

Immune biology of Ag-specific $\gamma\delta$ T cells in infections

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Abstract Accumulating evidence suggests that human $\gamma\delta$ T cells act as non-classical T cells and contribute to both innate and adaptive immune responses in infections. V γ 2 V δ 2 T (also termed V γ 9 V δ 2 T) cells exist only in primates, and in humans represent a dominant circulating $\gamma\delta$ T-cell subset. Primate V γ 2 V δ 2 T cells are the only $\gamma\delta$ T cell subset capable of recognizing microbial phosphoantigen. Since nonhuman primate V γ 2 V δ 2 T cells resemble their human counterparts, in-depth studies have been undertaken in macaques to understand the biology and function of human V γ 2 V δ 2 T cells. This article reviews the recent progress for immune biology of V γ 2 V δ 2 T cells in infections.

Keywords $\gamma\delta$ T cells · T cell receptor · T cell responses · Human infections · Tuberculosis · Phosphoantigen · (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) · HIV · Pneumonic plague

Introduction

Accumulating evidence suggests that human $\gamma\delta$ T cells function as non-classical T cells and contribute to both innate and adaptive immune responses in infections [1–4]. V γ 2 V δ 2 T (also termed V γ 9 V δ 2 T) cells exist only in primates, and in humans represent a major circulating $\gamma\delta$ T cell subset that normally constitutes up to 65–90% of total

peripheral blood $\gamma\delta$ T cells. Since macaque V γ 2 V δ 2 T cells resemble their human counterparts, in-depth studies have been undertaken in nonhuman primates to understand biology and function of human V γ 2 V δ 2 T cells. This article reviews the recent progress for immune responses of V γ 2 V δ 2 T cells in infections.

Infection-driven adaptive immune responses of phosphoantigen-specific human V γ 2 V δ 2 T cells

A number of laboratories including ours have demonstrated that V γ 2 V δ 2 T cells can be activated in vitro by certain low m.w. foreign- and self-nonpeptidic phosphorylated metabolites of isoprenoid biosynthesis [e.g., (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP), isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP)] [7–11] commonly referred to as phosphoantigens. Microbial phosphoantigen HMBPP is produced in the 2-C-methyl-Derythritol-4-phosphate (MEP) pathway of isoprenoid biosynthesis of a number of bacteria and protozoa [5]. Infections of humans or nonhuman primates with HMBPP-producing microbes can induce major expansion of V γ 2 V δ 2 T cells [5]. Typically, *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis* both produce HMBPP, and early infections of macaques with these mycobacteria can induce remarkable expansion of V γ 2 V δ 2 T cells [1]. Surprisingly, BCG re-infection or BCG infection followed by *M. tuberculosis* challenge can induce rapid recall expansion or adaptive immune responses in macaques [1]. Re-occurrence of clonotypic TCR sequences of V γ 2 V δ 2 T cells is also evident during rapid recall expansion, suggesting the memory-like responses [1]. Our new studies provide additional in vivo evidence indicating that rapid recall

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expansion or adaptive immune response of V γ 2 V δ 2 T cells can also be induced during re-infection of macaques with *Listeria monocytogenes* capable of producing HMBPP (Ryan et al., 2010 International $\gamma\delta$ T cell conference). In fact, increases in human $\gamma\delta$ T cells or V γ 2 V δ 2 T cells were reported in leprosy granulomatous disease and tuberculous meningitis, respectively (see review [6]). Examples for infection-driven expansion of human $\gamma\delta$ T cells also include salmonellosis, brucellosis, legionellosis, tularemia, brucellosis, Legionellosis, malaria, toxoplasmosis, Leishmaniasis and others (see review [6]). The notion that human V γ 2 V δ 2 T cells can mount adaptive immune response in infections is also supported by other human studies [5, 7–12]. However, like CD4+ or CD8+ T cells, V γ 2 V δ 2 T cells appear to be depressed in terms of frequency or effector function during chronically active tuberculosis or chronic phase of AIDS virus infections [13, 14]. It is still debatable as to whether such depressed V γ 2 V δ 2 T cells contribute to the development of active tuberculosis or result from immune dysfunction in chronic tuberculosis.

The extraordinary expansion and recall expansion of V γ 2 V δ 2 T cells in infections indeed argue against the old simple paradigm that human $\gamma\delta$ T cells completely function as innate cells. Notably, human $\gamma\delta$ T cells have been long considered innate cells due to the lack of convincing evidence indicating major clonal expansion and adaptive feature for these $\gamma\delta$ T cells. Nevertheless, the emerging evidence suggests that human V γ 2 V δ 2 T cells can have both innate and adaptive immune features in infections.

