


SPECIAL FEATURE REVIEW

Emerging role of $\gamma\delta$ T cells in vaccine-mediated protection from infectious diseases

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Abstract

$\gamma\delta$ T cells are fascinating cells that bridge the innate and adaptive immune systems. They have long been known to proliferate rapidly following infection; however, the identity of the specific $\gamma\delta$ T cell subsets proliferating and the role of this expansion in protection from disease have only been explored more recently. Several recent studies have investigated $\gamma\delta$ T-cell responses to vaccines targeting infections such as *Mycobacterium*, *Plasmodium* and influenza, and studies in animal models have provided further insight into the association of these responses with improved clinical outcomes. In this review, we examine the evidence for a role for $\gamma\delta$ T cells in vaccine-induced protection against various bacterial, protozoan and viral infections. We further discuss results suggesting potential mechanisms for protection, including cytokine-mediated direct and indirect killing of infected cells, and highlight remaining open questions in the field. Finally, building on current efforts to integrate strategies targeting $\gamma\delta$ T cells into immunotherapies for cancer, we discuss potential approaches to improve vaccines for infectious diseases by inducing $\gamma\delta$ T-cell activation and cytotoxicity.

Keywords: cytokines, infection, proliferation, vaccination, V γ 9V δ 2 T cells, $\gamma\delta$ T cells

INTRODUCTION

Although representing only a small percentage of T cells (generally 2–5% of peripheral blood T cells in healthy adults), $\gamma\delta$ T cells have increasingly been recognised for their unique roles in establishing and regulating the inflammatory response to infectious diseases. These unconventional T cells have antigen recognition capacity, tissue tropism and cytotoxic functions that are distinct from $\alpha\beta$ T cells. $\gamma\delta$ T cells are the first T cells to appear in the thymus during foetal thymic ontogeny and, following gene rearrangement, express different T-cell receptor (TCR) sequences.¹ TCR diversity is

different across different animals, but in humans, subsets expressing different V γ and V δ regions localise to different tissues and have differing effector functions. For example, the most abundant subset in human adult peripheral blood is V γ 9V δ 2 cells (also referred to as V γ 2V δ 2) while V δ 1⁺ cells are more common in mucosal tissues.² Existing only in primates, V γ 9V δ 2 cells recognise phosphoantigens induced by stress or pathogens in a process that is dependent on butyrophilin 3A1 (BTN3A1, CD277), a type I glycoprotein in the B7 family.³ Other signalling pathways for human $\gamma\delta$ T-cell activation involve TCR interaction with ligands such as F1-ATPase or endothelial protein C

receptor, or additional cell surface receptors such as natural killer group 2 member D (NKG2D) receptors or toll-like receptors (TLR).⁴ Unlike $\alpha\beta$ T cells, all of these pathways are independent of the major histocompatibility complex (MHC). In some animals (e.g. cattle, sheep, chickens), $\gamma\delta$ T cells express highly diverse TCRs regardless of tissue localisation, while in others (e.g. mice), almost all $\gamma\delta$ T cells in the epidermal layer of the skin (called 'dendritic epidermal T cells') express identical $\gamma\delta$ TCRs. Interestingly, $\gamma\delta$ TCRs are structurally more similar to immunoglobulins than $\alpha\beta$ TCRs; the CDR3 lengths of TCR δ chains are long and variable, whereas those of the TCR γ chains are short and constrained.¹ The presence of TCR chains that use antibody-like V domains is widely distributed in vertebrates, suggesting a selective pressure for TCR chains that recognise antigen in ways similar to that of antibodies.

Several $\gamma\delta$ T-cell subsets have long been known to rapidly increase in number following systemic infections and to perform numerous roles, including direct anti-microbial roles, recruitment of innate immune cells and activation of adaptive immune cells.⁴ In many situations, including most bacterial and parasitic infections in humans, it is the $V\delta 2^+$ T-cell subset that proliferates, while in some viral infections, $V\delta 1^+$ T cells expand and exert anti-microbial activities. Interestingly, $\gamma\delta$ T cells also appear to have some level of functional plasticity, enabling them to adapt their function at different points during infection based on TCR signalling and environmental cues. Animal models have further provided support that these cells are not simply biomarkers of infection, but can in fact mediate protection from disease and/or recurrent infection. Despite being known to have an important role in immunity to infectious diseases, $\gamma\delta$ T cells have, with the exception of the Bacillus Calmette–Guérin (BCG) vaccine for tuberculosis, largely been ignored in vaccine development. Whether $\gamma\delta$ T cells are stimulated directly by the antigen component of the vaccine or indirectly with an appropriate adjuvant, there may be many opportunities to improve vaccine effectiveness by targeting $\gamma\delta$ T cells. In this article, we will review the evidence for the role of $\gamma\delta$ T cells in vaccine-induced protection to bacterial, protozoan and viral infections. Many of these diseases, particularly those responsible for the highest mortality and morbidity worldwide – tuberculosis, malaria and HIV – do not yet have an effective vaccine because of rapid pathogen evolution and

