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REVIEW

# Depletion and dysfunction of Vγ2Vδ2 T cells in HIV disease: mechanisms, impacts and therapeutic implications

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**Infection with human immunodeficiency virus (HIV) disrupts the balance among γδ T cell subsets, with increasing Vδ1+ cells and substantial depletion of circulating Vδ2+ cells. Depletion is an indirect effect of HIV in CD4-negative Vδ2 cells, but is specific for phosphoantigen-responsive subpopulations identified by the Vγ2-Jγ1.2 (also called Vγ9-JγP) T cell receptor rearrangement. The extent of cell loss and recovery is related closely to clinical status, with highest levels of functional Vδ2 cells present in virus controllers (undetectable viremia in the absence of antiretroviral therapy). We review the mechanisms and clinical consequences for Vδ2 cell depletion in HIV disease. We address the question of whether HIV-mediated Vδ2 cell depletion, despite being an indirect effect of infection, is an important part of the immune evasion strategy for this virus. The important roles for Vδ2 cells, as effectors and immune regulators, identify key mechanisms affected by HIV and show the strong relationships between Vδ2 cell loss and immunodeficiency disease. This field is moving toward immune therapies based on targeting Vδ2 cells and we now have clear goals and expectations to guide interventional clinical trials.**
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## INTRODUCTION

Among the earliest steps in the thymic production of lymphocytes is the rearrangement of the T cell receptor γ chain. If the rearranged γ chain delivers a signal, then the δ chain is rearranged and cells are committed to the γδ T cell lineage. Only when T cell receptor γ signaling fails, do nascent T cells proceed to β chain rearrangement and enter the αβ T cell lineages. The order of appearance in the thymus and their primary function during T cell recognition of non-peptidic, major histocompatibility complex-unrestricted responses to non-peptidic antigens suggests that human γδ T cells are a primitive part of adaptive immunity, intermediate between pattern recognition by natural killer (NK) cells and the peptide epitope specificity of αβ T cells. The roles of these cells in immunity to infectious diseases are well established, and their contributions to immunoregulation are increasingly apparent. Our focus here is on the complex effects of human immunodeficiency virus type 1 (HIV) on γδ T cells.

Normally, γδ T cell responses involve cellular activation driven by host or pathogen-derived molecules followed by proliferation, which creates a larger population of effector cells with improved functionality. Elevated γδ T cell levels occur during acute *Plasmodium* infection[1] or in response to some bacterial pathogens.[2,3] The γδ T cell responses to HIV are more complex, involving expansion and contraction of individual subsets, each of which are diagnostic of virus infection and disease progression.

The earliest reported association between γδ T cells and HIV disease noted increasing levels of Vδ1 cells in patients;[4] normally, the Vδ2 subset predominates in peripheral blood with a Vδ2/Vδ1 ratio of 10 : 3 for healthy North American or European donors.[4,5] In HIV disease, Vδ1 cell expansion and a loss of circulating Vδ2 cells leads to an inverted Vδ2/Vδ1 ratio.[6,7] An important obstacle to studying Vδ1 cells is that the antigen specificity of this subset remains unclear. Earlier reports that *Candida*[8] is a Vδ1 cell antigen have not been confirmed, and identifying Vδ1-specific antigens in human beings remains a challenge. Without this knowledge, it will be difficult to design immunotherapies based on Vδ1 cells even if these therapies prove valuable for HIV suppression. Because Vδ1 cells are present in significant numbers at mucosal sites, activation of this subset could help control HIV replication at HIV portals of entry and should be studied in more detail.

As the level of Vδ1 cells increases, Vδ2 cell levels and function decrease in HIV disease; Vδ2 T cell numbers and function were correlated directly with CD4 T cell counts and inversely with viral loads.[9] We and others described a common defect among all patients with HIV, which is a lack of response to *in vitro* stimulation with phosphoantigens.[9–11] Phosphoantigen responses require the Vγ2-Jγ1.2 T cell receptor rearrangement and are depleted preferentially during HIV disease.[12] Thus, loss of the phosphoantigen response, which is normally ubiquitous in healthy populations, is a marker for HIV

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disease and one of only two examples of T cell receptor-specific lymphocyte depletion after HIV infection, the other being loss of invariant chain NKT cells.[13] We believe that HIV-mediated depletion of Vδ2 T cells is an important immune evasion strategy and a key step in the establishment of viral persistence.

