figure 1). Final numbers of subjects in each cohort were as follows: $n=31$ subjects in the Early HIV cohort, $n=30$ subjects in the Chronic HIV cohort (with 26 subjects matched to the Early HIV cohort), and $n=27$ subjects in the Healthy controls (HIV negative) cohort recruited from laboratory volunteers (Supplementary Figure 1). Ethics approval was obtained from the relevant institutional committee of St Vincent's Hospital, Sydney or the University of Melbourne.

Phenotyping of lymphocyte populations

PBMC were phenotyped with the following panel of markers: Live-dead-Aqua (Life Technologies, Grand Island, NY, USA), CD3-Pacific Blue clone SP34-2 (BD Biosciences, San Diego, CA, USA), CD8-APC-H7 clone SK1 (BD Biosciences), CD161-APC clone HP-3G10 (BioLegend, San Diego, CA, USA), Vα7.2-FITC clone 3C10 (BioLegend) CD4-qdot 605 clone T4/19Thy5D7 (NIH nonhuman primate reagent resource), CD38-Alexa Fluor 700 clone HIT2 (BioLegend), HLA-DR-PE-CF594 clone G46-6 (BD Biosciences) for 30 min, followed by washing with fluorescence-activated cell sorting wash buffer (0.5% w/v bovine serum albumin, 2 mM ethylene diamine tetraacetic acid in phosphate-buffered saline) and fixation with formaldehyde (1% v/v) prior to flow cytometry analysis.

When the second generation MR1tetramers[16] became available during this study, a second sample from 10 subjects in the Chronic HIV cohort was phenotyped with MR1-PE tetramers loaded with either 5-(2-oxopropyl-ideneamino)-6-D-ribitylaminouracil (5-OP-RU) (the MAIT activating ligand) or 6-formyl pterin (6-FP, MAIT non-activating ligand) in conjunction with Live-dead-Aqua, CD3, CD8, CD161, CD4, CD38, HLA-DR and Vα7.2 as above and CCR6-PE-Cy7 clone 11A9 (BD Biosciences) for 30 min on ice followed by washing and fixation as above.

Activation of PBMC

PBMC (4×10^5) were incubated with C1R cells expressing MR1 (2×10^5) as previously described,[13] and stimulated with 0.0152 μM reduced synthetic 6-hydroxymethyl-8-D-ribityllumazine (rRL-6-CH_2OH or 6-HM; activating ligand) or 0.0152 μM 6-FP (non-stimulatory ligand) for 12 h, 37 °C. Brefeldin A (5μg ml^{-1}) was added 1 h post activation. Prior to permeabilisation, PBMC were stained with Live-dead-Aqua, MR1-PE tetramers loaded with 5-OP-RU, CD3, CD8, CD161 and CD4 as above and fixed. Cells were permeabilised with fluorescence-activated cell sorting PERM (BD Biosciences) and intracellularly stained with IFNγ-Alexa Fluor 700 clone B27 (BD Biosciences), TNF-PE-Cy7 clone MAb11 (BD Biosciences) and CD69-PerCP clone L78 (BD Biosciences).

Flow cytometry

Polychromatic flow cytometry analysis was performed on an 18-parameter LSR-Fortessa instrument (BD Biosciences) and analysed with FlowJo version 9.6 (Tree Star, Ashland, OR, USA). The large number of samples were phenotyped and analysed by flow cytometry in batches on different days. The position of the majority of CD4+ Vα7.2+CD161+ MAIT cells of each sample was used to guide placement of the gates. Absolute numbers of MAIT cells and subsets in the blood of the HIV-infected subjects were calculated by multiplying the % of MAIT cells within the lymphocyte gate by the number of lymphocytes present in the same blood sample through a diagnostic full blood examination. As there was no full blood examination on the same sample of the control HIV-negative healthy donors, the average number of lymphocytes in blood on the basis of standard reference ranges (2750 μl^{-1}) was used to calculate the approximate number of MAIT cells and MAIT subsets.