other biological and technical challenges. However, considering the functional roles of $\gamma\delta$ T cells and incorporating them into a vaccine strategy could be an important step towards reducing the devastating impact of these diseases.

MYCOBACTERIA AND OTHER BACTERIAL INFECTIONS

A number of studies have shown expansion of $\gamma\delta$ T-cell populations in response to various bacterial infections, both in humans and in animal models. In humans, $\gamma\delta$ T cells accumulate at mucosal epithelial tissues, including the lungs,⁵ and have been shown to rapidly proliferate following infection with *Mycobacterium tuberculosis* (*Mtb*).^{6,7} These responding $\gamma\delta$ T cells primarily express $V\gamma 9V\delta 2^8$ and recognise *Mtb* phosphoantigen.^{6,9} Studies testing whether $\gamma\delta$ T cells expand in response to the *Mtb* heat shock protein HSP65 have had somewhat conflicting results, but suggest that while some $\gamma\delta$ T-cell clones can recognise HSP65, the majority of cells respond to other antigens.^{7,10,11} Several *in vitro* studies have suggested that $V\gamma 9V\delta 2$ T cells may mediate protection from *Mtb*. These cells appear to be capable of directly killing extracellular *Mtb* via release of granzysin and intracellular *Mtb* via granzysin and perforin.¹² Mycobacteria-specific $V\gamma 9V\delta 2$ T cells from individuals positive for the tuberculosis skin test also produce granzyme A, which indirectly leads to *Mtb* destruction by stimulating $TNF\alpha$ production by infected macrophages.¹³ In the mouse model, although $\gamma\delta$ T cells seem to be less essential to immunity against *Mtb*,^{14,15} GM-CSF production by $\gamma\delta$ T cells in the lungs seems to play a role in protection and an additive effect between GM-CSF and $IFN\gamma$ promoted macrophage control of intracellular bacterial replication.¹⁶ Clearly, the $V\gamma 9V\delta 2$ T-cell subset is important in the human immune response to *Mtb*, but further work is required to evaluate the role of various cytokines in protection from disease at different timepoints during infection.

$\gamma\delta$ T cells also seem to play a role in immunity induced by BCG, the only current vaccination against *Mtb*. Similarly to natural infection, $\gamma\delta$ T-cell populations expand and produce $IFN\gamma$ in response to BCG vaccination.^{17–19} In fact, $IFN\gamma$ production by these cells was greater than that of $CD4^+$ T cells.¹⁹ In adults, $V\delta 2^+$ $\gamma\delta$ T cells from BCG-vaccinated individuals expanded more than cells

from non-vaccinated individuals in response to *in vitro* *Mtb* restimulation; this memory-like phenotype could not solely be attributed to increased helper functions from mycobacteria-specific memory CD4⁺ T cells.²⁰ Given that BCG contains lower levels of phosphorylated nonpeptidic antigens compared to *Mtb*,²¹ it is unclear whether $\gamma\delta$ T cells responding to BCG are recognising the same or different antigens compared to natural infection. Further studies are needed to evaluate the functional role of $\gamma\delta$ T-cell expansion following BCG vaccination, including any role for memory-like subsets and whether expansion provides protection upon challenge or infection with *Mtb*. Considering the importance of granulysin, perforin and granzyme A in response to *Mtb*, it may also be useful to incorporate strategies that elicit these responses into vaccine design.