More recent studies have elucidate mechanisms for Vδ2 cell depletion in HIV disease[14] and expanded our understanding of how loss of these cells during early infection and potential reconstitution of the subset during prolonged therapy impacts a broad array of immune responses.[15]

## MECHANISMS OF Vδ2 T CELL DEPLETION IN HIV DISEASE

Loss of Vδ2 T cells is believed to be an early event in HIV disease. As discussed above, HIV infection alters blood levels for both the Vδ1 and Vδ2 subsets. The Vδ2/Vδ1 ratio is inverted in HIV disease due to the increased levels of Vδ1 and the depletion of Vδ2 T cells.[4,5] A recent study in SIV-infected macaques confirmed the Vδ1/Vδ2 inversion and argued that intestinal microbial translocation stimulated the increased number of Vδ1 cells and was responsible for the inverted ratio.[15] We showed earlier that functional responses of Vδ2 T cells to phosphoantigen were impaired in HIV disease,[16] and bulk depletion of this subset was an important cause of the inverted ratio. Functional responses to phosphoantigen were lost in numerous patients even while CD4 T cell counts remained within normal ranges,[16] indicating that the depletion of Vδ2 T cells occurs early after HIV infection. We also found extensive and ongoing depletion of Vδ2 T cells during progressing disease. In a unique Chinese cohort of individuals infected at roughly the same time and with very similar virus strains (due to contaminated blood-drawing equipment), we found a direct relationship between declining Vδ2 T cell levels, CD4 cell counts and plasma viremia.[9] In other clinical groups, Vδ2 cell levels were correlated with CD4 cell counts at the initiation of treatment.[17] These observations showed that Vδ2 cell depletion occurred during the early phase of HIV disease.

The most curious aspect of HIV-mediated Vδ2 cell loss is that these cells are highly resistant to HIV infection. The vast majority (>99%) of circulating Vδ2 T cells are CD4-negative. Despite one report that circulating γδ T cells can be productively infected with HIV-1,[18] we and others have been unable to demonstrate infection of this subset *in vitro* or in cells recovered from infected patients. The absence of cell surface CD4 is an important reason why Vδ2 T cells are non-permissive for virus infection. Understanding the effects of HIV on non-permissive Vδ2 T cells has revealed new mechanisms of viral pathogenesis.

A growing body of evidence shows that HIV envelope glycoproteins can induce apoptosis of uninfected, CD4-negative cells including neurons,[19] cardiomyocytes,[20] hepatocytes,[21] proximal renal tubular cells,[22] lung endothelial cells[23] and human vascular endothelial cells.[24]

Vδ2 cells express CCR5 and α4β7.[14,25] Chemokine receptor CCR5 binds the V3 loop of envelope glycoprotein (gp120). Envelope binding to CD4 increases the efficiency of the gp120–CCR5 interaction but is not required.[26,27] CCR5 is present at very high levels on activated Vδ2 cells, approaching 60 000 cell surface receptors per lymphocyte, which is approximately 10-fold higher than the density of CCR5 on activated CD4 T cells.[14] Vδ2 cells also express α4β7 integrin, which binds the V2 loop of envelope glycoprotein.[14,28] CCR5 and α4β7 exist in close physical proximity on the Vδ2 cell membrane, and the combination allows for high-avidity binding of envelope glycoprotein. Both soluble (monomeric) and membrane-anchored (trimeric) R5-tropic envelope induced significant killing of Vδ2 T cells.[14]

Previous studies showed that HIV envelope-mediated CD4 T cell death involved CCR5, Fas and caspase 8.[29] We found that gp120 glycoprotein signals, *via* the CCR5 receptor, the activation of p38 kinase and the initiation of a signaling cascade resulting in Fas-independent caspase activation and Vδ2 T cell death.[14] Blocking Fas did not prevent envelope-induced cell death, and gp120 did not change the expression of Fas or FasL on Vδ2 T cells. Actually, Fas-independent caspase activation has been observed[30,31] and required p38 MAPK, which is consistent with our results. Thus, direct binding of the HIV envelope glycoprotein to CCR5 on Vδ2 cells is one mechanism for Vδ2 cell death.

This model for envelope-mediated depletion of Vδ2 T cells is compatible with known events during HIV disease. As mentioned above, Vδ2 T cell depletion appears to occur early in HIV disease during intervals of higher viremia, and consequently, higher levels of circulating envelope glycoprotein. We also know that antibody responses, including neutralizing antibodies that might recognize the chemokine receptor binding site on envelope glycoprotein, are slow to develop after HIV infection.[32] This delay would allow more time for envelope-mediated Vδ2 cell depletion.

During *in vitro* studies of Vδ2 T cell killing mechanisms, we also noted a strong effect of antigen stimulation. When cells were exposed to phosphoantigen followed by the addition of envelope glycoprotein, the highest levels of cell death were observed.[14] This pattern is consistent with activation-induced cell death, as proposed by others, and may link the specific loss of phosphoantigen-reactive Vγ2-Jγ1.2 cells to the mechanism for envelope-mediated cell death signaling.

