SPECIAL FEATURE REVIEW

γδ T-cell responses during HIV infection and antiretroviral therapy

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Abstract
HIV infection is associated with a rapid and sustained inversion of the Vδ1:Vδ2 T-cell ratio in peripheral blood. Studies of antiretroviral therapy (ART)-treated cohorts suggest that ART is insufficient to reconstitute either the frequency or function of the γδ T-cell subset. Recent advances are now beginning to shed light on the relationship between microbial translocation, chronic inflammation, immune ageing and γδ T-cell immunology. Here, we review the impact of acute, chronic untreated and treated HIV infection on circulating and mucosal γδ T-cell subsets and highlight novel approaches to harness γδ T cells as components of anti-HIV immunotherapy.

Keywords: γδ, gut, HIV, SIV, Vδ1, Vδ2

INTRODUCTION

γδ T cells are a subset of T cells that express a distinct T-cell receptor (TCR) consisting of a γ and a δ-chain. This allows γδ T cells to respond rapidly to nonpeptide antigens without the requirement of MHC presentation. In humans, γδ T cells have a relatively restricted repertoire of V gene segments and the most commonly used Vδ gene segments are Vδ1, Vδ2 and Vδ3. Human Vδ1 T cells predominantly reside in tissue and can make up to 40% of the intraepithelial lymphocytes (IEL) in the gut epithelia.1 Vδ1 T cells are also distributed in other tissues including dermis, spleen and liver, where they are involved in sustaining homeostasis and maintaining epithelial tissue integrity.2 Although Vδ1 T cells are also present in peripheral blood, Vδ2 T cells constitute the majority of human blood γδ T cells3 where they almost exclusively associate with the Vγ9 chain. Unlike Vδ1 T cells, which typically recognise CD1c and CD1d via the TCR, the Vγ9Vδ2 T cells recognise intermediate metabolites from the isoprenoid biosynthesis pathway, such as the host molecule isopentenyl pyrophosphate (IPP) or the pathogen-associated molecule (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP).4–8 These phosphoantigens bind the protein BTN3A1, triggering an ‘inside-out’ conformational change that promotes BTN3A1 binding to the Vγ9Vδ2 TCR and subsequent T-cell activation.9 Human Vδ3 T cells have also been reported to recognise CD1d, but are usually only present in peripheral blood at low frequencies.10 However, they have been observed to expand in the blood in patients with cytomegalovirus infection, CD4 T-cell deficiency and B-cell leukaemia.11–15

HIV-1 (herein referred to as HIV) infection still remains one of the most challenging health issues worldwide. In 2017, an estimated 36.9 million people...
were currently infected with the virus of which 1.8 million were children under the age of 15. Despite increasing awareness of the disease and improved access to antiretroviral therapy (ART), approximately 5000 individuals become newly infected every day. In addition to infecting and depleting CD4+ T cells, HIV infection also has a wide overall effect on the immune system, mediated largely by the phenomenon of microbial translocation. Rapid replication of HIV in gut-associated lymphoid tissue (GALT) results in substantial damage to the gut epithelial barrier and the subsequent translocation of microbial products such as LPS into the circulation. Epithelial barrier and the subsequent translocation of microbial products such as LPS into the circulation. An alternative hypothesis suggests that microbial translocation associated with acute HIV infection could drive Vδ2 T-cell activation and apoptosis. Analysis of a cohort of 79 acutely infected men suggested, however, that there is no relationship between biomarkers of microbial translocation and Vδ2 frequency. While two studies have reported a correlation between microbial translocation and bulk Vδ T-cell activation, it is unclear whether this relationship reflects activation of the Vδ1 or Vδ2 population, or simply the change in Vδ1:Vδ2 ratio. Vδ2 depletion is also associated with a reduction of antigen-induced IFN-γ/TNF-α/TGF-β production and proliferative/cytotoxic capacity in the residual Vδ2 population. Changes in Vδ2 cytokine production may be related in part to the relative expansion of terminally differentiated memory (TEMRA) cells and loss of the central memory (TCM) subset during chronic infection. Although all Vδ2 memory subsets exhibit significant increases in activation during acute and chronic HIV infection, TEMRA cells tend to exhibit the highest levels of activation as measured by CD38 expression. However, no studies have conclusively demonstrated a causal relationship between memory differentiation or activation and loss of antigen-induced cytokine responses in HIV.

Interesting data regarding the relationship between HIV viremia and Vδ2 T cells come from a study of structured treatment interruption (STI), where participants receiving antiretroviral therapy (ART) ceased treatment for 4–6 weeks. Within the
The first month following viral rebound, the Vδ2 compartment lost nearly all capacity for IFNγ production in response to antigen stimulation. While slower, Vδ2 T-cell counts and the frequency of TCM cells both declined by the end of the STI. All perturbations in the Vδ2 population were restored within a month of resumption of ART, demonstrating the rapid and reversible nature of the Vδ2 response to viremia. Although a mechanistic explanation of this relationship is still lacking, it is possible that Vδ2 T-cell anergy is induced by productive HIV infection of DCs, which can inhibit Vδ2 responses to phoshooantigen in vitro in a contact-dependent manner.

**Peripheral Vδ1 T cells**

The destructive impact of HIV infection on Vδ2 T cells stands in direct contrast to the observed expansion of peripheral Vδ1 T cells, which occurs rapidly during acute infection (and prior to HIV seroconversion). Several studies have confirmed that the relative enrichment of Vδ1 cells as a proportion of the total γδ T-cell population is also reflected as an increase in absolute Vδ1 (or Vδ2) T-cell count in the periphery. Similar to Vδ2 cells, Vδ1 cells are significantly more activated in HIV-infected subjects compared with controls. In acute, chronic and naturally controlled infection, the majority of Vδ1 cells exhibit a T EMRA phenotype, which correlates with absolute Vδ1 T-cell counts and is suggestive of antigen-driven proliferation and activation. However, observations that Vδ1 T-cell frequency is increased even in elite or viremic controllers (with low or undetectable viral loads) suggest that HIV replication itself does not drive this expansion. Rather, Vδ1 expansion may be linked to microbial translocation, as nonhuman primate models have shown a correlation between lymph node *Eschericia coli* levels and peripheral Vδ1 frequency. Another potential mechanism for the accumulation of peripheral Vδ1 cells lies in the ability of Tat peptides to block Vδ1 chemotaxis, which may reduce Vδ1 cell recruitment from the periphery to mucosal sites, a phenomenon which is supported by some evidence from nonhuman models.
primates (NHPs). Notably, however, evidence exists for the simultaneous accumulation of Vδ1 cells in both the periphery and gut mucosa in humans, as well as the periphery and multiple tissues in NHP models, implying that the mechanisms underlying Vδ1 expansion are likely multifactorial.

Recently, studies have begun to assess comprehensively Vδ1 phenotype and function during HIV infection. Fenoglio and colleagues demonstrated that expanded Vδ1 cells in HIV-infected subjects respond to Candida albicans stimulation and coexpress IFNγ and IL-17. This is associated with TBX21 (Tbet), RORC, CD161, CCR4 and CCR6 expression. Interestingly, a substantial proportion (mean ~20%) of Vδ1 cells from this HIV-infected cohort expressed IFNγ directly ex vivo, suggesting that circulating Vδ1 cells exist in a highly activated state. These data are consistent with those of Olson et al., which demonstrated a 15-fold elevation in mitogen-induced IFNγ + TNFα + MIP-1β+ ‘proinflammatory’ Vδ1 cells in viremic HIV-infected subjects. Further characterisation of the reactivity of the expanded Vδ1 cells will be required, however, as Olson et al. failed to identify any IL-17 production or Candida albicans reactivity by the Vδ1 subset, in direct contrast to the results of Fenoglio et al. Furthermore, a study in SIV-infected NHPs found that Vδ1 cells did express low levels of IL-17 in response to mitogen, but that IL-17 production was significantly reduced in SIV+ animals compared with controls. Differences in antigen reactivity and IL-17 production may be related to the duration of stimulation and cell culture, particularly in the case of Candida, but will need to be resolved in future studies.

In addition to cytokine production, clinically relevant characteristics of Vδ1 cells include the expression of NK cell receptors (NKR) and the capacity to mediate cellular cytotoxicity of HIV-infected target and bystander cells. Assessment of NKG2A and NKG2C expression, which initiates inhibitory or activating signals in response to HLA-E binding, respectively, demonstrated that Vδ1 cells from HIV-infected subjects progressively lose NKG2A expression and acquire NKG2C. In vitro, these NKG2C+ Vδ1 cells can recognise and kill HIV-infected CD4+ T cells. The modulation of CD94, NKG2A and NKG2C on Vδ1 cells in HIV has interesting implications for the regulation of Vδ1 cell function. Studies of NK cells have shown that the NKG2A+ NK cell subset contains the highest frequency of NK cells capable of recognising autologous HIV-infected CD4+ T cells, possibly due to HLA-E presentation of a capsid-derived peptide that blocks NKG2A inhibitory signalling. Conversely, elevation of HLA-A, and subsequently HLA-E, expression during infection is associated with poor immunological control of HIV, which is speculated to occur due to NKG2A-mediated inhibition of NK cell function. Blockade of NKG2A in vitro suggests that Vδ1 cells, unlike NK cells, might be relatively resistant to NKG2A-mediated inhibitory signalling. The role for CD94/NKG2A+ Vδ1 cells to control HIV replication or to be inhibited by HLA-E expression during disease therefore remains to be determined.