Studies in non-human primates further support an important role for $\gamma\delta$ T cells in responding to *Mtb* infection and BCG vaccination. These studies may additionally provide insight into mechanisms driving immunity induced by $\gamma\delta$ T-cell expansion. Non-human primates serve as a useful model as they also express the V γ 9V δ 2 T-cell subset, which recognise *Mtb*, unlike murine $\gamma\delta$ T cells which do not recognise phosphoantigen or microbial antigens.¹⁵ Administration of an *Mtb* phosphoantigen analog combined with IL-2 expanded the V γ 9V δ 2 T-cell population during *Mtb* infection.²² Expanded V γ 9V δ 2 T cells differentiated into effector subpopulations, expressed cytokines such as IFN γ , perforin, granulysin and IL-12, and led to enhanced pulmonary responses of peptide-specific CD4⁺/CD8⁺ T cells.²² Importantly, diminished TB lesions and reduced *Mtb* proliferation were also observed, suggesting a role for expanded/differentiated V γ 9V δ 2 T cells in resistance to *Mtb* infection.²² In another approach, adoptive transfer of autologous V γ 9V δ 2 T cells 1 or 3 weeks after *Mtb* infection led to significant protection from *Mtb*, including a rapid recall-like increase in the pulmonary V γ 9V δ 2 T-cell subset, decreased *Mtb* infectious burdens (particularly in the lungs) and reduced pathology.²³ Following BCG vaccination, V γ 9V δ 2 T cells expanded as early as 4–6 days post-vaccination with peak levels at 3–5 weeks post-vaccination; this expansion further coincided with clearance of bacteraemia and immunity to fatal tuberculosis after challenge.²⁴ Finally, a prime-boost approach using

phosphoantigen followed by fusion proteins led to expansion of $\gamma\delta$ T cells displaying effector memory surface markers and producing cytokines such as IL-2, IL-6, IFN γ and TNF α following primary vaccination.²⁵ As these cells anergised following boosts whereas $\alpha\beta$ T cells expanded,²⁵ future studies could investigate whether anergy can be prevented and $\gamma\delta$ T-cell recall responses preserved. Together, the described studies in macaques provide evidence that $\gamma\delta$ T cells confer protection from symptomatic *Mtb* infection and support targeting these cells in vaccination approaches to *Mtb*.

The $\gamma\delta$ T-cell ontogeny is quite different in other mammals compared to humans and non-human primates; however, studies in cattle and pigs showed similar responses to those found in humans and macaques. Cattle and other ruminants express large proportions of $\gamma\delta$ T cells that decline with age, but remain high relative to human levels.^{26,27} In cattle, $\gamma\delta$ T cells rapidly proliferate following infection with *Mycobacterium bovis*^{28–30} or BCG vaccination.^{31,32} Similarly, in pigs, $\gamma\delta$ T cells proliferated following vaccination with BCG.³³

Other bacterial agents demonstrating $\gamma\delta$ T-cell expansion following infection and vaccination include *Leptospira borgpetersenii*, *Salmonella enterica*, *Francisella tularensis* and *Listeria monocytogenes*. Similarly to the described response to *Mtb*, human $\gamma\delta$ T-cell populations, in particular the V γ 9V δ 2 subset, expand following leptospirosis infection.^{34,35} In leptospirosis vaccination studies in cattle, IFN γ -producing $\gamma\delta$ T cells expressing the WC1 co-receptor expand post-vaccination and upon *in vitro* restimulation.^{36–38} $\gamma\delta$ T cells also expand following salmonella vaccination in chickens and macaques^{39,40} or following salmonella infection in humans.⁴¹ Furthermore, following salmonella or listeria vaccination in macaques, $\gamma\delta$ T cells displaying V γ 9V δ 2 were the major T-cell subset proliferating.^{40,42} Following subclinical *Listeria monocytogenes* infection, V γ 9V δ 2 T cells expanded, trafficked to the lungs and intestinal mucosa and evolved into effector cells producing IFN γ , TNF α , IL-4, IL-17 and/or perforin.⁴² These cells could then lyse infected target cells and inhibit intracellular bacterial growth, demonstrating a potential role in protection from listeria.⁴² Interestingly, $\gamma\delta$ T cells displaying V γ 9V δ 2 expanded in humans infected with *F. tularensis*,^{43,44} but did not expand following

vaccination, perhaps because of different phosphoantigens present.⁴³

In summary, a number of studies have not only demonstrated $\gamma\delta$ T-cell expansion in various bacterial infections, but also possible mechanisms of protection provided by this cell population, including both direct killing and recruitment of other cell types via production of pro-inflammatory cytokines. Although clear that $\gamma\delta$ T cells respond differently based on infectious agent, specific proliferation of the V γ 9V δ 2 subset in response to a number of bacterial pathogens correlates with protection from symptomatic disease. Consequently, upregulating activation and/or functional responses of this subset by vaccination may enhance protection against the agent targeted by immunisation. However, given the $\gamma\delta$ T-cell anergy observed in the described vaccine study combining phosphoantigen with a subunit anti-tuberculosis vaccine,²⁵ as well as prevalent examples of T-cell exhaustion in other contexts, further work is needed to assess potential mechanisms driving such processes. Timing of interventions could therefore be optimised to induce maximal $\gamma\delta$ T-cell recall responses and promote activation without causing exhaustion.