We also tested the effects of HIV envelope protein on Vδ1 T cells, which are present at higher levels in HIV disease. We confirmed an earlier result that Vδ1 cells do not express CCR5.[25] However, these cells express α4β7 integrin at levels sufficient to allow for detectable binding of gp120.[33] When envelope binds Vδ1 cells (in the absence of CCR5), no detectable p38/caspase signal was generated and there was no evidence for induced cell death.[33] These results indicate that α4β7 alone cannot mediate HIV envelope-induced death signaling. In Vδ2 cells, α4β7 may enhance killing by increasing the avidity for gp120 binding, but CCR5 is the key signaling receptor for cell death. These findings explain the differential effects of gp120 on Vδ2 *vs.* Vδ1 T cells, which are mirrored in HIV patients in whom Vδ2 T cells are depleted while the levels of Vδ1 T cells are increased.

## CLINICAL STUDIES OF Vδ2 T CELL DEPLETION IN HIV DISEASE

Studies of αβ T cells are aided by the knowledge of epitope specificity and major histocompatibility complex-restricting elements. The function of CD8-positive T cells in controlling virus replication can be analyzed by looking for escape variants in which the restricted epitope is modified within the viral genome or by correlating disease progression with 'elite' Class I haplotypes.[34,35] These approaches link changes in pathogen abundance or spread to the capacity for antigen-specific immunity. Studies of the roles for γδ T cells suffer from a lack of major histocompatibility complex restriction and the fact that all Vδ2 cells respond to the same or very similar phosphoantigens. Additionally, phosphoantigens are produced by many microbial pathogens, including protozoa and bacteria, and can be induced to higher than normal levels by virus infection in mammalian cells. Thus, all cells within the host have the capacity to produce phosphoantigens capable of stimulating Vδ2 cell responses. These unusual features of Vδ2 cell biology have impeded our ability to link the functions of these cells with known impacts on infectious disease, even though HIV-driven Vδ2 cell depletion is consistent with the definition of a viral immune evasion mechanism.

In a sense, HIV infection creates a natural 'knockout' of Vδ2 T cells and provides an opportunity to explore the functions of these cells. The challenge is to understand how and to what extent the loss of Vδ2 T cells contribute to immune deficiency in the context of changes to many other lymphocyte and innate immune cell subsets. An approach used extensively in our laboratory combines the molecular analysis of the TCR-γ2 repertoire, enumeration and phenotyping of circulating Vδ2 cells, and comparison to disease status using well-defined cohorts of $HIV^+$ patients and matched controls.

The earliest studies of HIV and γδ T cells (early 1990s) were conducted during a time when patients had access to single-drug therapy at best, and treatment was generally initiated only when CD4 counts dropped below 200.[36] Among patients with CD4 <200, we noted extreme Vδ2 cell defects, including decreased levels in circulation[16,37,38] and specific losses of Vγ2-Jγ1.2 chain rearrangements.[12] Both of these changes contributed to an overall loss of phosphoantigen responsiveness among HIV-positive patients, which had been reported earlier but was attributed incorrectly to T cell anergy.[4] Cross-sectional analyses of former blood/plasma donors from China who became infected with HIV at approximately the same time and with very similar strains of HIV provided the best evidence for relationships between Vδ2 cell depletion and viral RNA burden or CD4 cell count.[9] The positive correlation between CD4 T and Vδ2 T cell counts and the inverse correlation between the viral load and Vδ2 T cells observed in that study showed that Vδ2 T cell count and functionality are strongly associated with HIV infection, or in other words, rapid disease progression was related to rapid Vδ2 T cell depletion.

Another cross-sectional study examined HIV patients undergoing highly active antiretroviral therapy to define the effects of therapy on Vδ2 T cells.[39] Blood Vδ2 cell counts and the proportions of Vγ2-Jγ1.2 cells increased during prolonged and effective virus suppression. This cross-sectional study provided the first indication that treatment with virus suppression and CD4 T cell reconstitution might also increase both the Vδ2 cell counts and the repertoire complexity.

Subsequent longitudinal studies in patients with advanced HIV disease, who were the first to receive combination antiretroviral therapy, showed that up to 2 years of highly active antiretroviral therapy did not increase the levels of Vδ2 T cells in the blood or the proportion of cells expressing Vγ2-Jγ1.2 rearrangements.[40] In these studies, changes in Vδ2 counts and function only manifested when therapy intervals exceeded 22 months, a number that was close to the study duration. However, late-stage patients starting antiretroviral therapy with ⩽200 CD4 T cells may not recover Vδ2 cells, just as they often fail to reconstitute CD4.