**Mucosal γδ T-cell subsets**

While studies of peripheral blood samples provide important insights into γδ T-cell biology, Vδ1 cells are naturally enriched in the same mucosal tissues that support HIV replication (i.e. the gut mucosa and female reproductive tract). Numbers (and frequency) of duodenal γδ T cells (mostly Vδ1+) are significantly increased among HIV-infected subjects compared with controls. This was confirmed by a detailed study from Poles and colleagues, who compared Vδ1 and Vδ2 subset frequencies in the peripheral blood and rectal mucosa of healthy and HIV-infected participants. γδ T-cell dynamics in the gut reflected those of the peripheral blood, with significant increases in Vδ1 and decreases in Vδ2 frequency during infection. Despite the parallel dynamics of the γδ T-cell populations at these two sites, analysis of CDR3 length showed little overlap between the two anatomical sites for either Vδ1 or Vδ2 subsets, as well as evidence of private, polyclonal expansions. In contrast to these results, a study of 15 acutely and 14 chronically infected participants found a significant loss of Vδ1 cells in the duodenum during chronic infection, with no change in Vδ2 frequency. Duodenal Vδ1 cells of chronically-infected participants exhibited an increase in TEMRA differentiation compared with controls, although mucosal Vδ1 cells were predominately TEM phenotype, which is distinct from the peripheral blood. Beyond differences in anatomical sampling location (duodenum versus rectum), there are limited data available to explain the discrepancies in these studies.
To date, only one study has assessed the impact of HIV infection on γδ T cells at the female reproductive tract and involved mostly participants receiving ART. In this group, HIV infection was associated with a significant reduction in both Vδ1 and Vδ2 frequencies at the endocervix, but memory distribution, NKR expression or function was not assessed.

**IMPACT OF ART ON γδ T-CELL POPULATIONS**

**Vδ2 T cells**

Numerous studies have assessed Vδ1 and Vδ2 T-cell frequencies in ART-treated cohorts, although substantially fewer have provided more comprehensive data regarding phenotype and function. Both cross-sectional and longitudinal cohort studies find that ART fails to restore normal frequencies or numbers of Vδ2 T cells. This observation is corroborated by evidence that ART only partially restores the depletion of Jγ1.2 TCR repertoire, with almost no subjects exhibiting a typical frequency of Jγ1.2 chains within the Vδ2 subset and few intraparticipant changes in a longitudinal study. Phenotypically, more studies report residual activation of the Vδ2 subset during ART compared with healthy controls than normalisation of activation. Data on memory subset distribution is more controversial, with some evidence that the expanded TEMRA population persists during ART, while other studies show a reduction in TEMRA frequencies that closely resemble uninfected controls. Functionally, the majority of evidence suggests that Vδ2 cytokine production, GzmB expression/cytotoxicity and proliferative capacity remain compromised during ART, with only a single study showing a beneficial impact of ART on Vδ2 proliferation and TNFα secretion.

**Vδ1 T cells**

Cross-sectional data support the maintenance of an expanded Vδ1 cell population during viral suppression, an observation that was also confirmed in the longitudinal follow-up of 8 subjects from the day of ART initiation through day 540 on therapy. At mucosal sites, the population of expanded γδ T cells is maintained during ART, with only modest normalisation in some individuals. The peripheral Vδ1 subset in ART cohorts retains the TeMRA phenotype associated with untreated infection and is reported to express elevated levels of PD-1 compared with healthy controls. Whether ART reduces Vδ1 activation is unresolved but evidence suggests ongoing Vδ1 proliferation during viral suppression and the maintenance of a large proinflammatory IFNγ+ TNFα+ MIP-1β+ polyfunctional population. A single study of NKR expression reported elevated levels of CD94/NKG2A, CD158a, CD158b and NKB1 on Vδ1 cells compared with healthy controls, which was accompanied by a loss of CD28 expression and an upregulation of CD45RO.

**MICROBIAL TRANSLOCATION, IMMUNE ACTIVATION AND APOPTOSIS**

The relationship between microbial translocation, systemic immune activation and perturbations of γδ T-cell subsets has been a common and enduring thread throughout the studies described above. Microbial translocation occurs as a result of the massive depletion of CD4+ T cells at the gut mucosa during acute HIV or SIV infection. This includes the preferential loss of Th17 cells, which contribute to the maintenance of epithelial barrier integrity and wound healing in the gastrointestinal mucosa. The dysregulation of mucosal immunity and loss of epithelial integrity allow the translocation of microbial products such as LPS into the circulation, resulting in systemic immune activation and inflammation (recently reviewed in References 80 and 82). Unfortunately, suppressive ART is unable to fully restore the mucosal CD4+ T-cell compartment and abolish systemic inflammation.

The disruption of mucosal immunity not only allows microbial products to translocate into the circulation, but also alters the composition of the gut microbiota. A number of human cohort studies have consistently shown an enrichment of proinflammatory bacteria such as the Enterobacteriaceae family and a loss of clades such as Bacteroides. Enterobacteriaceae are particularly likely to translocate across the mucosal barrier and induce the production of reactive oxygen species by innate immune cells, driving inflammation. At least one member of this family, *E. coli*, activates Vδ2 T cells in vitro, raising the possibility that sustained exposure to
translocated microbes could drive apoptosis of γδ T cells, similar to the apoptosis of bystander CD4+ in the lamina propria that occurs by Fas-FasL interactions.79 While the impact of microbiome perturbations on the gut-resident Vδ1 T-cell population remains to be understood, data have shown a correlation between iNKT cells in the gut mucosa and the prevalence of *Bacteroides* and *Prevotella* microbes.83 Thus, γδ T cells may be directly impacted by changes in the microbial community at the gut mucosa, the translocation of proinflammatory products into the circulation, or dysregulation of innate and adaptive immune cells during both untreated and treated HIV infection.

**CLINICAL IMPACT OF γδ T-CELL PERTURBATIONS DURING HIV INFECTION**

**Contribution to HIV control**

Whether γδ T-cell activation and cytolytic capacity during HIV infection can actually contribute to control of viremia or disease progression remains an open question. γδ cells can undoubtedly control HIV replication through multiple mechanisms *in vitro*, including direct cytotoxicity of infected cells.43,84 Vδ2 T cells can be recruited to HIV-infected DCs via CCL4 production, where they control viral replication and reduce HIV transmission to bystander CD4+ T cells.61 β-Chemokine production by both Vδ185 and Vδ286 cells can block HIV infection of target cells. However, whether this *in vitro* activity translates into *in vivo* control of viremia remains unresolved. Although HIV elite/viral controllers exhibit Vδ2 depletion relative to healthy controls, they maintain Vδ2 frequencies that are significantly higher than either untreated or ART-treated subjects.44,87 These cells predominately exhibit a TCM phenotype87 and produce more IL-17 than cells from viremic patients.44 Unfortunately, such studies are confounded by an inability to determine whether viral control preserves γδ T-cell ratios and phenotypes, or whether maintenance of a TCM/IL-17-expressing Vδ2 population contributes to the control of viral replication. Nonhuman primates offer a unique opportunity to longitudinally compare preinfection γδ populations to infection susceptibility and viral load setpoint. Although Tuero et al.88 reported an inverse correlation between endocervical Vδ2 T-cell frequency and chronic viral load (VL) in SIV-infected macaques, supporting a potential protective role for γδ T cells in this animal model, these studies are still lacking in the literature and this should be further investigated in future studies.

**Impact on coinfection**

While suppressive ART successfully controls HIV replication regardless of γδ T-cell reconstitution, residual impairment of the Vδ2 subset likely has profound implications for immunity against a number HIV coinfections. Tuberculosis is currently the leading cause of death among HIV-infected individuals, and evidence suggests that active TB and HIV infection have additive effects on peripheral Vδ2 depletion89 and dysfunction.90 At the site of TB infection, however, it is unclear what impact HIV has on lung γδ T-cell populations. Only a single study has reported BAL γδ T-cell numbers in healthy and HIV-infected participants where there was a significant increase in total γδ cells during HIV infection, but the delta chain usage was not determined.91 However, the possible impact of HIV infection on Vδ2 responses to *Mycobacterium* has been clearly demonstrated in NHP models. Naïve macaques are able to induce robust primary and recall Vδ2 responses to BCG vaccination in the periphery and lung, while SIV-infected macaques showed no response to BCG in either site.92 Encouragingly, administration of ART improved NHP Vδ2 responses to BCG, possibly due to reconstitution of Mtβ-specific CD4+ T cells.93 NHP studies will be critical in determining whether host-directed therapy targeting Vδ2 T cells94 can enhance protection against TB reactivation in HIV-infected populations.