MALARIA INFECTION

In addition to long-standing evidence that $\gamma\delta$ T cells play a role in initial responses to parasitic infections, there is increasing evidence that $\gamma\delta$ T cells are important in vaccine-induced protection from malaria. Studies over the past few decades have shown that $\gamma\delta$ T cells (particularly the V δ 2⁺ subset) rapidly expand following infection with the most virulent human malaria parasite, *Plasmodium falciparum* (*Pf*), in children, malaria-naïve adults and malaria-experienced adults.^{45–48} Frequencies of $\gamma\delta$ T-cell subsets, including V δ 2⁺, V δ 2⁻, activated CD11c⁺ or CD16⁺/Tim-3⁺ $\gamma\delta$ T cells, have all been associated with malaria exposure.^{49–56} Higher frequencies and malaria-responsive cytokine production of V δ 2⁺ T cells correlate with protection against subsequent infection in children living in endemic settings,^{57,58} and *in vitro*, these cells perform cytotoxic, anti-parasitic functions.^{59,60} Furthermore, these cells can also act as antigen-presenting cells,^{61–64} which may further enhance the response to infection and/or vaccination. In malaria-naïve volunteers exposed to *Pf*-infected mosquitoes, while under chloroquine prophylaxis,

$\gamma\delta$ T cells expand after infection.⁶⁵ Elevated frequencies of $\gamma\delta$ T cells expressing effector memory surface markers and enhanced responsiveness to *Pf* stimulation persist for over 1 year following experimental infectious challenge.⁶⁵ A recent small study from the same group reported that vaccination with BCG changed the course of experimental malaria infection and that BCG vaccination was associated with altered innate immune activation (including $\gamma\delta$, NK and monocytes) following malaria challenge. Interestingly, expression of the activation marker CD69 on both NK cells and $\gamma\delta$ T cells was associated with reduced parasitaemia.⁶⁶ Trends towards increased degranulation and granzyme B production among $\gamma\delta$ T cells from BCG-vaccinated volunteers compared to unvaccinated were also observed.⁶⁶ Together, these results suggest an important role for $\gamma\delta$ T cells in mediating protective immunity to malaria.

Although there is not yet an effective vaccine for malaria, preliminary studies testing whole parasite vaccines in humans and mice suggest an important role for $\gamma\delta$ T cells in protection from subsequent infection. The malaria vaccine that has advanced farthest to date is the RTS,S vaccine, which is based on the *Pf* circumsporozoite (CSP) protein and targets the sporozoite and liver stages of infection. Interestingly, RTS,S phase 3 trials in African children detected no significant change in $\gamma\delta$ T-cell frequencies following vaccination and minimal cytokine production by these cells in response to *in vitro* CSP stimulation.⁶⁷ However, as the authors examined total $\gamma\delta$ T cells rather than V δ 2⁺ or other $\gamma\delta$ T-cell subsets, it will be important for future studies to determine whether specific subsets correlate with protection and if so, whether future RTS,S formulations can target these subsets. RTS,S trials in malaria-naïve populations have generally focused on anti-CSP antibody studies and CD4⁺/CD8⁺ T-cell responses without examining innate populations like $\gamma\delta$ T cells. One recent study utilising a systems approach identified natural killer (NK) cell signatures that correlated with and predicted protection,⁶⁸ suggesting that depending on the precise vaccine regimen, innate immune responses could be significant.

In contrast to RTS,S, vaccine formulations using sporozoites (the stage of the parasite injected by the mosquito into the human) have indicated a direct or indirect role for $\gamma\delta$ T cells in protection. In malaria-naïve individuals immunised with the

attenuated *Pf* sporozoite (PfSPZ) vaccine, $V\delta 2^+$ T cells expanded in a dose-dependent fashion and frequencies of these cells correlated with protection more significantly than any other cellular immune responses.^{69–71} Numbers of memory $V\delta 2^+$ T cells also correlated with protection in a recent PfSPZ trial in a malaria-endemic region in Mali.⁷² Finally, when malaria-naïve individuals were immunised with non-irradiated PfSPZ combined with chemoprophylaxis (PfSPZ-cVAC), the frequency of $V\delta 2^+$ T cells increased in a dose-dependent manner and memory $\gamma\delta$ T cells specifically increased expression of $IFN\gamma$ and the activation marker CD38.⁷³ Additional work is needed to further elucidate the mechanism of $V\delta 2^+$ T-cell-induced protection, as well as to determine whether frequencies of these cells could be used as a biomarker for protection in PfSPZ vaccinations in malaria-endemic regions.