One cohort study examined patients with natural control over HIV replication, designated as natural virus suppressors (NVS). This cohort had been defined earlier[41] and included approximately 65 patients who had confirmed HIV infection for 5 or more years with undetectable HIV viremia in the absence of antiretroviral therapy. Similar patients were designated as elite controllers or elite suppressors by other groups. We were surprised to find that NVS patients had circulating Vδ2 cell levels equivalent to those of healthy controls.[42] This conclusion was based on race-matched cohorts established by an earlier study[43] that reported a fourfold difference in Vδ2 cell levels between age and gender-matched HIV-negative African-American and Caucasian adults.

The similar levels of circulating Vδ2 T cells in NVS and race-matched controls were actually hiding an underlying repertoire defect. T cell receptor repertoire studies investigating the Vγ2 chain showed a defect in NVS that was similar to that observed in patients with progressing HIV disease. An important difference between the two cohorts was that phosphoantigen responsiveness, a marker of functional responses, was lost in the HIV-infected cohort but retained or recovered in the NVS cohort. Based on these studies, we hypothesized that NVS donors undergo initial HIV-mediated depletion of Vγ2-Jγ1.2-positive cells, which stops when viremia is controlled. Residual (surviving) Vδ2 cells proliferate and replenish the circulating population. Such an unusual situation, in which antigen-specific depletion can be overcome by replenishing the levels of surviving cells, is a unique characteristic of the Vδ2 cell population. Because all cells with the Vγ2-Jγ1.2 rearrangement are capable of responding to phosphoantigen, any of these cells that survive the initial impact of HIV can expand to reconstitute similar levels of functionality even with a substantial repertoire defect.[44]

The situation is not true for αβ T cells because only one or a small number of clones respond to a single antigen, and when these clones are depleted, the antigen response is lost. Redundancy in the Vδ2 cell response to phosphoantigen allows this population to sustain a significant amount of clonal depletion and still retain the capacity to proliferate and reconstitute a functional subset. This pattern of reconstitution among NVS donors, combined with the observation that Vδ2 cells in these patients appeared more activated and had a higher frequency for expressing the CD56 marker of cytotoxic cells, strongly suggested that Vδ2 cell responses in NVS patients help control viremia and may be important for the natural suppression of HIV.

The NVS cohort lacks measurable viremia, and clinical studies designed to activate γδ T cells would lack facile, short-term markers for efficacy. Consequently, we studied individuals with persistent HIV infection and chronic low-level viremia. In the absence of overt disease, these patients have elected not to receive antiretroviral therapy. At the extreme in such groups are individuals who have been infected for more than 20 years with chronic viremia in the range of 2000 RNA copies per ml of plasma but who maintain acceptable CD4 cell counts and have no overt disease progression.[45] The γδ T cells of patients in this group are chronically exposed to viral proteins, which is in contrast to those of NVS patients with natural mechanisms for complete virus suppression or patients receiving antiretroviral therapy. Studies of chronic viremia patients showed that Vδ2 cell counts were lower than those found in NVS patients but higher than those of patients who were infected and received antiretroviral therapy.[45] However, the Vγ2 repertoire defect in chronic viremia was far greater than that found in either the NVS or treated cohorts. Chronic exposure to viral proteins deepened the impact on Vδ2 T cells in terms of the Vγ2 repertoire. In this group of chronic viremia patients, the repertoire defect was not correlated with the CD4 count, suggesting that viremia is the key factor affecting Vδ2 T cells.

Most of the work mentioned so far was based on TCR spectratyping to study the Vδ2 T cell repertoire. It is now possible to compare clinical cohorts on the basis of detailed TCR sequencing studies, which is a quantitative method that can be used to view changes in the complexity of circulating lymphocyte populations. Here, special features of γδ T cells work greatly to our advantage in that the Vγ2 chain repertoire in healthy adults is much smaller than the overall repertoire for any individual Vβ chain and is amenable to 'shallow sequencing'. We analyzed the Vγ2 sequences obtained from healthy or HIV-infected patients and focused on abundance and variety of public and private clonotypes or nucleotypes found in each cohort (Chaudhry *et al*., manuscript in preparation). Public clonotypes have been observed[48] and defined for αβ T cells as 'amino acid sequences of TCR that are present and dominant in immune response to a specific epitope in a majority of individuals'.

Public Vβ chains use common V and J regions but might differ within the hypervariable CDR3 region. We define public Vγ2 chains more stringently and require that they have identical V, CDR3 and J region amino acid or nucleotide sequences.