Similarly, the expansion of Vδ1 cells during HIV infection may impact coinfection with several herpesviruses. Cytomegalovirus (CMV) infection is a widespread pathogen that usually causes asymptomatic infections. However, in HIV-infected individuals, this pathogen can result in clinical manifestations including chorioretinitis and CMV enterocolitis. Similar to HIV infection, there is a selective expansion of Vδ2+ cells during CMV infection.95 These cells are suggested to participate in the control of CMV replication and display potent anti-CMV responses *in vitro*.12 Although the expansion of Vδ1 cells as a result of HIV infection would presumably be beneficial for
control of CMV infection, CMV replication is enhanced by inflammatory stimuli. Since it is reported that the functional characteristics of the expanded Vδ1 cells in HIV+ individuals are skewed towards a proinflammatory profile, this may instead contribute to CMV-associated morbidity, although this remains to be determined.96–99 Furthermore, it is unclear whether the high prevalence of CMV infection among HIV+ individuals is a driver of the Vδ1 T-cell expansion observed during chronic infection.

Human herpesvirus 8 (HHV8) is also a virus which has increased seroprevalence in HIV-infected individuals and can cause significant disease in the form of Kaposi’s sarcoma (KS).100 Although the effector populations involved in control of this virus remain elusive, HHV8 infection is also associated with an expansion of Vδ1 cells that respond to HHV8-infected cells and prevent virus release in immunocompetent hosts.101 The role γδ T cells play in promoting HHV-8 to progress to KS is currently unknown, but considering that inflammatory cytokines including IFN-γ, IL-6, IL-1β and TNF-α are produced by infiltrating cells in lesions of KS, expanded Vδ1 cells in HIV+ individuals may potentially contribute to progression of clinical symptoms in a similar way as with CMV.102

Other common coinfections among HIV-infected populations include Cryptococcus, viral hepatitis and malaria. There are little data available regarding γδ T-cell responses to Cryptococcus infection in HIV-infected human cohorts, but murine studies have established a role for γδ T cells in Cryptococcal immunity in the lung.103,104 Hepatitis B and C infections have a deleterious impact on γδ T cells, similar to HIV infection, which is discussed more fully below. Studies in malaria-endemic populations have revealed a substantial role for γδ T cells in immunity to Plasmodium spp. This topic has been recently reviewed in References 105 and 106, which highlighted the protective and immunoregulatory roles of both Vδ2 and Vδ1 γδ subsets. Surprisingly, however, there are no studies of HIV/malaria coinfection that report on γδ T cells, which would represent an interesting focus for future clinical cohorts.

Impact on immune cell crosstalk

γδ T cells can not only exert direct antimicrobial activity, but can also orchestrate and regulate the activation, maturation and recruitment of a variety of other immune cells.107,108 Activated Vδ2 cells from healthy individuals can induce the maturation of neutrophils,109 DCs and B cells into APCs.110 Some evidence that this function is compromised during HIV infection, as HIV-infected APCs cannot undergo full γδ T cell-induced maturation in vitro, leading to high-residual CCR5 expression and low CD86 and HLA-DR expression.62 This impairment of crosstalk may enhance DC susceptibility to infection through CCR5 expression, as well as compromise CD4+ T-cell responses that rely on DC-mediated antigen presentation. To date, however, follow-up on these observations is lacking. Additionally, Vδ2 cells can themselves present antigen and act as APCs for conventional αβ T cells.111 Antigen-activated Vδ2 cells express HLA-DR, CD80, CD86, CD40 and CD54 at levels comparable to LPS-matured DCs and can induce primary αβ T-cell responses.111 Transient activation-induced upregulation of CCR7 implies that Vδ2 may home to draining lymph nodes during infection initiate adaptive immune responses. Considering the residual depletion, terminal differentiation and dysfunction that characterise Vδ2 cells in ART-treated subjects, it is likely that acquisition of APC function following antigen stimulation is compromised in the context of HIV infection. The contribution of such dysfunction to poor antimicrobial immunity or vaccination in ART-treated subjects should be further explored, as in vivo Vδ2 immunotherapy could be considered to address these defects in immune function.

More recently, Vδ2 cells have been recognised to provide CD40L-dependent help to B cells.112–116 As noted above, the transient expression of CCR7 after activation allows Vδ2 cells to traffic to secondary lymphoid tissues, where they cluster within the germinal centre of mucosal B-cell follicles.116 Stimulation with the phosphoantigen IPP is sufficient to elicit the delayed but robust expression of surface molecules involved in B-cell help; 36–84 h poststimulation, Vδ2 T cells express CD40L, ICOS, OX-40 and CD70.116 In vitro coculture assays indicate that activated Vδ2 cells can promote B-cell antibody secretion to a similar, or even increased, degree as Tfh cells.116 Further studies have suggested that antigen exposure in the presence of IL-21 is required to induce the expression of CD40L and ICOS on circulating Vδ2 cells.113,114 Interestingly, treatment of macaques with intravenous HMBPP and IL-2 during chronic
SHIV infection resulted a prolonged boosting of SHIVenv-specific antibody titres, suggesting that Vδ2 cells can contribute to humoral immunity in vivo. Despite these fascinating observations, further data regarding the impact of Vδ2 depletion on humoral immunity during HIV infection are lacking.

**γδ T CELLS IN ANTI-HIV IMMUNOTHERAPY**

Interest is increasing in developing host-directed immunotherapies to either supplement or replace current ART. Studies which have investigated the use of Vδ2 cells for anti-HIV immunotherapy are summarised in Table 1. At the simplest level, methods for recovering Vδ2 responses to phosphoantigen among HIV-infected donors include cytokine supplementation with IL-18 or IL-12. Such an approach may improve Vδ2-mediated immune responses against *Mycobacterium* or other bacterial infections. More complex interventions designed to specifically target HIV-infected cells include an effort to harness the ability of Vδ2 T cells to perform antibody-dependent cellular cytotoxicity (ADCC) via CD16 expression. CD16 Vδ2 cells exhibit poor responses to phosphoantigen, but respond robustly to antibody-coated target cells. These responses are largely maintained, if not slightly enhanced, in ART-treated subjects, suggesting that Vδ2 cells derived from HIV-infected individuals could contribute to the killing of HIV-infected target cells. More encouragingly, ex vivo expansion of Vδ2 cells from HIV-infected subjects results in upregulation of CD16 expression and quantifiable ADCC of antibody-coated targets.

### Table 1. Summary of HIV immunotherapy studies using Vδ2 T cells

| Outcome                  | Study and Species | HIV Status  | Cell Type | Results                                                                                                                   |
|--------------------------|-------------------|-------------|-----------|---------------------------------------------------------------------------------------------------------------------------|
| Antigen responses        | Murday et al.     | Human HIV+ ART | *Ex vivo* Vδ2 | IL-18 stimulation improves IPP-induced Vδ2 proliferation in HIV+ individuals                                               |
|                          | Cardone et al.    | Human Healthy | *Ex vivo* Vδ2 and HIV-infected monocyte-derived DC | Vδ2 cell phosphoantigen responses in the presence of HIV-infected DC are inhibited due to poor IL-12 secretion by the DCs. Responses can be restored by addition of IL-12 to Vδ2/DC cocultures |
| ADCC                     | He et al.         | Human HIV+ ART | *Ex vivo* Vδ2 | Vδ2 cells from ART-treated individuals exhibit CD16 expression and degranulate in response to CD16-mediated activation |
| Direct cytotoxicity      | Poonia et al.     | Human Healthy, HIV+ ART | IPP/zoledronate + IL-2 expanded PBMC | Expanded Vδ2 cells expressed CD16 and were capable of killing antibody-coated target cells |
|                          | Garrido et al.    | Human HIV+ ART | Bisphosphonate pamidronate (PAM) + IL-2 expanded PBMC | Vδ2 cells exhibited direct cytotoxicity against Daudi cells. IPP-expanded cells were more potent killers than zoledronate-expanded cells |
| In vivo expansion        | Ali et al.        | Macaque HIV-infected (acute or chronic) | Injection of HMBPP + IL-2 in vivo | Treatment resulted in expansion and activation of Vδ2 cells. Treatment during acute infection exacerbated viral replication and disease progression in an IL-2-dependent manner. Treatment during chronic infection boosted Env-specific antibody titres but did not impact viral load or disease progression |
|                          | Poonia et al.     | Humanised mice HIV+ | Adoptive transfer of zoledronate + IL-2 expanded PBMC | No impact of expanded Vδ2 T-cell transfer on CD4+ T-cell loss, CD4:CD8 T-cell ratio or viral load |
T-cell expansion appears to be a viable and reproducible strategy for the production of large numbers of autologous \( \gamma \delta \) T cells from ART-treated HIV-infected individuals, although optimal expansion culture conditions may differ between healthy and infected groups.\(^{120}\) Expanded cells express low levels of inhibitory surface receptors and can kill latently infected \( CD4^+ \) T cells after latency reversal with vorinostat \textit{in vitro}.\(^{120}\) \textit{In vivo}, however, results of \( \gamma \delta \) T-cell immunotherapy are varied. Administration of HMBPP and IL-2 to chronically SIV-infected macaques expanded and activated \( \gamma \delta \) T cells, transiently boosted SHIV-specific CD8\(^+ \) T-cell responses and resulted in a sustained increase of SHIV-specific antibody titres.\(^{121}\) Nonetheless, there was no impact of \( \gamma \delta \) expansion on viral load or disease progression during chronic infection, and a negative impact of \( \gamma \delta \) expansion during acute infection. These results were mirrored in a study of humanised mice treated with expanded \( \gamma \delta \) cells, which similarly observed no protective effect of \( \gamma \delta \) treatment on viral replication or \( CD4^+ \) T-cell depletion.\(^{122}\)

**SIMILARITIES WITH OTHER CHRONIC INFLAMMATORY DISEASES**

The hallmark impacts of HIV infection on \( \gamma \delta \) T cells (\( \gamma \delta: \gamma \alpha \) ratio inversion, activation and terminal differentiation, functional defects) are, in fact, not unique to HIV infection itself. Other chronic inflammatory diseases are associated with similar effects, including kidney disease, viral hepatitis and obesity. Understanding the commonalities in pathogenesis between these diverse conditions may provide further insight into the mechanisms of \( \gamma \delta \) perturbation and identify useful therapeutic targets.