In the mouse model, results have depended somewhat on the parasite strain used, but generally support $\gamma\delta$ T cells as a correlate of natural and vaccine-induced protection. In the lethal *Plasmodium berghei* ANKA model, $\gamma\delta$ T cells were not required to prevent infection upon blood-stage challenge following sporozoite vaccination, but did contribute to pre-erythrocytic immunity by recruiting dendritic cells and $CD8^+$ T cells.⁷² These cells may also be important in modulating functional T follicular helper (Tfh) cell and germinal centre B-cell responses.⁷⁴ In contrast to these indirect roles in protection, $\gamma\delta$ T cells appear to act as important effector cells following vaccination with nonlethal *Plasmodium yoelii* sporozoites.⁷⁵ Results from mice lacking $\alpha\beta$ T cells further suggest that $\gamma\delta$ T-cell cytotoxicity may become more effective after interaction with $CD4^+$ T cells.⁷⁵ Mice lacking $\gamma\delta$ T cells further reveal that these cells may be particularly important in immunity targeting the liver stages of the parasite (before it enters the bloodstream).⁷⁶ Clearly, it will be important to evaluate whether these differing results between murine parasite strains are solely because of differences in the type of immunity induced (i.e. *P. berghei*-irradiated sporozoite vaccination induces sterile immunity, while *P. yoelii* vaccination does not). Interestingly, a vaccine using whole lysate of the promastigote stage of a related parasite, *Leishmania amazonensis*, led to protection against subsequent infection that was dependent on the presence of $\gamma\delta$ T cells.⁷⁷ The

mechanisms driving this protection and implications for malaria vaccines, however, are unknown.

In sum, results from vaccination studies targeting malaria (and potentially other parasitic infections such as leishmaniasis) strongly suggest that $\gamma\delta$ T cells play an important role in protection from future infection. However, future work is required to definitively show that $\gamma\delta$ T cells directly mediate protection rather than act as a biomarker of infection, as well as to determine the mechanism of protection and the role of $V\delta 2^-$ subsets (if any). In particular, it will be important to assess whether protection is mediated via direct $\gamma\delta$ T-cell cytotoxicity and/or more indirect effects such as antigen presentation, recruitment of other cell types, or stimulation of functional Tfh cells and antibodies. Given that most malaria vaccines in trials, including the leading RTS,S vaccine, use specific antigens rather than whole sporozoites, vaccine effectiveness may be improved by the addition of an adjuvant or other vaccine component that stimulates $\gamma\delta$ T-cell responses. BCG vaccination may be a potential approach based on recent results of increased activation of innate cell populations following CHMI in BCG-vaccinated individuals;⁶⁶ however, given that this response only occurred in half of the vaccinated volunteers and the sample size was small, further study is warranted.

VIRAL INFECTIONS

There is evidence that $\gamma\delta$ T cells may play a role in response to viral infections, including influenza virus, HIV and cytomegalovirus (CMV), and that they can directly kill virally infected cells. There is also evidence that these cells can expand *in vivo* in response to bisphosphonate stimulation and viral vaccination strategies and may contribute to improved outcomes, thereby raising the possibility that these cells could be targeted to play an important role in vaccine-mediated protection.

Regarding influenza, several studies have shown that phosphoantigen or pamidronate-activated $\gamma\delta$ T cells are capable of inhibiting virus replication by killing influenza-infected macrophages⁷⁸ and/or lung alveolar epithelial cells.⁷⁹ Phosphoantigen-activated cells also have non-cytolytic activities in response to pandemic H1N1, producing $IFN\gamma$ and expressing inflammatory chemokines.⁸⁰ Relatedly, it was also recently shown that $V\gamma 9V\delta 2$ T cells can promote $CD4^+$ T follicular helper cell

differentiation, B-cell class switching and influenza virus-specific antibody production in an *in vitro* co-culture assay,⁸¹ suggesting that these cells may provide both a direct cytotoxic and potential synergistic role in the adaptive immune response to influenza.