Viewing the TCR repertoire in terms of public clonotype abundance provides a deeper understanding of which components from the Vγ2Vδ2 cell population are lost, preserved or recovered upon HIV infection and subsequent virus suppression. Sequencing studies also revealed significantly lower proportions of public Vγ2 chains in NVS and chronic low-level viremia patients compared to healthy controls, consistent with an initial HIV-mediated Vδ2 cell depletion and prolonged exposure to viral proteins in the latter group. The surprise came in analyzing the Vγ2 repertoire from patients with long-term (greater than 5 years) virus suppression due to antiretroviral therapy. Despite having low levels of Vδ2 cells, these patients showed surprisingly higher proportions of public Vγ2 chains, suggesting that the Vγ2 chain repertoire had been reconstituted. Indeed, among some patients in this group, the complexity and characteristics of the Vγ2 repertoire were similar if not better than what was observed in uninfected controls. Considering our earlier observations about the relationship between Vδ2 cells and prolonged therapy, we now have specific evidence for T cell receptor reconstitution in long-term treated patients.

Overall, the mechanisms for immune cell reconstitution in treated HIV disease remain obscure. Investigators studying CD4 T cell reconstitution argue that thymic output is ongoing among adults, and once HIV-mediated damage is arrested by antiretroviral therapy, *de novo* T cell synthesis will eventually replace the missing CD4 T cells.[47–49] What is not known from CD4 T cell studies is whether increases in cell count are accompanied by increasing repertoire complexity, as would be predicted for *de novo* thymic output. For Vδ2 cells, there is now clear evidence that prolonged treatment leads to reconstitution of the Vγ2 chain repertoire, and presumably, to recovery of normal or near-normal functionality. Due to concerns about the dangers of treatment interruption and viral recrudescence, there is little incentive to attempt treatment interruptions in patients with reconstituted Vγ2 repertoires. However, with more studies on both Vδ2 and CD4 T cells, along with Vγ2 and Vβ repertoires, we expect that questions about the mechanisms for immune reconstitution and the possibility of acquiring a normal or near-normal immune capacity will be resolved, and treatment cessation studies may resume again to determine whether TCR repertoire reconstitution restores the ability for natural immune control over HIV.

These detailed investigations of Vδ2 T cells in well-defined cohorts reinforce the concept that better functioning γδ cells are related to an improved health status in HIV patients. However, proof of this conjecture will only be obtained once intervention studies are undertaken with the specific intent of manipulating Vδ2 T cell function.

## HOW DOES Vδ2 CELL DEPLETION IMPACT HIV DISEASE?

The relationships between Vδ2 T cell depletion and HIV disease are now well established. However, we lack a deep appreciation for the specific functions of Vδ2 T cells that are compromised during HIV and how they contribute to immune deficiency and AIDS. Here, we have divided the potential functions of Vδ2 cells into immunoregulation or effector mechanisms that might impact HIV disease.

Recent studies have focused on the capacity for Vδ2 cells to regulate innate immunity. Reciprocal interactions between Vδ2 cells and immature dendritic cells (DCs) are required both to activate Vδ2 T cells and to promote DC maturation.[50] Immature DCs may potentiate Th1 and Th2 cytokine production by Vδ2 cell clones, which are, in turn, required for the maturation of DC.[51] When modeled *in vitro* with purified DC and γδ T cells, this interaction promotes antigen presentation and potentially contributes to inflammation. However, there are reciprocal interactions between Vδ2 cells and NK cells that complicate the picture. We showed that Vδ2 cells interact with NK cells *via* the costimulatory molecule CD137 to increase NK cytotoxicity against normally NK-resistant tumor cell lines.[52] When Vδ2 cells are removed from peripheral blood mononuclear cell (PBMC) and standard conditions are used to activate NK, the NK cell cytotoxic potential was reduced, meaning that NK–γδ T cell interactions are needed to achieve full NK effector function. These interactions depended on costimulatory markers, including CD137/CD137L and ICOS/ICOSL (unpublished). After contacting Vδ2 cells, newly educated NK cells have an increased capacity for cytotoxicity of autologous, mature DC (unpublished). Thus, interactions between Vδ2 and NK cells produce NK effectors capable of eliminating antigen-presenting DC and decreasing inflammation. We can envision that this type of mechanism works best when NK and Vδ2 cells are activated and both are present at high levels where they can interact and control inflammation. During HIV disease, Vδ2 cell levels are greatly reduced, which may contribute to NK cell defects.[53] With insufficient numbers of Vδ2 cells, and consequently, fewer functional NK cells, we might expect an accumulation of mature DCs with an increased potential for chronic inflammation. A chronic inflammatory or hyperactivated state is characteristic of HIV disease and is a major target for the development of new therapies.