Chronic kidney disease (CKD) is a progressive condition in which the loss of renal function results in the accumulation of uraemic toxins and proinflammatory cytokines (reviewed in Reference 123). End-stage renal disease (ESRD), the final stage of CKD, is associated with high levels of immune activation, poor responses to immunisation and high susceptibility to infection. Matsumoto first reported a significant loss of \( \gamma \delta \) T cells among CKD patients requiring hemodialysis.\(^{124}\) They speculated that \( \gamma \delta \) depletion likely occurred because of Fas- and LFA-1-dependent apoptosis related to uraemia. Similarly, we observed a significant loss of phosphoantigen-reactive \( \gamma \delta \) T cells in ESRD patients compared with healthy controls.\(^{125}\) Surprisingly, however, there was no relationship between plasma proinflammatory cytokine levels and \( \gamma \delta \) frequency or dysfunction,\(^ {125}\) raising the question of what drives \( \gamma \delta \) T-cell loss during ESRD. Similar to HIV infection, it is unclear whether low peripheral \( \gamma \delta \) frequencies truly reflect apoptosis or, instead, recruitment to inflamed tissues. \( \gamma \delta \) frequencies only partially normalise following kidney transplantation, with \( \gamma \delta \) frequencies remaining significantly lower than healthy controls.\(^{126}\) The fact that uraemia-associated changes in the \( \gamma \delta \) repertoire are not effectively reversed upon transplantation suggests that transplant patients may exhibit long-term susceptibility to some infections, similar to HIV ART-treated patients.

Viral hepatitis is also associated with changes in the circulating \( \gamma \delta \) repertoire that are highly reminiscent of HIV infection. Chronic HCV infection is associated with peripheral \( \gamma \delta \) depletion,\(^ {127,128}\) acquisition of an activated/TEMRA phenotype,\(^ {128,129}\) upregulation of CD16 and granzyme\(^ {129}\) and functional impairment.\(^ {128,129}\) HBV-infected subjects exhibit loss of peripheral \( \gamma \delta \) cells\(^ {130,131}\) and/or expansion of peripheral \( \gamma \alpha \) cells,\(^ {132}\) which correlates with serum ALT levels.\(^ {130,131}\) Residual \( \gamma \delta \) cells from these individuals are impaired for IFN\( \gamma \) production, cytotoxicity\(^ {130}\) and proliferation\(^ {131}\) and exhibit an activated, TEMRA surface phenotype.\(^ {131}\) Expression of granzyme and cytotoxic markers is particularly enhanced in HCV-infected subjects with greater degrees of liver damage, suggesting the potential involvement of \( \gamma \delta \) T cells in mediating immunopathology during infection. Despite this, phosphoantigen-activated \( \gamma \delta \) cells can restrict \textit{in vitro} HCV replication in an IFN\( \gamma \)-dependent manner,\(^ {133}\) making them potential immunotherapeutic targets for HCV treatment. \textit{In vitro} studies suggested that \( \gamma \delta \) dysfunction may be at least partially abrogated by treatment with IFN\( \alpha \), which boosts phosphoantigen responses in \( \gamma \delta \) T cells from both healthy and HCV-infected subjects\(^ {128}\) (although this effect was not replicated by others\(^ {129}\) ). \textit{In vivo}, however, a standard course of Peg-IFN\( \alpha \) and ribavirin therapy resulted in pronounced \( \gamma \delta \) anergy after 4 weeks of treatment in two studies of chronically HCV-infected patients.\(^ {129,134}\) Notably, although \( \gamma \delta \) IFN\( \gamma \) responses were decreased/almost absent after treatment, perforin and degranulation responses were elevated, suggesting the
possibility that IFN-α drives a transition of Vδ2 cells away from cytokine responses and towards cytotoxicity.129 The results of these clinical trials highlight important considerations for the in vivo use of drugs to promote Vδ2 activation and proliferation during treated HIV infection, and the need to assess anergy at multiple timepoints after therapy.

Similar to CKD patients, obese adults exhibit increased susceptibility to infection associated with chronic inflammation.135,136 Peripheral Vδ2 T cells are depleted in obese individuals (in a BMI-dependent manner),137,138 are more likely to exhibit a TEMRA phenotype and respond poorly to influenza-infected APCs,138 mirroring the effects of HIV infection, HBV/HCV infection and renal disease on this compartment. As expected, cytokine supplementation overcomes some of the function defect, with IL-2 boosting in vitro Vδ2 function among obese subjects.138

Interestingly, these three conditions, as well as HIV infection, all involve some degree of gut dysbiosis. As previously discussed, HIV infection results in substantial damage to the gut epithelium and microbial translocation, which causes widespread immune activation.139 Microbial translocation has also been reported in ESRD/CKD cohorts140–142 and is increasingly being recognised as an important driver of T-cell dysfunction and chronic inflammation.143,144 Indeed, we found that plasma sCD14 levels in ESRD patients correlate with HMBPP-induced IFNg production by γδ T cells, suggesting a possible link between microbial translocation and Vδ2 T-cell function.125 Similar observations have been made in the context of chronic HCV/HBV infection and liver cirrhosis, where overgrowth of pathogenic gut bacteria increases gut permeability and allows translocation of bacterial products into the liver via the portal vein.139,145,146 Finally, obese individuals also exhibit elevated levels of LPS and other markers of microbial translocation, supporting a putative link between gut permeability, low-grade inflammation, and γδ T-cell depletion and dysfunction.147,148

UNRESOLVED QUESTIONS AND FUTURE DIRECTIONS

As the field of HIV immunology moves forward from studies of HIV pathogenesis towards a focus on inflammation and immune ageing among ART-treated populations,149 there are several key questions surrounding γδ T-cell immunology that remain unanswered. First, the link between poor reconstitution of the γδ compartment and persistent innate immune activation and inflammation during ART is poorly understood. In a fascinating study, Belkina et al. comprehensively assessed the expression of inhibitory surface receptors on a wide range of lymphocyte subsets including two NK cell populations, conventional T cells, Tregs, iNKT cells and γδT cells in ART-treated and control participants.150 Importantly, these cohorts were stratified for age, allowing for a simultaneous assessment of immune ageing in each group. Among all lymphocyte subsets, only γδ phenotype was sufficient to distinguish between the control and infected groups. A transition of the γδ compartment from ‘resting’ CD160+ phenotype to an ‘activated/exhausted’ TIGIT+PD-1+ phenotype was associated with plasma-derived proinflammatory profile.150 While an inversion of the Vδ2:Vδ1 ratio was confirmed for a subset of HIV-infected study participants, no data were available to assess the separate contribution of Vδ1 and Vδ2 cells to the HIV-associated inflammation and ageing. Such information will be critical to understanding which γδ subset primarily expressed the TIGIT+PD-1+ phenotype and/or correlates mostly strongly with plasma inflammatory biomarkers. In addition to these data, a transcriptomics study of mitogen-activated lymphocyte responses identified γδ T-cell differentiation as a differentially regulated pathway between healthy control and long-term ART cohorts.149

Second, the field will benefit from a better understanding of the composition of the expanded Vδ1 subset in ART cohorts. Vδ1 T cells include CD1-restricted, lipid-reactive T cells, as well as cells with undefined antigen specificity and numerous mechanisms to sense host cell stress. As studies undertake novel approaches to defining Vδ1 T-cell subsets with different functions and phenotypes,151 we will move closer to understanding what drives the dramatic peripheral expansion and proinflammatory cytokine profile of these cells. It is interesting to note that the phenomena of microbial translocation and gut dysbiosis are common to many chronic inflammatory diseases associated with changes in the Vδ1:Vδ2 T-cell ratio. Recent data from murine models highlight the physiologic importance of gut-derived γδ cells and their ability to traffic to inflamed tissues,
including the brain.\textsuperscript{152} Whether gut dysbiosis is a predominante driver of $\gamma\delta$ dysfunction and accumulation during HIV or other viral infections remains to be fully investigated.