It is likely that the infections with HMBPP-producing pathogens are the driving forces for adaptive immune response of V γ 2 V δ 2 T cells. Our new in vivo studies suggest that both HMBPP and infection-driven cytokines are needed for clonal expansion and recall expansion (Ryan et al., unpublished data). This helps to explain at least in part why repeated in vivo treatments of macaques by a phospholigand and IL-2 can induce expansion of V γ 2 V δ 2 T cells each time, but not necessarily stimulate greater magnitudes of recall expansion each time after the treatment [15]. The finding derived from small phospholigand and IL-2 treatment cannot rule out the possibility that V γ 2 V δ 2 T cells can contribute to adaptive immune response in infections in that V γ 2 V δ 2 T cells do not exactly resemble their $\alpha\beta$ T cell counterpart mounting the typical recall or memory responses when exposed to the simple treatment comprised only of very small phospholigand molecule and IL-2. It is also possible that the initial treatment with phospholigand and IL-2 has already reached the maximum activation/expansion potential of V γ 2 V δ 2 T cells (up to 80% of T cells are HMBPP/IL2-expanded $\gamma\delta$ T cells [15, 16]), and a subsequent treatment will not be able to go beyond the saturated points due to the host self-

control mechanism for shutting-down over-expanding T cell clones. A human in vitro study also implicates that HMBPP is different from infection in activation and function of V γ 2 V δ 2 T cells, as mycobacteria-stimulated V γ 2 V δ 2 T cells, but not HMBPP-activated V γ 2 V δ 2 T cells, are able to suppress *M. tuberculosis* replication [17].

Molecular mechanism by which V γ 2 V δ 2 T cells interact with microbial phosphoantigen HMBPP during infections

Primate V γ 2 V δ 2 T cell subset remains the only $\gamma\delta$ T cells capable of recognizing a microbial phosphoantigen, whereas no defined microbial antigens can be recognized by $\gamma\delta$ T cells from mice and other species [1–4]. While the chemistry of phosphoantigens and their ability to activate V γ 2 V δ 2 T cells have been well described, molecular mechanisms by which HMBPP interacts with $\gamma\delta$ T cells remain poorly characterized [18–21]. Most studies done to date have been focused on prenyl pyrophosphates, particularly IPP, but rarely the microbial phosphoantigen HMBPP [22]. Earlier experiments using V γ 2 V δ 2 T cell activation as readouts demonstrated that IPP does not need to undergo cell-entry or processing and that phosphoantigen activation of V γ 2 V δ 2 T cells requires cell–cell contact [18, 23]. A putative molecule, but not MHC class I, class II, or CD1, appears to be required to present IPP for immune activation of V γ 2 V δ 2 T cells [18]. Despite decade-long studies, however, there has been no direct evidence indicating that human or macaque V γ 2 V δ 2 TCR (instead of human macaque hybrids) can directly bind to HMBPP or HMBPP complex [24, 25].

We presumed that the development of soluble, tetrameric V γ 2 V δ 2 TCR would provide a useful approach to explore the molecular mechanism by which V γ 2 V δ 2 T cells interact with phosphoantigen HMBPP. We therefore took advantage of our decades-long TCR expertise and MHC tetramer technology [26, 27] to develop high-affinity-binding soluble V γ 2 V δ 2 TCR tetramer [28]. We demonstrated that soluble V γ 2 V δ 2 TCR tetramer, once labeled with fluorescence, makes it possible to visualize APC presentation of phosphoantigen HMBPP to V γ 2 V δ 2 TCR [28]. We show that exogenous HMBPP is associated with APC membrane in an appreciable affinity, and that the membrane-associated HMBPP is readily recognized by the V γ 2 V δ 2 TCR tetramer (Fig. 1) [28]. In fact, the V γ 2 V δ 2 TCR tetramer stably binds HMBPP presented on membrane by various APC cell lines from humans and non-human primates but not those from mouse, rat or pig [28]. This finding suggests that V γ 2 V δ 2 TCR is one of the important elements dictating the specific recognition of microbial HMBPP by V γ 2 V δ 2 T cells [28]. The V γ 2 V δ 2