Knowing that activated Vδ2 cells express costimulatory molecules raises questions about the potential interactions of these cells with other lymphocyte or innate immune cell populations. Cross-talk involving Tregs, DC and CD8 αβ T cells, which is promoted by γδ T cells that were activated with aminobisphosphonate, has been described in multiple myeloma.[54] Vδ2 cells might also interact with CD4 T cells to affect the maturation and differentiation of these latter cells. It has been shown that phosphoantigen-dependent cross-talk between Vγ2Vδ2 T cells and autologous monocytes induces acute inflammation during bacterial infection, resulting in the immediate production of cytokines and chemokines and enabling inflammatory DC to trigger the generation of $CD4^+$ effector αβ T cells expressing interferon-gamma (IFN-γ) and/or IL-17.[55] The HIV-mediated depletion of Vδ2 T cells will impact DC differentiation and the capacity for protective cellular immune responses.

The release of chemokines and cytokines is another important immunoregulatory function of γδ T cells. The Vδ2 subset responds to phosphoantigen stimulation by producing type I cytokines including tumor necrosis factor-α and IFN-γ.[56,57] These cytokines polarize toward type I immunity and may increase the capacity for controlling the virus. We have shown that circulating Vδ2 cells contain chemokines in cytoplasmic granules, most notably CCL-5 (RANTES).[58] Upon antigen stimulation, CCL-5 is rapidly released from circulating cells before gene expression and *de novo* protein synthesis. CCL-5 can bind and downmodulate CCR5, thereby inhibiting HIV-1 entry into host cells.[59] Vδ2 T cell activation with phosphoantigens causes rapid induction of other C-C chemokines, including MIP-1α and MIP-1β.[60] Additionally, soluble factors from phosphoantigen-activated Vδ2 T cells have the capacity to inhibit CXCR4-tropic virus replication[61] and may be similar to other factors defined as inhibitors of X4-tropic viruses (Cocchi *et al.*, in press). Depletion of Vδ2 cells may reduce the cytokine or chemokine production that is normally important for suppressing HIV infection.

A new subset of human $IL\text{-}17^+$ Vδ2 T lymphocytes was implicated in inflammatory responses against infectious microorganisms.[62] The

impact of HIV on this subset of Vδ2 cells is not known, but the levels of IL-17-producing Vδ1 T cells were also increased during HIV infection.[10] Recently, Vδ2 T cells were shown to express the B cell-attracting chemokine CXCL13 (BCA-1) when stimulated by IL-21.[63] This model predicts that IL-21 from germinal center Tfh cells will increase CXCL13 production by Vδ2 cells, attract B cells and generate high-affinity, class-switched antibodies. A defect in Vδ2 cells that occurs during HIV disease may compromise antibody responses to HIV.

Human γδ T cell responses to viruses have been characterized extensively for cytomegalovirus and influenza virus. Acute cytomegalovirus infection in pregnant women elicits specific gamma delta T cell expansion and differentiation in the fetus, but the affected cells are mainly Vδ1 cells and use mostly germline-encoded TCR-γ8 chains with limited diversity.[64] In renal allograft recipients who develop cytomegalovirus infection, sustained Vδ3 and Vδ1 activation was observed.[65,66] Protective Vδ2 T cell responses have also been defined for the human influenza virus. In humanized mice, aminobisphosphonates were used to stimulate human Vδ2 T cells, which then suppressed the influenza virus and improved the survival of infected mice. Protection in this model was mediated by a Vγ2Vδ2 T cell-dependent mechanism because mice reconstituted with PBMCs lacking Vγ2Vδ2 T cells did not show protective effects of aminobisphosphonate against influenza virus challenge.[67] In an *in vitro* system, the cytotoxicity of Vγ2Vδ2 T cells against influenza virus-infected monocyte-derived macrophages was dependent on NKG2D activation and was mediated by Fas–Fas ligand and perforin–granzyme B pathways.[68] Vδ2 T cells from human PBMCs can be activated by influenza virus to induce IFN-γ, and this activation depends on the mevalonate pathway,[69] which is the source of phosphoantigens and the target for aminobisphosphonate drugs.

Direct cytotoxicity against HIV-infected cells *in vivo* is another function of human γδ T cells. For Vδ1 T cells, the induction of NKG2C expression plays a key role in the destruction of HIV-infected CD4 T cells during HIV disease.[70] Additionally, NKp30 signaling results in an increase in chemokine release. Vδ2 cells were shown to have cytolytic activity against HIV-infected cells in several *in vitro* systems.[72,73] Whether this subset plays a significant role in the killing of infected cells *in vivo* is still not clear.