Finally, the question of whether productive HIV infection of V$\delta$2 T cells occurs in vivo remains a matter of debate. Commonly, V$\delta$2 T cells from healthy individuals are reported to be CD4$^+$\textsuperscript{51,53,75,153,154}, ostensibly rendering them impermissible to infection. Surprisingly, however, Wallace et al.\textsuperscript{58} reported in 1997 that V$\delta$2 T-cell lines could be productively infected with HIV in vitro over the course of 18 days. Similarly, coinfection with human herpesvirus 6 can induce CD4 expression on $\gamma\delta$ T cells in vitro, rendering them susceptible to HIV infection.\textsuperscript{52} Since then, some studies have reported low-level CD4 expression on peripheral $\gamma\delta$ T cells ex vivo,\textsuperscript{155} which is sufficient to mediate CD4-dependent productive HIV infection. Similarly, humanised mice produce thymic $\gamma\delta$ T cells that express CD4, CCR5 and CXCR4 and are susceptible to infection by multiple HIV isolates.\textsuperscript{156} Perhaps most intriguingly, mucosal V$\delta$2 T cells at the female reproductive tract are reported to be predominately CD4$^+$.\textsuperscript{42} The relevance of V$\delta$2 T-cell infection by HIV was recently highlighted by data indicating that circulating V$\delta$2 cells are a reservoir for replication-competent HIV in ART-suppressed patients.\textsuperscript{154} In this study, exposure of V$\delta$2 T cells to IL-2 was sufficient to induce CD4 expression on an average of 15% of isolated V$\delta$2 cells. Three acutely HIV-infected subjects (~23 days postinfection) also exhibited a similar level of CD4 expression on their V$\delta$2 T cells, suggesting that immune activation is sufficient to promote productive infection of V$\delta$2 T cells in vivo. What impact this might have on V$\delta$2-based immunotherapies and latency reactivation-based HIV cure strategies is currently unknown, but should be considered in future studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

JAJ and EME wrote and revised the manuscript.

REFERENCES

1. Marsal J, Agace WW. Targeting T-cell migration in inflammatory bowel disease. J Intern Med 2012; 272: 411–429.
2. Bonneville M, O’Brien RL, Born WK. $\gamma\delta$ T cell effector functions: a blend of innate programming and acquired plasticity. Nat Rev Immunol 2010; 10: 467–478.
3. Vantourout P, Hayday A. Six-of-the-best: unique contributions of $\gamma\delta$ T cells to immunology. Nat Rev Immunol 2013; 13: 88–100.
4. Spada FM, Grant EP, Peters PJ et al. Self-recognition of CD1 by $\gamma\delta$ T cells: implications for innate immunity. J Exp Med 2000; 191: 937–948.
5. Porcelli S, Brenner MB, Greenstein JL, Balk SP, Terhorst C, Bleicher PA. Recognition of cluster of differentiation 1 antigens by human CD4$^+$CD8$^+$ cytolytic T lymphocytes. Nature 1989; 341: 447–450.
6. Faure F, Jitsukawa S, Miossec C, Hercend T. CD1d-sulfatide-specific T cells in human blood use a semiinvariant V$\delta$1 TCR. J Exp Med 2013; 206: 2505–2510.
7. Bai L, Picard D, Anderson B et al. The majority of CD1d-sulfatide-specific T cells in human blood use a semiinvariant V$\delta$1 TCR. J Exp Med 2012; 206: 2505–2510.
8. Gober HJ, Li L, Yuan L et al. A structural change in butyrophilin upon phosphoantigen binding underlies phosphoantigen-mediated V$\delta$9V$\delta$2 T cell activation. Immunity 2019; 50: 1043–1053.e5.
9. Yang Y, Li L, Yuan L et al. A structural change in butyrophilin upon phosphoantigen binding underlies phosphoantigen-mediated V$\delta$9V$\delta$2 T cell activation. Immunity 2019; 50: 1043–1053.e5.
10. Mangan BA, Dunne MR, O’Reilly VP et al. Cutting edge: CD1d restriction and Th1/Th2/Th17 cytokine secretion by human V$\delta$3 T cells. J Immunol 2013; 191: 30–34.
11. Dechanet J, Merveille P, Lim A et al. Implication of $\gamma\delta$ T cells in the human immune response to cytomegalovirus. J Clin Investig 1999; 103: 1437–1449.
12. Halary F, Pitard V, Dubek D et al. Shared reactivity of V$\delta$2$^{\text{neg}}$ $\gamma\delta$ T cells against cytomegalovirus-infected cells and tumor intestinal epithelial cells. J Exp Med 2005; 201: 1567–1578.
13. Knight A, Madrigal AJ, Grace S et al. The role of V$\delta$2-negaitive $\gamma\delta$ T cells during cytomegalovirus reactivation in recipients of allogeneic stem cell transplantation. Blood 2010; 116: 2164–2172.
14. Bartkowiak J, Kulczyck-Wojdala D, Blonski JZ, Robak T. Molecular diversity of $\gamma\delta$ T cells in peripheral blood from patients with B-cell chronic lymphocytic leukaemia. Neoplasma 2002; 49: 86–90.
15. Kabelitz D, Hinz T, Dobmeyer T et al. Clonal expansion of V$\gamma$2V$\delta$3-expressing $\gamma\delta$ T cells in an HIV-1-negative patient with CD4 T-cell deficiency. Br J Haematol 1997; 96: 266–271.
16. UNAIDS. UNAIDS Data 2018. 2018 ed2018. p. 1–376.
17. Brenchley JM, Price DA, Schacker TW et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 2006; 12: 1365–1371.
18. Dillon SM, Frank DN, Wilson CC. The gut microbiome and HIV-1 pathogenesis: a two way street. AIDS 2016; 30: 2737–2751.
19. Deeks SG, Kitchen CM, Liu L et al. Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. *Blood* 2004; 104: 942–947.

20. Giorgi JV, Hultin LE, McKeating JA et al. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *J Infect Dis* 1999; 179: 859–870.

21. Giorgi JV, Lyles RH, Matud JL et al. Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. *J Acquir Immune Defic Syndr* 2002; 29: 346–355.

22. Liu Z, Cumberland WG, Hultin LE, Kaplan AH, Detels R, Giorgi JV. CD8+ T-lymphocyte activation in HIV-1 disease reflects an aspect of pathogenesis distinct from viral burden and immunodeficiency. *J Acquir Immune Defic Syndr Hum Retroviral* 1998; 18: 332–340.

23. Brenchley JM, Karandikar NJ, Betts MR et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. *Blood* 2003; 101: 2711–2720.

24. Day CL, Kaufmann DE, Kiepiela P et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* 2006; 443: 350–354.

25. Petrovas C, Casazza JP, Brenchley JM et al. PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. *J Exp Med* 2006; 203: 2281–2292.

26. Petrovas C, Chaon B, Ambrozak DR et al. Differential association of programmed death-1 and CD57 with ex vivo survival of CD8+ T cells in HIV infection. *J Immunol* 2009; 183: 1120–1132.

27. Eriksson EM, Milush JM, Ho EL et al. Expansion of CD8+ T cells lacking Sema4D/CD100 during HIV-1 infection identifies a subset of T cells with decreased functional capacity. *Blood* 2012; 119: 745–755.

28. Wykes MN, Lewin SR. Immune checkpoint blockade in infectious diseases. *Nat Rev Immunol* 2018; 18: 91–104.

29. Autran B, Triebel F, Katlama C, Rozenbaum W, Hercend T, Debere P. T cell receptor γδ+ lymphocyte subsets during HIV infection. *Clin Exp Immunol* 1989; 75: 206–210.

30. De Maria A, Ferrazin A, Ferrini S, Ciccone E, Terragna A, Moretta L. Selective increase of a subset of T cell receptor γδ T lymphocytes in the peripheral blood of patients with human immunodeficiency virus type 1 infection. *J Infect Dis* 1992; 165: 917–919.

31. Hinz T, Wesch D, Freise K, Reckziegel A, Arden B, Kabelitz D. T cell receptor γδ repertoire in HIV-1-infected individuals. *Eur J Immunol* 1994; 24: 3044–3049.

32. Li Z, Li W, Li N et al. γδ T cells are involved in acute HIV infection and associated with AIDS progression. *PLoS One* 2014; 9: e106064.

33. Li Z, Jiao Y, Hu Y et al. Distortion of memory Vδ2 γδ T cell contributes to immune dysfunction in chronic HIV infection. *Cell Mol Immunol* 2015; 12: 604–614.

34. De Paoli P, Gennari D, Martelli P et al. A subset of γδ lymphocytes is increased during HIV-1 infection. *Clin Exp Immunol* 1991; 83: 187–191.

35. Wesch D, Hinz T, Kabelitz D. Analysis of the TCR Vγ repertoire in healthy donors and HIV-1-infected individuals. *Int Immunol* 1998; 10: 1067–1075.

36. Poggi A, Carosio R, Fenoglio D et al. Migration of Vδ1 and Vδ2 T cells in response to CXCR18 and CXCR18 ligands in healthy donors and HIV-1-infected patients: competition by HIV-1 Tat. *Blood* 2004; 103: 2205–2213.