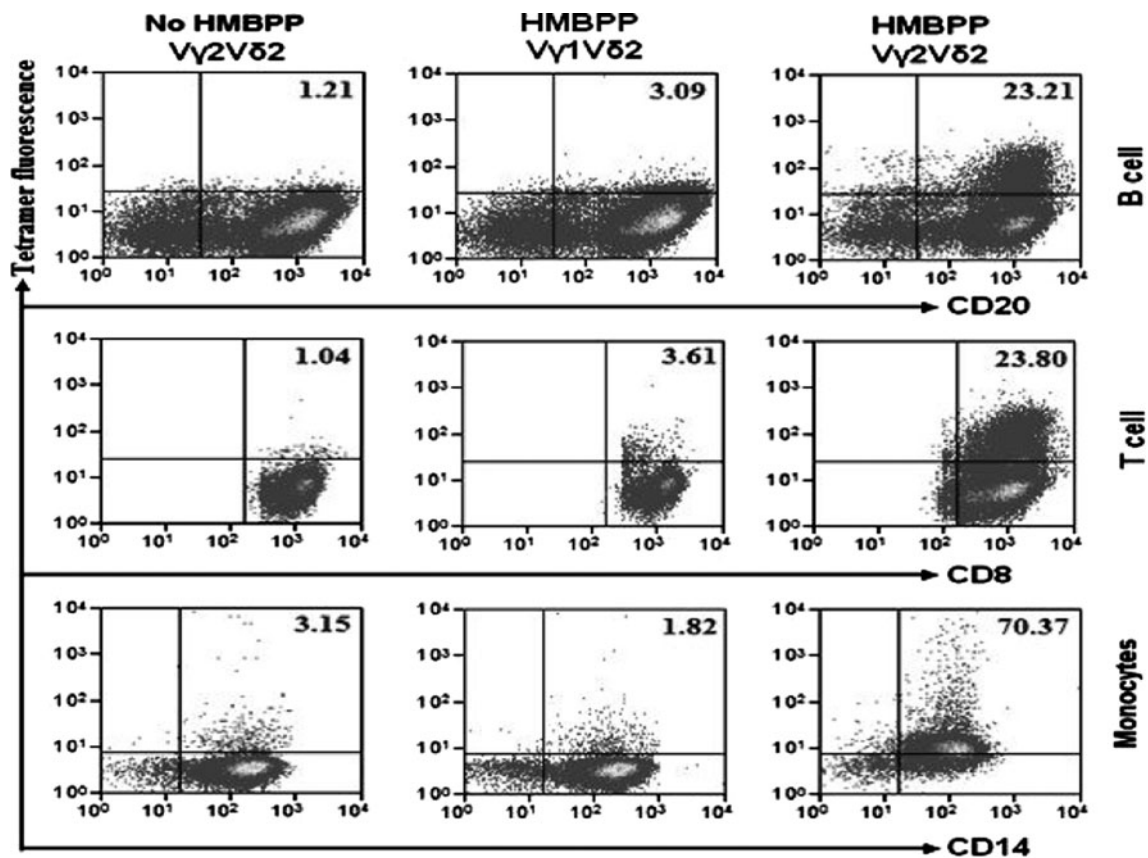


Fig. 1 Flow cytometry histograms showed that FITC-labeled V γ 2 V δ 2 TCR tetramer bound to the membrane-associated HMBPP presented by macaque primary B cells, T cells and monocytes. Large proportions of these cell populations were stained positive by the V γ 2 V δ 2 TCR tetramer. No staining was seen for the two controls:

(1) cells not pulsed with HMBPP and directly incubated with FITC-labeled V γ 2 V δ 2 TCR tetramer (no HMBPP/V γ 2 V δ 2); and (2) cells pulsed with HMBPP but incubated with the FITC-labeled V γ 1 V δ 2 TCR tetramer control (HMBPP/V γ 1 V δ 2)

TCR tetramer also binds the membrane-associated HMBPP on primary monocytes, B cells and some T cells, suggesting that various host immune cells can present HMBPP for activation of V γ 2 V δ 2 T cells during infections with HMBPP-producing pathogens [28]. Consistently, endogenous phosphoantigen produced in mycobacterium-infected DC is transported and presented on membrane, and stably bound to the V γ 2 V δ 2 TCR tetramer. This is particularly significant for host responses to infections with HMBPP-producing intracellular pathogens, as the infected APC, such as DC or macrophages, can rapidly present HMBPP to V γ 2 V δ 2 TCR for early activation and expansion of V γ 2 V δ 2 T cells. Interestingly, the capability of APC to present HMBPP for recognition by V γ 2 V δ 2 TCR is diminished after protease treatment of APC. Thus, our studies elucidate that an affinity HMBPP–APC association confers binding to V γ 2 V δ 2 TCR, and that the putative APC membrane molecule presenting HMBPP appears to be a protein or protein-associated component existing in primate APC or T cells but not rodent APC [28]. Thus, the V γ 2 V δ 2 TCR tetramer-based studies enhance our

understanding of molecular aspects for the interaction between V γ 2 V δ 2 T cells and APC presenting microbial phosphoantigen HMBPP in infections.