Activated Vδ2 T cells also express CD16, the Fc receptor for IgG that mediates antibody-dependent cellular cytotoxicity (ADCC). Exploiting the ADCC effector function of Vδ2 T cells has become an important strategy in cancer treatments in which therapeutic targeting antibodies are already used for tumor reduction. Adding potent effector cells may increase tumor reduction and treatment efficacy. Indeed, the clinical development of this approach for cancer treatment is ongoing and promises to improve the function of already existing therapeutic antibodies *via* the aminobisphosphonate activation of $CD16^+$ Vδ2 cell effectors.[74] The CD16-dependent antiviral activities of human γδ cells are currently being discovered. During a human cytomegalovirus infection, $CD16^+V\delta2^-$ T cells produced IFN-γ when incubated with IgG-opsonized virions, an effect that led to the inhibition of Human cytomegalovirus (HCMV) multiplication *in vitro*.[75] A $V\delta2^+$ fraction that was cytotoxic against influenza virus-infected cells was defined by CD56 expression, and the cytotoxicity was due to CD16-dependent degranulation.[76] The $CD56^+$ cytotoxic subset of Vδ2 cells expresses significantly higher levels of CD16,[76,77] and this CD16 is believed to perform the cytotoxic functions of Vδ2 cells *via* ADCC, the CD16-mediated degranulation pathway, or by acting as a lysis receptor to mediate direct cytotoxicity.[76] Activated Vδ2 T cells are potent ADCC effectors against target cells displaying HIV envelope glycoprotein on their surface (our unpublished data). When the HIV protein is targeted by human monoclonal antibodies, CD16 Vδ2 T cells effectively recognize and kill these targets. Similar to the approach being developed for cancer, we can imagine combined therapies for HIV disease including potent targeting antibodies delivered in the context of *in vivo* Vδ2 T cell activation that will promote the efficacious reduction of infected cell burden *via* ADCC.

Surveillance against malignant disease is another important function of Vδ2 T cells. Indeed, a useful target cell for measuring Vδ2 cytotoxicity is Daudi Burkitt's lymphoma cell line.[78] Many other (but not all) non-Hodgkin's lymphomas are also facile targets for cytolysis by phosphoantigen-stimulated Vδ2 T cells, and our group showed that Vδ2 cells were potent cytotoxic effectors against Kaposi's sarcoma (KS) cell lines (unpublished). We can find healthy donors capable of lysing B-cell lymphoma or KS cell lines at very low effector/target cell ratios, sometimes below 1 : 1. Many other tumors are also susceptible to Vδ2 cytotoxicity, but higher effector/target cell ratios are needed to achieve significant killing.[52] During HIV disease, there is a rapid loss of tumor effector capacity against Daudi cell targets, which is disproportionately greater than the overall loss of Vδ2 cells,[10] indicating that natural tumor surveillance is lost during HIV disease. Given the potent activity of Vδ2 cells against B-cell lymphoma and KS tumor cell lines, it is not surprising that the HIV-mediated depletion of this effector cell population is associated with dramatic increases in the risk for malignant disease, including B-cell lymphoma (>10 increase in relative risk) and KS (>1000 increase in relative risk).[79,80] Only approximately 1% of HIV-associated lymphomas are T cells,[81] and Vδ1 cells are the likely effector population for these cancers.[82,83] Cancers that are potentially controlled by Vδ2 cell surveillance (B-cell lymphoma and KS) are greatly increased during HIV disease, while the effect is less for cancers that are recognized by the Vδ1 cell subset. Thus, specific effects of HIV infection (decreasing Vδ2 cell levels and increasing Vδ1 cell levels) are reflected in the pattern of hematopoietic malignancies, indicating the critical role for Vδ2 in surveillance against lymphoma and KS.

## TREATMENT APPROACHES TARGETING Vδ2 CELLS IN HIV DISEASE

We recently reviewed objectives and potential strategies for exploiting Vδ2 T cells in HIV disease.[44] Aminobisphosphonate compounds, including pamidronate or zoledronate, can be used in conjunction with IL-2 to expand and activate human Vδ2 T cells *in vivo*. Most research on *in vivo* γδ T cell activation seeks to activate direct cytotoxicity against tumor cells. In some cases, aminobisphosphonate/IL-2 treatments are combined with therapeutic monoclonal antibodies to target tumor cells for killing *via* ADCC.[74] Such approaches depend on the patient having sufficient levels of responsive Vδ2 T cells, which can be activated *in vivo* to become tumor effectors. We would like to develop similar strategies for Vδ2 cell activation as an immunotherapy for HIV disease.

Earlier, we discussed the features of Vδ2 T cells in NVS and patients with chronic, low viral loads. In both of these groups, the levels of Vδ2 T cells are increased and the cells are activated, apparently in response to virus infection. Despite repertoire defects, including substantial depletion of the Vγ2-Jγ1.2 chains, circulating Vδ2 T cell levels are sufficient to warrant therapeutic intervention studies. Therapeutic intervention studies may be difficult for the virus suppressor group in which the viral load is undetectable and patients are healthy without opportunistic infections. Treatment studies in this group would likely be directed toward reducing the provirus burden, which is another

measure of infected cell frequency. However, it is less likely that clinical endpoints can be defined for NVS treatment studies, and this group is unlikely to be an early target for clinical research. The therapeutic activation of Vδ2 T cells with aminobisphosphonate/IL-2 could be evaluated in patients with low viral load, using reduction in viremia as an endpoint.