37. Rossol R, Dobmeyer JM, Dobmeyer TS et al. Increase in Vδ1 γδ T cells in the peripheral blood and bone marrow as a selective feature of HIV-1 but not other virus infections. *Br J Haematol* 1998; 100: 728–734.

38. Poles MA, Barsoum S, Yu W et al. Human immunodeficiency virus type 1 induces persistent changes in mucosal and blood γδ T cells despite suppressive therapy. *J Virol* 2003; 77: 10456–10467.

39. Li Z, Lu X, Hu Z et al. Syphilis infection differentially regulates the phenotype and function of γδ T cells in HIV-1-infected patients depending on the HIV-1 disease stage. *Front Immunol* 2017; 8: 991.

40. Olson GS, Moore SW, Richter JM et al. Increased frequency of systemic pro-inflammatory Vδ1 γδ T cells in HIV elite controllers correlates with gut viral load. *Sci Rep* 2018; 8: 16471.

41. Cimini E, Agrati C, D’Offizi G et al. Primary and chronic HIV infection differently modulates mucosal Vδ1 and Vδ2 T-cells differentiation profile and effector functions. *PLoS One* 2015; 10: e0129771.

42. Strbo N, Alcaide ML, Romero L et al. Loss of intraepithelial endocervical γδ (GD) 1 T cells in HIV-infected women. *Am J Reprod Immunol* 2016; 75: 134–145.

43. Fausther-Bovendo H, Wauquier N, Cherfils-Vicini J, Cremer I, Debere P, Vieillard V. NKG2C is a major triggering receptor involved in the Vδ1 T cell-mediated cytotoxicity against HIV-infected CD4 T cells. *AIDS* 2008; 22: 217–226.

44. Chevalier MF, Bhatnagar N, Didier C et al. γδ T-cell subsets in HIV controllers: potential role of Tγδ17 cells in the regulation of chronic immune activation. *AIDS* 2019; 33: 1283–1292.

45. Enders PJ, Yin C, Martini F et al. HIV-mediated γδ T cell depletion is specific for Vγ2+ cells expressing the Jγ1.2 segment. *AIDS Res Hum Retroviruses* 2003; 19: 21–29.

46. Li H, Peng H, Ma P et al. Association between Vγ2/Vδ2 T cells and disease progression after infection with closely related strains of HIV in China. *Clin Infect Dis* 2008; 46: 1466–1472.

47. Bhatnagar N, Girard PM, Lopez-Gonzalez M et al. Potential role of Vδ2 γδ T cells in regulation of immune activation in primary HIV infection. *Front Immunol* 2017; 8: 1189.

48. Poccia F, Boullier S, Leceur H et al. Peripheral Vγ9/Vδ2 T cell deletion and anergy to nonpeptidic mycobacterial antigens in asymptomatic HIV-1-infected persons. *J Immunol* 1996; 157: 449–461.

49. Chaudhry S, Cairo C, Venturi V, Pauza CD. The γδ T-cell receptor repertoire is reconstituted in HIV patients after prolonged antiretroviral therapy. *AIDS* 2013; 27: 1557–1562.

50. Davodeau F, Peyrat MA, Hallett MM et al. Close correlation between Daudi and mycobacterial antigen recognition by human γδ T cells and expression of V9JPC1 γδV2DJC δ-encoded T cell receptors. *J Immunol* 1993; 151: 1214–1223.

51. Brenner MB, McLean J, Scheft H et al. Two forms of the T-cell receptor γ protein found on peripheral blood cytotoxic T lymphocytes. *Nature* 1987; 325: 689–694.
52. Lusso P, Garzino-Demo A, Crowley RW, Mainanti MS. Infection of γ9/δ T lymphocytes by human herpesvirus 6: transcriptional induction of CD4 and susceptibility to HIV infection. J Exp Med 1995; 181: 1303–1310.

53. Li H, Pauza CD. HIV envelope-mediated, CCR5/4α/7-dependent killing of CD4-negative γ9/δ T cells which are lost during progression to AIDS. Blood 2011; 118: 5824–5831.

54. Calenda G, Keawvichit R, Arrode-Bruses G et al. Integrin 4αβ2 blockade preferentially impacts CCR6+ lymphocyte subsets in blood and mucosal tissues of naïve rhesus macaques. J Immunol 2018; 200: 810–820.

55. Byrareddy SN, Arthos J, Cicala C et al. Sustained viremic control in SIV+ macaques after antiretroviral and 4αβ7 antibody therapy. Science 2016; 354: 197–202.

56. Santangelo PJ, Cicala C, Byrareddy SN et al. Early treatment of SIV+ macaques with an 4αβ7 mAb alters virus distribution and preserves CD4+ T cells in later stages of infection. Mucosal Immunol 2018; 11: 932–946.

57. Kosub DA, Lehrman G, Milush JM et al. γδ T-cell functional responses differ after pathogenic human immunodeficiency virus and nonpathogenic simian immunodeficiency virus infections. J Virol 2008; 82: 1155–1165.

58. Wallace M, Scharko AM, Pauza CD et al. Functional γδ T-lymphocyte defect associated with human immunodeficiency virus infections. Mol Med 1997; 3: 60–71.

59. Martini F, Urso R, Gioia C et al. γδ T-cell anergy in human immunodeficiency virus-infected persons with opportunistic infections and recovery after highly active antiretroviral therapy. Immunology 2000; 100: 481–486.

60. Martini F, Poccia F, Goletti D et al. Acute human immunodeficiency virus replication causes a rapid and persistent impairment of Vγ9Vδ2 T cells in chronically infected patients undergoing structured treatment interruption. J Infect Dis 2002; 186: 847–850.

61. Cardone M, Ikeda KN, Varano B, Gessani S, Conti L. HIV-1-induced impairment of dendritic cell cross talk with γ9/δ T lymphocytes. J Virol 2015; 89: 4798–4808.

62. Sacchi A, Rinaldi A, Tumino N et al. HIV infection of monocytes-derived dendritic cells inhibits Vγ9Vδ2 T cells functions. PLoS One 2014; 9: e111095.

63. Harris LD, Klatt NR, Vinton C et al. Mechanisms underlying γδ T-cell subset perturbations in SIV-infected Asian rhesus macaques. Blood 2010; 116: 4148–4157.

64. Kosub DA, Durudas A, Lehrman G et al. γδ T cell mRNA levels decrease at mucosal sites and increase at lymphoid sites following an oral SIV infection of macaques. Curr HIV Res 2008; 6: 520–530.

65. Nilssen DE, Muller F, Oktedalen O et al. Intraepithelial γδ T cells in duodenal mucosa are related to the immune state and survival time in AIDS. J Virol 1996; 70: 3545–3550.

66. Fenoglio D, Poggi A, Catellani S et al. Vδ1 T lymphocytes producing IFN-γ and IL-17 are expanded in HIV-1-infected patients and respond to Candida albicans. Blood 2009; 113: 6611–6618.

67. Sindhu ST, Ahmad R, Morisset R, Ahmad A, Menezes J. Peripheral blood cytotoxic γδ T lymphocytes from patients with human immunodeficiency virus type 1 infection and AIDS lyse uninfected CD4+ T cells, and their cytolic potential correlates with viral load. J Virol 2003; 77: 1848–1855.

68. Lisovsky I, Sitman G, Song R et al. A higher frequency of NKG2A+ than of NKG2A- NK cells responds to autologous HIV-infected CD4+ cells irrespective of whether or not they coexpress KIR3DL1. J Virol 2015; 89: 9909–9919.

69. Davis ZB, Cogswell A, Scott H et al. A conserved HIV-1-derived peptide presented by HLA-E renders infected T-cells highly susceptible to attack by NKG2A/CD94-bearing natural killer cells. PLoS Pathog 2016; 12: e1005421.

70. Ramsuran V, Naranbhai V, Horowitz D et al. Elevated HLA-A expression impairs HIV control through inhibition of NKG2A-expressing cells. Science 2018; 359: 86–90.

71. Negash M, Tsegaye A, Wassie L, Howe R. Phenotypic and functional heterogeneity of peripheral γ9 T cells in pulmonary TB and HIV patients in Addis Ababa, Ethiopia. BMC Infect Dis 2018; 18: 464.

72. Casetti R, De Simone G, Sacchi A et al. Vγ9Vδ2 T-cell polyfunctionality is differently modulated in HAART-treated HIV patients according to CD4 T-cell count. PLoS One 2015; 10: e0132291.

73. Cummings JS, Cairo C, Armstrong C, Davis CE, Pauza CD. Impacts of HIV infection on Vγ9Vδ T cell phenotype and function: a mechanism for reduced tumor immunity in AIDS. J Leukoc Biol 2008; 84: 371–379.

74. Bordon J, Evans PS, Propp N, Davis CE Jr, Redfield RR, Pauza CD. Association between longer duration of HIV-suppressive therapy and partial recovery of the Vγ2 T cell receptor repertoire. J Infect Dis 2004; 189: 1482–1486.