Statistical analyses

Graphs were created using Prism GraphPad Version 6 (La Jolla, CA, USA). Statistical analyses were done with SPSS Statistics Version 20 (IBM, Armonk, NY, USA) and described in each figure legend.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Drs Lars Kjer-Nielsen and Alexandra Corbett, University of Melbourne, for providing the new generation MR1 tetramers, MAIT ligands and MR1:C1R cells, and for sharing MAIT-cell activation methodologies. We thank Drs David Fairlie and Ligong Liu, Institute for Molecular Biology, University of Queensland for providing the MAIT cell ligands. We thank the doctors and nurses who recruited the subjects in the early HIV-infection cohort and the study subjects themselves. Supported by Australian National Health and Medical Research Council (NHMRC) awards 510448, 455350, 1013667, 454309 and 508309, NHMRC Research Fellowships to DIG and SJK and an NHMRC postgraduate scholarship (629002) to CSF.

1 Brenchley JM. Mucosal immunity in human and simian immunodeficiency lentivirus infections. *Mucosal Immunol* 2013; **6**: 657–665.
2 Klatt NR, Funderburg NT, Brenchley JM. Microbial translocation, immune activation, and HIV disease. *Trends Microbiol* 2013; **21**: 6–13.
3 Fevrier M, Dorgham K, Rebollo A. CD4+ T cell depletion in human immunodeficiency virus (HIV) infection: role of apoptosis. *Viruses* 2011; **3**: 586–612.
4 Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ *et al.* CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med* 2004; **200**: 749–759.
5 Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C *et al.* Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. *J Exp Med* 2004; **200**: 761–770.
6 Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S *et al.* Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006; **12**: 1365–1371.
7 Jiang W, Lederman MM, Hunt P, Sieg SF, Haley K, Rodriguez B *et al.* Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. *J Infect Dis* 2009; **199**: 1177–1185.
8 Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE *et al.* Plasma levels of soluble CD14 independently predict mortality in HIV infection. *J Infect Dis* 2011; **203**: 780–790.
9 El-Sadr WM, Lundgren J, Neaton JD, Gordin F, Abrams D, Arduino RC *et al.* CD4+ count-guided interruption of antiretro`l treatment. *N Engl J Med* 2006; **355**: 2283–2296.
10 Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC *et al.* Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med* 2008; **5**: e203.
11 Le Bourhis L, Martin E, Peguillet I, Guihot A, Froux N, Core M *et al.* Antimicrobial activity of mucosal-associated invariant T cells. *Nat Immunol* 2010; **11**: 701–708.
12 Gold MC, Cerri S, Smyk-Pearson S, Cansler ME, Vogt TM, Delepine J *et al.* Human mucosal associated invariant T cells detect bacterially infected cells. *PLoS Biol* 2010; **8**: e1000407.
13 Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L *et al.* MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* 2012; **491**: 717–723.
14 Birkinshaw RW, Kjer-Nielsen L, Eckle SB, McCluskey J, Rossjohn J. MAITs, MR1 and vitamin B metabolites. *Curr Opin Immunol* 2014; **26**: 7–13.
15 Gold MC, Lewinsohn DM. Co-dependents: MR1-restricted MAIT cells and their antimicrobial function. *Nat Rev Microbiol* 2013; **11**: 14–19.
16 Corbett AJ, Eckle SB, Birkinshaw RW, Liu L, Patel O, Mahony J *et al.* T-cell activation by transitory neo-antigens derived from distinct microbial pathways. *Nature* 2014; **509**: 361–365.
17 Arstila T, Arstila TP, Calbo S, Selz F, Malassis-Seris M, Vassalli P *et al.* Identical T cell clones are located within the mouse gut epithelium and lamina propia and circulate in the thoracic duct lymph. *J Exp Med* 2000; **191**: 823–834.
18 Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F *et al.* Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. *Nature* 2003; **422**: 164–169.
19 Dusseaux M, Martin E, Serriari N, Peguillet I, Premel V, Louis D *et al.* Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. *Blood* 2011; **117**: 1250–1259.
20 Martin E, Treiner E, Duban L, Guerri L, Laude H, Toly C *et al.* Stepwise development of MAIT cells in mouse and human. *PLoS Biol* 2009; **7**: e54.
21 Cosgrove C, Ussher JE, Rauch A, Gartner K, Kurioka A, Huhn MH *et al.* Early and nonreversible decrease of CD161++ /MAIT cells in HIV infection. *Blood* 2013; **121**: 951–961.
22 Wong EB, Akilimali NA, Govender P, Sullivan ZA, Cosgrove C, Pillay M *et al.* Low levels of peripheral CD161++CD8+ mucosal associated invariant T (MAIT) cells are found in HIV and HIV/TB co-infection. *PLoS ONE* 2013; **8**: e83474.
23 Hansen TH, Huang S, Arnold PL, Fremont DH. Patterns of nonclassical MHC antigen presentation. *Nat Immunol* 2007; **8**: 563–568.
24 Patel O, Kjer-Nielsen L, Le Nours J, Eckle SB, Birkinshaw R, Beddoe T *et al.* Recognition of vitamin B metabolites by mucosal-associated invariant T cells. *Nat Commun* 2013; **4**: 2142.
25 Reantragoon R, Kjer-Nielsen L, Patel O, Chen Z, Illing PT, Bhati M *et al.* Structural insight into MR1-mediated recognition of the mucosal associated invariant T cell receptor. *J Exp Med* 2012; **209**: 761–774.
26 Porcelli S, Yockey CE, Brenner MB, Balk SP. Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4-8- alpha/beta T cells demonstrates preferential use of several V beta genes and an invariant TCR alpha chain. *J Exp Med* 1993; **178**: 1–16.
27 Tilloy F, Treiner E, Park SH, Garcia C, Lemonnier F, de la Salle H *et al.* An invariant T cell receptor alpha chain defines a novel TAP-independent major histocompatibility complex class Ib-restricted alpha/beta T cell subpopulation in mammals. *J Exp Med* 1999; **189**: 1907–1921.