Although both inactivated and live attenuated influenza vaccine reduce influenza illness and disease complications, live attenuated influenza vaccine has been shown to have superior efficacy in children.⁸² Influenza-responsive $\gamma\delta$ T cells were found to expand following live attenuated, but not inactivated, influenza vaccination,^{83,84} suggesting a potential immunologic correlate for this observation. Despite not proliferating after vaccination, $\gamma\delta$ T cells in elderly individuals receiving the inactivated vaccine did increase perforin production and, after *in vitro* restimulation, proliferated and produced IFN γ and IL-4.⁸⁴ Similarly, the $\gamma\delta$ T-cell response in the nasal mucosa was attenuated in cigarette smokers relative to non-smokers,⁸⁵ suggesting these cells may represent a correlate for why smokers respond less well to influenza vaccination. In a murine model of influenza, $\gamma\delta$ T cells significantly expand in bronchial alveolar fluid following infection,⁸⁶ and in a humanised mouse model, pamidronate administration to mice reconstituted with human PBMC reduced disease severity and mortality following H1N1 and H5N1 influenza infection. However, pamidronate had no effect in mice reconstituted with V δ 2⁻depleted cells.⁸⁷ Together, these studies suggest that $\gamma\delta$ T cells may not only represent an immunologic correlate of protection from influenza infection and vaccination, but that they might also be a mediator of protection.

Regarding HIV, it has long been known that both the V δ 1⁺ and V δ 2⁺ subsets of $\gamma\delta$ T cells have cytotoxic capacity against HIV⁸⁸⁻⁹⁰ and can inhibit viral replication *in vitro*. HIV-infected elite controllers have elevated levels of V δ 2⁺ T cells compared with HIV-negative controls or HIV-infected individuals on antiretroviral therapy,^{91,92} suggesting a potential role for these cells in inhibiting viral replication *in vivo*. $\gamma\delta$ T cells may also play a role in controlling viral infection at mucosal barriers. A recent study reported that higher levels of pro-inflammatory V δ 1⁺ T cells correlated with lower gut-associated HIV viral load,⁹³ and another study in rhesus macaques found that levels of CD8⁺ V δ 2⁺ T cells in the female reproductive tract correlated with lower

SIV viral loads.⁹⁴ V δ 1⁺ T cells expanding in HIV-infected individuals may also protect from other infections. For example, V δ 1⁺ T cells producing IFN γ and IL-17A responded to *Candida albicans*⁹⁵ and further expanded upon influenza vaccination combined with the MF59 adjuvant.⁹⁶

Individuals with chronic HIV infection have been found to have V δ 2⁺ T-cell depletion and dysfunction in response to phosphoantigenic stimulation.⁹⁷ It is possible, however, that some of these cells are not dysfunctional but rather have different functions. For example, He *et al.* identified a population of CD16⁺ V δ 2⁺ T cells that had decreased responses to phosphoantigens but increased capacity for antibody-dependent cellular cytotoxicity (ADCC). A decline in this population was associated with faster disease progression, while no decline was observed in individuals with controlled infection.⁹⁸ Administration of zoledronic acid with IL-2 in HIV-infected, antiretroviral naïve patients was associated with V δ 2⁺ T-cell expansion, dendritic cell activation and increased HIV-specific CD8⁺ T-cell responses.⁹⁹ It was also recently shown that $\gamma\delta$ T cells can be isolated from antiretroviral suppressed, HIV-infected individuals and that these cells can kill autologous HIV-infected CD4⁺ T cells. In addition, these cells could expand *ex vivo* following pamidronate stimulation and could significantly reduce viral replication, suggesting a potential role for these cells to clear HIV infection from latent reservoirs.¹⁰⁰

Even though HIV vaccine trials to date have not investigated any changes in $\gamma\delta$ T-cell populations, an intriguing study looked at canarypox as a vector for HIV antigens and, after *in vitro* expansion, identified a V γ 9⁺ population (specific for canarypox, not HIV antigens) that produced IFN γ .¹⁰¹ These results suggest that in addition to adjuvants, vaccine vectors could be used to target $\gamma\delta$ T-cell responses.