75. Boudova S, Li H, Sajadi MM, Redfield RR, Pauza CD. Impact of persistent HIV replication on CD4 negative Vγ2Vδ2 T cells. J Infect Dis 2012; 205: 1448–1455.

76. Hebbeler AM, Propp N, Cairo C et al. Failure to restore the Vγ2-Jγ1.2 repertoire in HIV-infected men receiving highly active antiretroviral therapy (HAART). Clin Immunol 2008; 128: 349–357.

77. Nilssen DE, Brandtzæg P. Intraepithelial γδ T cells remain increased in the duodenum of AIDS patients despite antiretroviral treatment. PLoS One 2012; 7: e29066.

78. Wesh D, Kabelitz D. Differential expression of natural killer receptors on Vδ1 γδ T cells in HIV-1-infected individuals. J Acquir Immune Defic Syndr 2003; 33: 420–425.

79. Li Q, Duan L, Estes JD et al. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature 2005; 434: 1148–1152.

80. Vujkovic-Cvijin I, Somsouk M. HIV and the gut microbiota: composition, consequences, and avenues for amelioration. Curr HIV/AIDS Rep 2019; 16: 204–213. e-pub ahead of print Apr 29; https://doi.org/10.1007/s11904-019-00441-w.

81. Brenchley JM, Paiardini M, Knox KS et al. Differential Th17 CD4 T-cell depletion in pathogenic and nonpathogenic lentiviral infections. Blood 2008; 112: 2826–2835.
82. Tincat C, Douek DC, Marchetti G. Gut barrier structure, mucosal immunity and intestinal microbiota in the pathogenesis and treatment of HIV infection. *AIDS Res Ther* 2016; 13: 19.

83. Ibarrondo FJ, Wilson SB, Hultin LE et al. Preferential depletion of gut CD4-expressing iNKT cells contributes to systemic immune activation in HIV-1 infection. *Mucosal Immunol* 2013; 6: 591–600.

84. Poccia F, Cipriani B, Vendetti S et al. CD94/NKG2 inhibitory receptor complex modulates both anti-viral and anti-tumoral responses of polyclonal phosphoantigen-reactive Vγ9Vδ2 T lymphocytes. *Immunology* 2014; 141: 596–608.

85. Omi K, Shimizu M, Watanabe E et al. Inhibition of R5-tropic HIV type-1 replication in CD4+ natural killer T cells by γδ T lymphocytes. *Immunology* 1999; 100: 858–861.

86. Poccia F, Battistini L, Cipriani B et al. Phosphoantigen-reactive Vγ9Vδ2 T lymphocytes suppress *in vitro* human immunodeficiency virus type 1 replication by cell-released antiviral factors including CC chemokines. *J Infect Dis* 1999; 180: 555–561.

87. Riedel DJ, Sajadi MM, Armstrong CL et al. Natural viral suppressors of HIV-1 have a unique capacity to maintain γδ T cells. *AIDS* 2009; 23: 1955–1964.

88. Tuero I, Venzon D, Robert-Guroff M. Mucosal and systemic γδ T cells associated with control of simian immunodeficiency virus infection. *J Immunol* 2016; 197: 4686–4695.

89. Shao L, Zhang W, Zhang S et al. Potent immune responses of Ag-specific Vγ2Vδ2 T cells and CD8+ T cells associated with latent stage of *Mycobacterium tuberculosis* coinfection in HIV-infected humans. *AIDS* 2008; 22: 2241–2250.

90. Rojas RE, Chervenak KA, Thomas J et al. Vδ2-γδ T cell function in *Mycobacterium tuberculosis*- and HIV-1-positive patients in the United States and Uganda: application of a whole-blood assay. *J Infect Dis* 2005; 192: 1806–1814.

91. Mwale A, Hummel M, Mvaya L et al. The B cell, CD8+ T cell and γδ T cell infiltration alters alveolar cell homeostasis in HIV-infected Malawian adults. *Wellcome Open Res* 2017; 2: 105.

92. Zhou D, Lai X, Shen Y et al. Inhibition of adaptive Vγ2Vδ2 T-cell responses during active mycobacterial coinfection of simian immunodeficiency virus SIVmac-infected monkeys. *J Virol* 2003; 77: 2998–3006.

93. Shen L, Shen Y, Huang D et al. Development of Vγ2Vδ2 T cell responses during active mycobacterial coinfection of simian immunodeficiency virus-infected macaques requires control of viral infection and immune competence of CD4+ T cells. *J Infect Dis* 2004; 190: 1438–1447.

94. Shen L, Frencher J, Huang D et al. Immunization of Vγ2Vδ2 T cells programs sustained effector memory responses that control tuberculosis in nonhuman primates. *Proc Natl Acad Sci USA* 2019; 116: 6371–6378.

95. Pitard V, Roumanes D, Lafarge X et al. Long-term expansion of effector/memory Vδ2 γδ T cells is a specific blood signature of CMV infection. *Blood* 2008; 112: 1317–1324.

96. Soderberg-Naucler C. Treatment of cytomegalovirus infections beyond acute disease to improve human health. *Expert Rev Anti Infect Ther* 2014; 12: 211–222.

97. Dobmeyer TS, Dobmeyer R, Wesch D, Helm EB, Hoelzer D, Kabelitz D. Reciprocal alterations of Th1/Th2 function in γδ T-cell subsets of human immunodeficiency virus-1-infected patients. *Br J Haematol* 2002; 118: 282–288.

98. Boullier S, Dadaglio G, Lafeuillade A, Debord T, Gougeon ML. Vδ1 T cells expanded in the blood throughout HIV infection display a cytotoxic activity and are primed for TNF-α and IFN-γ production but are not selected in lymph nodes. *J Immunol* 1997; 159: 3629–3637.

99. Hudspeth K, Fogli M, Correa DV et al. Engagement of Nkp30 on Vδ1 T cells induces the production of CCL3, CCL4, and CCL5 and suppresses HIV-1 replication. *BLOOD* 2012; 119: 4013–4016.

100. Rohner E, Wyss N, Heg Z et al. HIV and human herpesvirus 8 co-infection across the globe: systematic review and meta-analysis. *Int J Cancer* 2016; 138: 45–54.

101. Bercy S, De Rosa SC, Vieira J et al. γδ T cells involvement in viral immune control of chronic human herpesvirus 8 infection. *J Immunol* 2008; 180: 3417–3425.

102. Ensoli B, Barbiliari G, Buonaguro L, Gallo RC. Molecular mechanisms in the pathogenesis of AIDS-associated Kaposis's sarcoma. *Adv Exp Med Biol* 1991; 303: 27–38.

103. Uezu K, Kawakami K, Miyagi K et al. Accumulation of γδ T cells in the lungs and their regulatory roles in Th1 response and host defense against pulmonary infection with *Cryptococcus neoformans*. *J Immunol* 2004; 172: 7629–7634.

104. Wozniak KL, Kolls JK, Wormley FL Jr. Depletion of γδ T lymphocytes suppress *in vitro* antiviral factors including CC chemokines. *Blood* 2002; 100: 3693–3700.

105. Peters C, Kabelitz D, Wesch D. Regulatory functions of γδ T cells is a cytotoxic activity and are primed for TNF-α and IFN-γ production but are not selected in lymph nodes. *J Immunol* 2002; 168: 1411–1417.

106. Dantzler KW, Jagannathan P. Regulatory functions of γδ T cells involvement in viral immune control of chronic human herpesvirus 8 infection. *J Immunol* 2008; 180: 3417–3425.

107. He Y, Wu K, Hu Y et al. γδ T cell and other immune cells crosstalk in cellular immunity. *J Immunol* 2014; 190: 4601–4611.

108. Peters C, Kabelitz D, Wesch D. Regulatory functions of γδ T cells involvement in viral immune control of chronic human herpesvirus 8 infection. *J Immunol* 2008; 180: 3417–3425.

109. Dantzler KW, Jagannathan P. γδ T cells in antigen-specific immunity: new insights into their diverse functions in protection and tolerance. *Front Immunol* 2018; 9: 2445.

110. Kabelitz D. Reciprocal alterations of Th1/Th2 responses that control tuberculosis in nonhuman primates. *J Infect Dis* 2003; 188: 2641–2647.

111. Brandes M, Willimann K, Moser B. Professional antigen-presentation function by human γδ T Cells. *Science* 2005; 309: 264–268.

112. Born WK, Huang Y, Reinhardt RL, Huang H, Sun D, O'Brien RL. γδ T cells and B cells. *Adv Immunol* 2017; 134: 1–45.

113. Caccamo N, Todaro M, La Manna MP, Sireci G, Stassi G, Dieli F. IL-21 regulates the differentiation of a human γδ T cell subset equipped with B cell helper activity. *PLoS One* 2012; 7: e41940.
114. Bansal RR, Mackay CR, Moser B, Eberl M. IL-21 enhances the potential of human γδ T cells to provide B-cell help. *Eur J Immunol* 2012; 42: 110–119.

115. Caccamo N, Battistini L, Bonneville M et al. CXCR1 identifies a subset of Vγ9Vδ2 T cells which secrete IL-4 and IL-10 and help B cells for antibody production. *J Immunol* 2006; 177: 5290–5295.