V γ 2 V δ 2 TCR appears to play a fundamental role in recognition of HMBPP and subsequent clonal activation/expansion of V γ 2 V δ 2 T cells during infections

Since V γ 2 V δ 2 TCR dictates the specific interaction with microbial phosphoantigen HMBPP, it would be interesting to see the HMBPP-engaged TCR molecule events driving sequential activation and expansion of V γ 2 V δ 2 T cells in early infections. We presume that molecular imaging of HMBPP-triggered TCR response would be a best approach to elucidate TCR molecule responses. We have innovatively combined near-field optical microscopy (NSOM) and fluorescent quantum dot (QD) nanotechnology (NSOM/QD), and developed a best-optical-resolution nanoscale molecular imaging (<50 nm) system

for visualizing molecule events in immune cells. Such a best-optical-resolution molecular imaging system allows us to show that non-stimulating $V\gamma 2$ $V\delta 2$ TCR molecules in resting $\gamma\delta$ T cells are distributed individually from each other on the cell surface, with mean sizes of <50 – 70 nm (Fig. 2) [29]. However, HMBPP pulsation of APC for engaging $V\gamma 2$ $V\delta 2$ TCR rapidly induces formation of ~ 120 – 300 nm TCR nanoclusters [29]. Such TCR molecule events coincide with the translocation of $PKC\theta$ to the membrane for signal transduction and activation (data not shown). Surprisingly, such $V\gamma 2$ $V\delta 2$ TCR nanoclusters can be sustained on the membrane during an in vivo

clonal expansion of $V\gamma 2$ $V\delta 2$ T cells after HMBPP/IL-2 treatment and mycobacterial infection. The TCR nanoclusters can array to form nanodomains or microdomains on the membrane of clonally-expanded $V\gamma 2$ $V\delta 2$ T cells (Fig. 2) [29]. Interestingly, expanded $V\gamma 2$ $V\delta 2$ T cells bearing TCR nanoclusters or nanodomains were able to re-recognize phosphoantigen and to exert better effector function [29]. In contrast, non-specific stimulants cannot induce $V\gamma 2$ $V\delta 2$ TCR nanoclustering or nanodomain formation. These nanoscale novel findings suggest that $V\gamma 2$ $V\delta 2$ TCR plays a fundamental role in recognition of HMBPP and subsequent clonal activation and expansion