Finally, in the context of CMV infection, oligoclonal $\gamma\delta$ (primarily V δ 2⁻) T cells expand and differentiate into effector/memory cells.¹⁰²⁻¹⁰⁵ Expansion of V δ 2⁻ T cells is associated with viral clearance both in immunosuppressed^{102,106,107} and in healthy populations.^{102,107} These cells likely contribute to viral clearance via effector functions such as cytotoxicity and IFN γ /TNF α production,¹⁰⁸ 'antibody-dependent cell-mediated inhibition',¹⁰⁹ and enhanced cytotoxicity via sensing of IL-18 from virus-infected cells.¹¹⁰ During secondary infection, cells proliferate and resolve infection

Table 1. Human γδ T-cell responses to bacterial, protozoan and viral infections and corresponding vaccinations

Author, year	Agent	Cohort	γδ T-cell subset	Impact of infection/vaccination on γδ T-cell activation	Associations between γδ T-cell features and function/clinical outcomes
Bacterial Barnes et al. 1992 ⁶	<i>Mycobacterium tuberculosis</i> (<i>Mtb</i>)	Adults with tuberculous infection	All γδ	Strong correlation between expansion of γδ T cells and <i>Mtb</i>	<i>Mtb</i> -reactive γδ T cells produced IFNγ, GM-CSF, IL-3 and TNFα; secretion of macrophage-activating cytokines may contribute to resistance against mycobacterial infection
Dieli et al. 2001 ¹²	<i>Mtb</i>	PPD-positive adults	Vγ9Vδ2		Vγ9Vδ2 T lymphocytes efficiently kill extracellular and intracellular <i>Mtb</i> through release of granulysin and perforin
Spencer et al. 2013 ¹³	<i>Mtb</i>	PPD-positive, HIV-negative adults	Vγ9Vδ2		Infected macrophages co-cultured with γδ T cells produced TNFα and inhibited intracellular mycobacterial growth
Hoft et al. 1998 ²⁰	Bacille Calmette–Guérin (BCG)	Adults	All γδ	γδ T-cell expansion after vaccination; memory-like immune responses after <i>in vitro</i> restimulation	Enhanced responsiveness after BCG vaccination suggests that γδ T cells are important to secondary immune response
Mazzola et al. 2007 ¹⁷	BCG	Infants	All γδ	Remarkable expansion of γδ T cells in response to vaccination	
Tastan et al. 2005 ¹⁸	BCG	Infants	All γδ	Significant increase in γδ T cells following vaccination at birth	
Zufferey et al. 2013 ¹⁹	BCG	Adults, children and infants	All γδVδ2 ⁺	γδ T cells (particularly Vδ2 ⁺ subset) from infants and children immunised with BCG expand after <i>in vitro</i> restimulation	Vδ2 ⁺ T cells produce IFNγ following BCG vaccination
Barry et al. 2006 ³⁴	Unknown <i>Leptospira</i> species	Adult case study	All γδ	Patient had an almost tenfold increase of γδ T cells above baseline following infection	γδ T-cell expansion parallels patient's symptoms; unable to determine whether γδ T cells play role in resolution of or exacerbation of symptomatic disease
Klimpel et al. 2003 ³⁵	<i>Leptospira interrogans</i>	Adults	All γδ	Preferential <i>in vitro</i> expansion of TCR+ γδ T cells in cultures exposed to high numbers of <i>Leptospira</i>	
Workalemahu et al. 2014 ⁴⁰	lytB-aroA- <i>Salmonella enterica</i> serovar Typhimurium SL7207	Adults	Vγ9Vδ2	LytB negative vaccines stimulated large <i>ex vivo</i> expansions of Vγ9Vδ2 T cells from human donors	
Poquet et al. 1998 ⁴³	<i>Francisella tularensis</i> and <i>F. tularensis</i> live vaccine strain (LVS)	Adults	Vγ9Vδ2	Massive increase in Vγ9Vδ2 T cells during infection; minor or no increase in Vγ9Vδ2 T cells after live strain vaccination	
Protozoan Ho et al. 1990 ⁴⁵	<i>Plasmodium falciparum</i> (Pf)	Individuals (age not reported) with acute infection	All γδ	γδ T cells expand after infection and remain elevated for at least 4 weeks	

(Continued)

Table 1. Continued.