116. Brandes M, Willimmann K, Lang AB et al. Flexible migration program regulates γδ T-cell involvement in humoral immunity. *Blood* 2003; 102: 3693–3701.

117. Murday AS, Chaudhry S, Pauza CD. Interleukin-18 activates Vγ9Vδ2 T cells from HIV-positive individuals: recovering the response to phosphoantigen. *Immunology* 2017; 151: 385–394.

118. He X, Liang H, Hong K et al. The potential role of CD16+Vγ2/Vδ2 T cell-mediated antibody-dependent-cell-mediated cytotoxicity in control of HIV type 1 disease. *AIDS Res Hum Retroviruses* 2013; 29: 1562–1570.

119. Poonia B, Pauza CD. γδ T cells from HIV+ donors can be expanded in vitro by zoleodronate-interleukin-2 to become cytotoxic effectors for antibody-dependent cellular cytotoxicity. *Cytotechnology* 2012; 14: 173–181.

120. Garrido C, Clohosey ML, Whitworth CP, Hudgens M, Margolis DM, Sorianno-Sarabia N. γδ T cells: an immunotherapeutic approach for HIV cure strategies. *JCI Insight* 2018; 3: e120121.

121. Ali Z, Yan L, Plagman N et al. γδ T cell immune manipulation during chronic phase of simian-human immunodeficiency virus infection [corrected] confers immunological benefits. *J Immunol* 2009; 183: 5407–5417.

122. Poonia B. Adoptive transfer of aminobisphonate-expanded Vγ9Vδ2 T cells does not control HIV replication in a humanized mouse model. *Immunotherapy* 2016; 8: 521–526.

123. Betjes MG. Immune cell dysfunction and inflammation in end-stage renal disease. *Nat Rev Nephrol* 2013; 9: 255–265.

124. Matsumoto Y, Shinzato T, Takai I et al. Peripheral deletion of γδ T cells in haemodialysis patients. *Nephrol Dial Transplant* 1998; 13: 2861–2866.

125. Juno JA, Waruk JLM, Harris A et al. γδ T-cell function is inhibited in end-stage renal disease and impacted by latent tuberculosis infection. *Kidney Int* 2017; 92: 1003–1014. e-pub ahead of print Jun 24; https://doi.org/10.1016/j.kint.2017.03.036

126. Zhuang Q, Peng B, Wei W et al. The detailed distribution of T cell subpopulations in immune-stable renal allograft recipients: a single center study. *PeerJ* 2019; 7: e6417.

127. Par G, Rukavina D, Podack ER et al. Decrease in CD3-negative-CD8dim+ and Vδ2/Vγ9 TeR+ peripheral blood lymphocyte counts, low perforin expression and the impairment of natural killer cell activity is associated with chronic hepatitis C virus infection. *J Hepatol* 2002; 37: 514–522.

128. Cimini E, Bonnafous C, Bordoni V et al. Interferon-α improves phosphoantigen-induced-Vγ9Vδ2 T-cells interferon-γ production during chronic HCV infection. *PLOS One* 2012; 7: e37014.

129. Yin W, Tong S, Zhang Q et al. Functional dichotomy of Vδ2 γδ T cells in chronic hepatitis C virus infections: role in cytotoxicity but not for IFN-γ production. *Sci Rep* 2016; 6: 26296.

130. Chen M, Zhang D, Zhen W et al. Characteristics of circulating T cell receptor γδ T cells from individuals chronically infected with hepatitis B virus (HBV): an association between Vδ2 subtype and chronic HBV infection. *J Infect Dis* 2008; 198: 1643–1650.

131. Wu X, Zhang JY, Huang A et al. Decreased Vδ2 γδ T cells associated with liver damage by regulation of Th17 response in patients with chronic hepatitis B. *J Infect Dis* 2013; 208: 1294–1304.

132. Chen M, Hu P, Peng H et al. Enhanced peripheral γδT cells cytotoxicity potential in patients with HBV-associated acute-on-chronic liver failure might contribute to the disease progression. *J Clin Immunol* 2012; 32: 877–885.

133. Agrati C, Alonzi T, De Santis R et al. Activation of Vγ9Vδ2 T cells by non-peptidic antigens induces the inhibition of subgenomic HCV replication. *Int Immunol* 2006; 18: 11–18.

134. Cimini E, Bonnafous C, Sicard H et al. In vivo interferon-α/ribavirin treatment modulates Vγ9Vδ2 T-cell function during chronic HCV infection. *J Interferon Cytokine Res* 2013; 33: 136–141.

135. Nay NS, Larson EC, Jameson JM. Chronic Inflammation and γδ T cells. *Front Immunol* 2016; 7: 210.

136. Lackey DE, Olefsky JM. Regulation of metabolism by the innate immune system. *Nat Rev Endocrinol* 2016; 12: 15–28.

137. Donnemelli G, Del Corno M, Pierdominici M et al. Distinct blood and visceral adipose tissue regulatory T cell and innate lymphocyte profiles characterize obesity and colorectal cancer. *Front Immunol* 2017; 8: 643.

138. Costanzo AE, Taylor KR, Dutt S, Han PP, Fujioka K, Jameson JM. Obesity impairs γδ T cell homeostasis and antiviral function in humans. *PLoS One* 2015; 10: e0120918.

139. Brenchley JM, Douek DC. Microbial translocation across the GI tract. *Annu Rev Immunol* 2012; 30: 149–173.

140. Shi K, Wang F, Jiang H et al. Gut bacterial translocation may aggravate microinflammation in hemodialysis patients. *Dig Dis Sci* 2014; 59: 2109–2117.

141. Wang F, Jiang H, Shi K, Ren Y, Zhang P, Cheng S. Gut bacterial translocation is associated with microinflammation in end-stage renal disease patients. *Nephrology (Carlton)* 2012; 17: 733–738.

142. Anders HJ, Andersen K, Stecher B. The intestinal microbiota, a leaky gut, and abnormal immunity in kidney disease. *Kidney Int* 2013; 83: 1010–1016.

143. Pan W, Kang Y. Gut microbiota and chronic kidney disease: implications for novel mechanistic insights and therapeutic strategies. *Int Urol Nephrol* 2018; 50: 289–299.

144. Vaziri ND, Zhao YY, Pahl MV. Altered intestinal microbial flora and impaired epithelial barrier structure and function in CKD: the nature, mechanisms, consequences and potential treatment. *Nephrol Dial Transplant* 2016; 31: 737–746.

145. Yang R, Xu Y, Dai Z, Lin X, Wang H. The immunologic role of gut microbiota in patients with chronic HBV infection. *J Immunol Res* 2018; 2018: 2361963.

146. Milosevic I, Vujovic A, Barac A et al. Gut-liver axis, gut microbiota, and its modulation in the management of liver diseases: a review of the literature. *Int J Mol Sci* 2019; 20: E395.
147. Troseid M, Nestvold TK, Rudi K, Thoresen H, Nielsen EW, Lappegard KT. Plasma lipopolysaccharide is closely associated with glycemic control and abdominal obesity: evidence from bariatric surgery. *Diabetes Care* 2013; 36: 3627–3632.

148. Tuomi K, Logomasino JV. Bacterial lipopolysaccharide, lipopolysaccharide-binding protein, and other inflammatory markers in obesity and after bariatric surgery. *Metab Syndr Relat Disord* 2016; 14: 279–288.

149. Rhoades N, Mendoza N, Jankeel A et al. Altered immunity and microbial dysbiosis in aged individuals with long-term controlled HIV infection. *Front Immunol* 2019; 10: 463.

150. Belkina AC, Starchenko A, Drake KA et al. Multivariate computational analysis of γδ T cell inhibitory receptor signatures reveals the divergence of healthy and ART-suppressed HIV+ aging. *Front Immunol* 2018; 9: 2783.

151. Dunne PJ, Maher CO, Freeley M et al. CD3ε expression defines functionally distinct subsets of Vδ1 T cells in patients with human immunodeficiency virus infection. *Front Immunol* 2018; 9: 940.

152. Benakis C, Brea D, Caballero S et al. Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. *Nat Med* 2016; 22: 516–523.

153. Bank I, Marcu-Malina V. Quantitative peripheral blood perturbations of γδ T cells in human disease and their clinical implications. *Clin Rev Allergy Immunol* 2014; 47: 311–333.

154. Soriano-Sarabia N, Archin NM, Bateson R et al. Peripheral Vγ9Vδ2 T cells are a novel reservoir of latent HIV infection. *PLoS Pathog* 2015; 11: e1005201.

155. Imlach S, Leen C, Bell JE, Simmonds P. Phenotypic analysis of peripheral blood γδ T lymphocytes and their targeting by human immunodeficiency virus type 1 *in vivo*. *Virology* 2003; 305: 415–427.

156. Gurney KB, Yang OO, Wilson SB, Uittenbogaart CH. TCRγδ+ and CD161+ thymocytes express HIV-1 in the SCID-hu mouse, potentially contributing to immune dysfunction in HIV infection. *J Immunol* 2002; 169: 5338–5346.

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