28 Reantragoon R, Corbett AJ, Sakala IG, Gherardin NA, Furness JB, Chen Z *et al.* Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. *J Exp Med* 2013; **210**: 2305–2320.
29 Walker LJ, Marrinan E, Muenchhoff M, Ferguson J, Kloverpris H, Cheroutre H *et al.* CD8alphaalpha expression marks terminally differentiated Hhman CD8+ T cells expanded in chronic viral infection. *Front Immunol* 2013; **4**: 223.
30 Leeansyah E, Ganesh A, Quigley MF, Sonnerborg A, Andersson J, Hunt PW *et al.* Activation, exhaustion, and persistent decline of the antimicrobial MR1-restricted MAIT-cell population in chronic HIV-1 infection. *Blood* 2013; **121**: 1124–1135.
31 Eggena MP, Barugahare B, Okello M, Mutyala S, Jones N, Ma Y *et al.* T cell activation in HIV-seropositive Ugandans: differential associations with viral load, CD4+ T cell depletion, and coinfection. *J Infect Dis* 2005; **191**: 694–701.
32 Kjer-Nielsen L, Borg NA, Pellicci DG, Beddoe T, Kostenko L, Clements CS *et al.* A structural basis for selection and cross-species reactivity of the semi-invariant NKT cell receptor in CD1d/glycolipid recognition. *J Exp Med* 2006; **203**: 661–673.
33 Crowe NY, Uldrich AP, Kyparissoudis K, Hammond KJ, Hayakawa Y, Sidobre S *et al.* Glycolipid antigen drives rapid expansion and sustained cytokine production by NK T cells. *J Immunol* 2003; **171**: 4020–4027.
34 Fernandez CS, Cameron G, Godfrey DI, Kent SJ. *Ex vivo* alpha-Galactosylceramide activation of NKT cells in humans and macaques. *J Immunol Methods* 2012; **382**: 150–159.
35 Wilson MT, Johansson C, Olivares-Villagomez D, Singh AK, Stanic AK, Wang CR *et al.* The response of natural killer T cells to glycolipid antigens is characterized by surface receptor down-modulation and expansion. *Proc Natl Acad Sci USA* 2003; **100**: 10913–10918.
36 Le Bourhis L, Mburu YK, Lantz O. MAIT cells, surveyors of a new class of antigen: development and functions. *Curr Opin Immunol* 2013; **25**: 174–180.
37 Greathead L, Metcalf R, Gazzard B, Gotch F, Steel A, Kelleher P. CD8+/CD161++ mucosal-associated invariant T-cell levels in the colon are restored on long-term antiretroviral therapy and correlate with CD8+ T-cell immune activation. *AIDS (London, England)* 2014; **28**: 1690–1692.

The Supplementary Information that accompanies this paper is available on the Immunology and Cell Biology website (http://www.nature.com/icb)