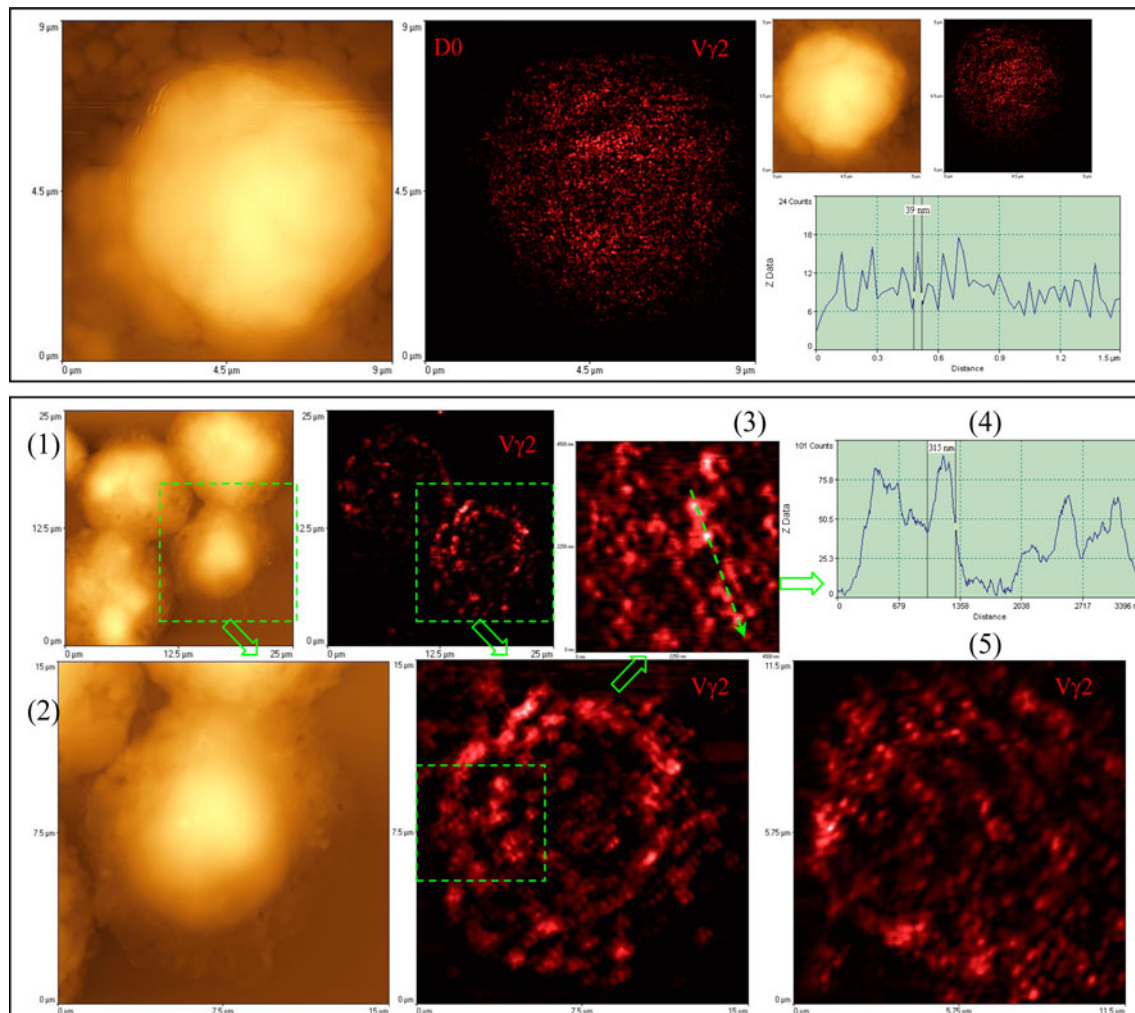


Fig. 2 NSOM/QD-based nanoscale imaging shows that $V\gamma 2$ $V\delta 2$ TCR arrayed to form high-density TCR nanoclusters, nanodomains and microdomains during the in vivo clonal expansion of $V\gamma 2$ $V\delta 2$ T cells after HMBPP/IL-2 treatment. *Upper panel* shows representative NSOM topographic (left) and fluorescence (middle) images indicating the dominance of non-engaging fluorescence TCR dots on the membrane of unstimulated $V\gamma 2$ T cells on day 0. The fluorescence intensity profile graph (right) is extracted from a random cross section in the fluorescence image (middle) showing that predominant

fluorescence TCR dots here displayed FWHM of ~ 50 nm. *Lower panel* shows representative NSOM images of TCR nanoclusters, nanodomains and microdomains on the membrane of clonally expanded $V\gamma 2$ $V\delta 2$ T cells on day 4. (2) is enlarged from the boxed area in the low-magnification NSOM image (1). The fluorescence intensity profile (4) is extracted from the cross section part (dashed arrow) in (3) that is enlarged from the boxed area in (2). (5) is the NSOM fluorescence image of another activated/expanded $V\gamma 2$ $V\delta 2$ T cell collected on day 4

of V γ 2 V δ 2 T cells during infections with HMBPP-producing pathogens.

A critical role of cytokines in adaptive immune responses of V γ 2 V δ 2 T cells in infections

Some cytokines produced in infections may contribute to the development of adaptive immune responses of V γ 2 V δ 2 T cells during infections with HMBPP-producing pathogens. This scenario is supported by the studies from us [2] and others [30]. We found that *M. tuberculosis* and BCG infections of macaques induced major expansion of V γ 2 V δ 2 T cells and coincident expression of variant IL-4 (VIL-4) mRNA encoding a protein comprised of N-terminal 97 amino acids (a.a.) identical to IL-4, and unique C-terminal 96 a.a. including a signaling-related proline-rich motif. We then expressed and purified VIL-4 to test the possibility that this variant cytokine can contribute to major expansion of V γ 2 V δ 2 T cells. The purified VIL-4 induces apparent expansion of phosphoantigen HMBPP-specific V γ 2 V δ 2 T cells in dose- and time-dependent manners [2]. The unique C-terminal 96 a.a. bearing the proline-rich motif (PPPCPP) of VIL-4 appears to confer the ability to expand V γ 2 V δ 2 T cells, since simultaneously produced IL-4 has only a subtle effect on these $\gamma\delta$ T cells. Moreover, VIL-4 seems to utilize IL-4 receptor α for signaling and activation, as the VIL-4-induced expansion of V γ 2 V δ 2 T cells can be blocked by anti-IL-4R α mAb but not anti-IL-4 mAb [2]. Surprisingly, VIL-4-expanded V γ 2 V δ 2 T cells after HMBPP stimulation appear to be heterologous effector cells capable of producing IL-4, IFN- γ and TNF- α [2]. Thus, mycobacterial infections of macaques induced variant mRNA encoding VIL-4 that functions as growth factor promoting expansion of HMBPP-specific V γ 2 V δ 2 T effector cells [2]. We presume that other cytokines may also exert similar effects facilitating major expansion of V γ 2 V δ 2 T cells during mycobacterial infections. In fact, it has been reported that IL-21 can also stimulate marked expansion of HMBPP-specific V γ 2 V δ 2 T cells [30]. Our new studies also demonstrate that IL-17, IL-22 and IL-23 can contribute to significant expansion of V γ 2 V δ 2 T cells after mycobacterial infection (Ryan et al., 2010 International $\gamma\delta$ T cell conference). Human in vitro studies also showed that IL-15 could help to develop antimicrobial function of V γ 2 V δ 2 T cells in *M. tuberculosis* infection [31]. Since HMBPP alone or cytokine alone is not able to induce major expansion of V γ 2 V δ 2 T cells, IL-2 or other cytokines may function as “second or third signal” for sustaining HMBPP/TCR-mediated activation and clonal expansion in infections.