If we can activate Vδ2 cells in HIV patients, what effects will this have on disease? Activation might enhance the cytotoxic effector activity, but there are potential obstacles to this outcome. The pronounced loss of CD56-positive cells, including NK, NKT, γδ and CD8 T cells, is an important and poorly understood aspect of HIV disease. Patients with chronic viremia have lower CD56 expression on Vδ2 cells and low responses to phosphoantigen stimulation.[45] Whether *in vivo* activation will be sufficient to increase CD56 expression and increase the effector activity remains to be tested in clinical studies.

We are most concerned with patients who receive antiretroviral therapy to arrest normally progressing HIV disease. Until a few years ago, we would have considered these individuals to be poor candidates for aminobisphosphonate/IL-2 therapy. However, we now know that long-term treatment in this group leads to reconstitution of the TCR-γ2 repertoire and should increase the response to immunotherapy.[45] One complication is that we only studied patients who reconstitute CD4 T cell counts after treatment initiation. Our cohort of treated patients excludes individuals with CD4 counts below 300 cells/mm$^3$; these patients were excluded to ensure that we had clinical specimens with sufficient numbers of Vδ2 T cells for studies on repertoire and functional responses. We have not yet studied that minority of patients who fail to reconstitute CD4 T cells after the initiation of treatment. We have insufficient data about γδ T cells in the 'non-reconstituting' group and especially about the TCR-γ2 repertoire in this group. Understanding the relationships between CD4 T cell reconstitution and TCR-γ2 repertoire changes is an important objective for future clinical studies, especially in a CD4 non-reconstituting population that represents approximately 15% of all individuals receiving long-term therapy for HIV disease.

During prolonged antiretroviral therapy, CD56 expression on Vδ2 cells increased to normal levels among individuals selected for CD4 counts >300 mm$^2$.[45] In HIV-infected, untreated patients, zoledronate plus IL-2 induced *in vivo* Vδ2 T-cell expansion and maturation,[84] suggesting a role for activation in restoring effector function. We demonstrated the possibility of expanding and improving the ADCC effector activity of Vδ2 T cells from HIV patients using *in vitro* treatment with zoledronate/IL-2.[85] Although these cells continue to have poor responses to phosphoantigens, PBMC cultures from patients receiving antiretroviral therapy respond to zoledronate and increase the number of Vδ2 cells with high cytotoxic potential. These experiments provide the first indication that immunotherapy targeting Vδ2 T cells might be an effective mechanism for controlling virus replication. For clinical studies, it may be necessary to select patients on the basis of the Vδ2 cell phenotype, possibly including functional or repertoire characterization to identify the individuals that are most likely to have positive responses.

## CONCLUSIONS

The targeted depletion of Vγ2-Jγ1.2 Vδ2 T cells is the best example of the TCR-specific effects of HIV on the immune system. A likely mechanism for this depletion involves HIV envelope glycoprotein signaling through CCR5 on Vδ2 cells, which phosphorylates p38 kinase, induces caspase activity and causes cell death. The absence of CCR5 on Vδ1 cells makes these cells insensitive to gp120-mediated depletion; thus, these cells may be stimulated to increase in number by bacterial products crossing the damaged gut mucosal epithelium in HIV patients. By eliminating the crucial subset of Vδ2 T cells, HIV cripples an important antiviral effector subset, modulates the antiviral activity of NK cells and removes a normal control over inflammation. These changes promote a chronic, hyperactive immune state, resulting in HIV persistence and progressing disease. The disease mechanisms of HIV impact many aspects of innate and acquired immunity. However, no other infectious disease triggers such a deep and long-lasting impact on γδ T cells. Depleting the Vγ2Vδ2 subset seems to be important for HIV immune evasion.

In some patients, prolonged antiretroviral therapy can lead to a partial reconstitution of Vδ2 cells and the recovery of normal complexity in the Vγ2 repertoire. Treatment improves both the quality and quantity of γδ T cells and may create opportunities for activating this T cell subset to improve viral immunity and reverse the negative impacts of HIV. There are few examples in which approved drugs, including the aminobisphosphonates discussed here, target a major subset of T cells, trigger abundant proliferation and enhance effector activity. We have ample evidence that damage to Vδ2 cells is important in HIV disease. The imperative now is to test these concepts in human clinical trials using γδ-targeted immunotherapies as an adjunct therapy for patients with HIV.

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