V γ 2 V δ 2 T effector cells may potentially enhance Ag-specific Ab response and $\alpha\beta$ CD4+ or CD8+ T cell responses in infections

It has been long postulated that $\gamma\delta$ T cells may bridge innate and adaptive immune responses in infection presumably due to the enriched distribution of $\gamma\delta$ T cells in mucosa. Such a hypothesis has not been well tested in mice since mouse $\gamma\delta$ T cells do not recognize phosphoantigen HMBPP or other microbial antigens. To address this, we treat macaques with HMBPP compound plus IL-2 regimen to investigate if activation/expansion of V γ 2 V δ 2 T cells can impact immune responses in infections. We first defined the utility and immune kinetics of the single-dose HMBPP plus 5-day IL-2 treatment regimen in macaques, and found that this regimen could expand macaque V γ 2 V δ 2 T cells from baseline 1 up to 80% in the T cell pool without detectable side effects [16, 29]. The HMBPP-activated V γ 2 V δ 2 T cells produced IFN γ and other cytokines, readily trafficked to lung, oral and gut mucosa, and sustained their pulmonary accumulation for months [16]. We then investigated whether this well-defined HMBPP/IL-2 therapeutic regimen could overcome HIV-mediated immune suppression to massively expand polyfunctional V γ 2 V δ 2 T cells, and whether such activation/expansion could impact AIDS pathogenesis in simian HIV (SHIV)-infected macaques. HMBPP/IL-2 coadministration during chronic phase of SHIV infection induced massive activation/expansion of V γ 2 V δ 2 T cells. HMBPP/IL-2 cotreatment during chronic infection did not exacerbate disease, and more importantly it could confer immunological benefits. Surprisingly, although viral antigenic loads were decreased upon HMBPP/IL-2 cotreatment during chronic SHIV infection, HMBPP activation of V γ 2 V δ 2 T cells boosted HIV Env-specific Ab titers. Such increases in Abs were sustained for >170 days and were immediately preceded by increased production of IFN- γ , TNF- α , IL-4, and IL-10 during peak expansion of V γ 2 V δ 2 T cells displaying memory phenotypes, as well as the short-term increased effector function of V γ 2 V δ 2 T cells and CD4+ and CD8+ $\alpha\beta$ T cells producing antimicrobial cytokines. The significance for these findings are twofold: (1) HMBPP/V γ 2 V δ 2 T cell-based intervention is potentially useful for combating neoplasms and HMBPP-producing opportunistic pathogens in chronically HIV-infected individuals; and (2) HMBPP-activated V γ 2 V δ 2 T cells may function as adjuvant and facilitate B cells producing Abs, and enhance CD4+ and CD8+ $\alpha\beta$ T cells producing antimicrobial cytokines during infections. In fact, these observations in macaques are consistent with the findings in the in vitro human studies. It has been shown that activation of human $\gamma\delta$ T cells can stimulate DC maturation [32, 33]. The HMBPP-dependent cross-talk

between monocytes and human $\gamma\delta$ T cells can drive fast inflammatory cytokine responses and monocyte differentiation to DC, and facilitate development CD4 T helper cells in the presence of additional microbial stimulants [34]. Activated human $V\gamma 2 V\delta 2$ T cells appear to function as APC in vitro, presenting peptide antigens to CD4+ and CD8+ T cells [35, 36]. $V\gamma 2 V\delta 2$ T cells have also been shown to improve B cells' ability to produce antibodies in vitro [32]. Further studies are needed to prove the tentative concept that $V\gamma 2 V\delta 2$ T effector cells may potentially enhance Ag-specific Ab response and $\alpha\beta$ CD4+ or CD8+ T cell responses in infections.

$V\gamma 2 V\delta 2$ T effector cells may function as immune regulators influencing other immune cells and their responses in infections

Activated $V\gamma 2 V\delta 2$ T cells produce Th1 cytokines and many other cytokines, and therefore may influence other effector cells in host responses to infections. To assess this possibility, we sought to determine whether $V\gamma 2 V\delta 2$ T cells could have direct impact on other T cell populations in vivo during infections or vaccination. Our studies were focused on two aspects: (1) interplay between CD4+CD25+Foxp3+ T regulatory cells (Treg) and HMBPP-activated $V\gamma 2 V\delta 2$ T effector cells during mycobacterial infection; and (2) $V\gamma 2 V\delta 2$ T cell-driven immune regulation of potentially inflammatory IL-22+ T cells in tuberculosis.

Foxp3+ Treg cells control immune responses to self- and foreign-antigens and play a major role in maintaining the balance between immunity and tolerance [37–39]. While Treg are beneficial for controlling autoimmunity, allograft rejection, or hypersensitivity, their over-responses in infections may lead to suppression of host anti-microbial immunity [37]. Development of a useful model system may help to identify potential mutual regulatory effects of Treg and other immune cells or elements. Interestingly, recent studies have shown that human recombinant IL-2 administration can lead to an increase in the frequency of circulating CD4+CD25+ regulatory T cells in cancer patients [40–43]. We and others have also shown that IL-2 plus phospholigand treatment can induce remarkable expansion of $V\gamma 2 V\delta 2$ T cells in nonhuman primates [15, 16, 44]. We therefore took advantage of the IL-2-based in vivo model systems to assess potential interplay or mutual regulations between $V\gamma 2 V\delta 2$ T cells and Treg during early mycobacterial infection in nonhuman primates. A short-term IL-2 treatment regimen induced marked expansion of CD4+CD25+Foxp3+ T cells, and subsequent suppression of mycobacterium-driven increases $V\gamma 2 V\delta 2$ T cells in acutely BCG-infected macaques. Surprisingly, activation

of $V\gamma 2 V\delta 2$ T cells by adding phosphoantigen Picostim (similar to HMBPP) to the IL-2 treatment regimen apparently down-regulates IL-2-induced expansion of CD4+CD25+Foxp3+ T cells [44]. The down-regulation of IL-2-induced expansion of Treg coincides with the sustained increases in numbers of $V\gamma 2 V\delta 2$ T cells through 42 days after the Picostim/IL-2 treatment and superimposed BCG infection. Consistently, in vitro activation of $V\gamma 2 V\delta 2$ T cells in PBMC by phosphoantigen+IL-2 can down-regulate IL-2-induced expansion of CD4+CD25+Foxp3+ T cells, but HMBPP-mediated antagonizing effect appears to require APC (monocytes) or other lymphocytes [44]. Since activated $V\gamma 2 V\delta 2$ T cells alone had no inhibiting effect on CD4+CD25+Foxp3+ T cells in the culture without APC and other cells in PBMC, we sought to determine whether some cytokines produced by phosphoantigen-activated $V\gamma 2 V\delta 2$ T cells contributed to the down-regulation of the IL-2-induced proliferation of Treg. We set up cytokine-neutralizing experiments in the proliferation assays using anti-IFN- γ , anti-IL-4, or anti-TGF- β neutralizing antibodies because HMBPP phosphoantigen stimulation could up-regulate many genes including those encoding those cytokines (Wang et al., data not shown). Surprisingly, while anti-TGF- β or anti-IL-4 neutralizing antibodies did not affect the HMBPP-mediated down-regulation of Treg, anti-IFN- γ neutralizing antibody significantly reduced the ability of HMBPP-activated $V\gamma 2 V\delta 2$ T cells to antagonize Treg expansion [44]. This suggests that Th1 network contributed to $V\gamma 2 V\delta 2$ T cells' antagonizing effects. Furthermore, activation of $V\gamma 2 V\delta 2$ T cells by Picostim + IL-2 treatment appears to reverse Treg-driven suppression of immune responses of phosphoantigen-specific IFN γ + or perforin+ $V\gamma 2 V\delta 2$ T cells and PPD-specific IFN γ + $\alpha\beta$ T cells [44]. Thus, phosphoantigen-activation of $V\gamma 2 V\delta 2$ T cells can antagonize IL-2-induced expansion of Treg and subsequent suppression of and anti-microbial T cell responses in mycobacterial infections. The findings from phosphoantigen/IL-2 treatment of macaques in the context of mycobacterial infection provide the first evidence suggesting that certain T cell subsets in the immune system can antagonize Foxp3+Treg and their suppression of Ag-specific T cell responses in infections.

Given the possibility that some over-reacting T effector cells may contribute to inflammation or tissue damages in infections, we sought to determine if HMBPP-activated $V\gamma 2 V\delta 2$ T cells can regulate those potentially inflammatory cells. In this end, we focused on IL-22+ T cells and their interplay with $V\gamma 2 V\delta 2$ T cells in infection as IL-22 has been shown to play a role in inflammation or autoimmunity [45–47]. We first demonstrated in a nonhuman primate model that *M. tuberculosis* infection results in apparent increases in numbers of T cells capable of

producing IL-22 de novo without in vitro Ag stimulation, and drives distribution of these cells more dramatically in lungs than in blood and lymphoid tissues [18, 19]. Consistently, IL-22+ T cells are visualized in situ in lung tuberculosis (TB) granulomas by confocal microscopy and immunohistochemistry, indicating that mature IL-22+ T cells are present in TB granuloma [18, 19]. Surprisingly, HMBPP activation of V γ 2 V δ 2 T cells down-regulates the capability of T cells to produce IL-22 de novo in lymphocytes from blood, lung/BAL fluid, spleen and lymph node [18]. Up-regulation of IFN γ -producing V γ 2 V δ 2 T effector cells after HMBPP stimulation coincides with the down-regulated capacity of these T cells to produce IL-22 de novo. Importantly, anti-IFN γ neutralizing Ab treatment reversed the HMBPP-mediated down-regulation effect on IL-22+ T cells, suggesting that V γ 2 V δ 2 T cell-driven IFN γ -networking function was the mechanism underlying the HMBPP-mediated down-regulation of the capability of T cells to produce IL-22 [18]. These novel findings raise the possibility to ultimately investigate the function of IL-22+ T cells and to target V γ 2 V δ 2 T cells for balancing potentially hyper-activating IL-22+ T cells in severe TB.

V γ 2 V δ 2 T effector cells can readily traffic to lungs and contribute to host resistance to pathogen-induced lung damages during acute pulmonary infection

Our studies have demonstrated that one of the remarkable immune features for HMBPP-specific V γ 2 V δ 2 T cells is their capability to traffic to lungs and other mucosal surface once they are activated and expanded [16, 48]. However, the possibility that V γ 2 V δ 2 T effector cells can confer protection against pulmonary infectious diseases has not been tested. Since we reproducibly showed that the HMBPP plus IL-2 treatment can induce prolonged accumulation of V γ 2 V δ 2 T effector cells in lungs [16, 29, 44, 49], we investigated whether the expanded V γ 2 V δ 2 T cells can mediate protection against acutely fatal pneumonic plague in the macaque model of inhalational *Yersinia pestis* infection. A delayed HMBPP/IL-2 administration after inhalational *Yersinia pestis* infection overcame acute infection and induced marked expansion of V γ 2 V δ 2 T cells [49]. Expanded V γ 2 V δ 2 T cells failed to control extracellular plague bacterial replication/infection, which led to extrathoracic dissemination, septicemia and fatal shock. This appeared to be understandable since an extracellular bacterial infection would usually be controlled by neutralizing antibodies but not T effector cells. Surprisingly, despite the absence of infection control, expansion of V γ 2 V δ 2 T cells after HMBPP/IL-2 treatment led to the attenuation of inhalation plague lesions in lungs [49]. Consistently, HMBPP-activated V γ 2 V δ 2 T cells

accumulated and localized in pulmonary interstitials surrounding small blood vessels and airway mucosa in the lung tissues with no or mild plague lesions [49]. These infiltrating V γ 2 V δ 2 T cells produced FGF-7, a homeostatic mediator against tissue damages [50]. In contrast, control macaques treated with glucose plus IL-2 or glucose alone exhibited severe hemorrhages and necrosis in most lung lobes, with no or very few V γ 2 V δ 2 T cells detectable in lung tissues. The findings are consistent with the paradigm that circulating V γ 2 V δ 2 T cells can traffic to lungs for homeostatic protection against tissue damages in infection. This finding indeed provides the first in vivo evidence in humans and primates indicating that V γ 2 V δ 2 T cells can contribute to anti-microbial immunity.

Concluding remarks

In conclusion, much progress has been made over the past years. While infections with phosphoantigen-producing microbes can drive adaptive immune responses of V γ 2 V δ 2 T cells in humans and nonhuman primates, V γ 2 V δ 2 TCR appears to be responsible for recognition of phosphoantigen HMBPP presented by APC and subsequent clonal activation/expansion of V γ 2 V δ 2 T cells during infections. IL-2 and other cytokines produced in infections play a critical role in mounting adaptive immune responses of HMBPP-specific V γ 2 V δ 2 T cells. Preliminary data suggest that V γ 2 V δ 2 T effector cells may potentially enhance Ag-specific Ab response and $\alpha\beta$ CD4+ or CD8+ T cell responses in infections. In addition, V γ 2 V δ 2 T effector cells may function as immune regulators antagonizing CD4+CD25+Foxp3+ T cells and influencing IL-22+ T effector cells in infections. Importantly, V γ 2 V δ 2 T effector cells can readily traffic to lungs and contribute to host resistance to pathogen-induced lung damage during an acute pulmonary infection.

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