Author, year	Agent	Cohort	γδ T-cell subset	Impact of infection/vaccination on γδ T-cell activation	Associations between γδ T-cell features and function/clinical outcomes
Roussilhon et al. 1994 ⁴⁸	<i>Pf</i>	Malaria-naïve adults with acute infection	All γδ	γδ T cells expand and remain elevated for months; subset proliferates <i>in vitro</i> in response to <i>Pf</i> schizont extract	
Hviid et al. 2001 ⁴⁶	<i>Pf</i>	Children with acute infection	Vδ1 ⁺	Vδ1 ⁺ T cells increase after treatment	Expanded Vδ1 ⁺ T cells produce pro-inflammatory cytokines
D'Ombain et al. 2008 ⁵⁷	<i>Pf</i>	Children in malaria-endemic region	All γδ		Production of IFNγ following <i>in vitro Pf</i> stimulation associated with immunity to symptomatic infection
Cairo et al. 2014 ⁵¹	<i>Pf</i>	Neonates in malaria-endemic region	Vδ2 ⁺	Neonates exposed to placental malaria had increased proportions of central memory Vγ2Vδ2 cells in cord blood	
Jaganathan et al. 2014 ⁵⁴	<i>Pf</i>	Children in malaria-endemic region	Vδ2 ⁺	Repeated infection associated with loss and dysfunction of Vδ2 ⁺ cells, including increased expression of immunoregulatory genes (Tim3, CD57, CD16)	Loss and dysfunction of pro-inflammatory Vδ2 ⁺ cells associated with clinical tolerance to infection
Farrington et al. 2016 ⁵²	<i>Pf</i>	Children in malaria-endemic region	Vδ2 ⁺	High prior malaria exposure leads to increased CD16 expression on Vδ2 ⁺ T cells	High prior malaria exposure leads to lower Vδ2 ⁺ T-cell functional responses; antimalarial chemoprevention associated with enhanced Vδ2 ⁺ cytokine production
Jaganathan et al. 2017 ⁵⁸	<i>Pf</i>	Children in malaria-endemic region	Vδ2 ⁺	Repeated infection associated with loss and dysfunction of Vδ2 ⁺ cells, including reduced proliferation	Higher pro-inflammatory cytokine production associated with protection from subsequent infection and increased odds of symptoms once infected
Schofield et al. 2017 ⁵⁵	<i>Pf</i>	Children in malaria-endemic region	All γδ	Tim-3 upregulated on γδ T cells following acute infection; frequency of Tim-3 ⁺ γδ T cells higher among malaria-exposed individuals compared to healthy controls	Individuals with asymptomatic malaria infection have higher proportions of Tim-3 ⁺ γδ T cells
Taniguchi et al. 2017 ⁵⁶	<i>Pf</i>	Adults and children with uncomplicated malaria	Non-Vδ2	Non-Vδ2 T cells expand during infection	Non-Vδ2 T cells produce IL-10 and IFNγ
Bediako et al. 2019 ⁴⁹	<i>Pf</i>	Malaria-exposed adults	All γδ	CD11c ⁺ γδ T cells expanded in individuals with high numbers of malaria episodes and distinguished between high vs. low malaria episode groups	
Teirlinck et al. 2011 ⁶⁵	Controlled human malaria infection (CHMI) [†] chemoprophylaxis	Malaria-naïve adults	All γδ	γδ T cells express effector memory phenotype	γδ T cells produce IFNγ even a year after infection

(Continued)

Table 1. Continued.

Author, year	Agent	Cohort	$\gamma\delta$ T-cell subset	Impact of infection/vaccination on $\gamma\delta$ T-cell activation	Associations between $\gamma\delta$ T-cell features and function/clinical outcomes
Seder et al. 2013 ⁷¹	Attenuated PfSPZ vaccination	Malaria-naïve adults	All $\gamma\delta$	$\gamma\delta$ T cells expanded following vaccination	Higher frequencies of $\gamma\delta$ T cells correlate with protection after controlled human malaria infection
Ishizuka et al. 2016 ⁶⁹	Attenuated PfSPZ vaccination	Malaria-naïve adults	V δ 2 ⁺	$\gamma\delta$ T cells expanded following immunisation	Higher frequencies of $\gamma\delta$ T cells correlate with protection after controlled human malaria infection
Mordmuller et al. 2017 ⁷³	Non-irradiated PfSPZ vaccination + chemoprophylaxis	Malaria-naïve adults	All $\gamma\delta$ /V γ 9V δ 2	Dose-dependent increase in the frequency of circulating $\gamma\delta$ T cells (primarily the V γ 9V δ 2 subset)	Memory $\gamma\delta$ T cells increase IFN γ secretion and expression of the activation marker CD38 post-vaccination
Zaidi et al. 2017 ⁷²	Irradiated PfSPZ vaccination	Malaria-exposed adults	All $\gamma\delta$ /V δ 2 ⁺	V δ 2 ⁺ T cells expanded following vaccination	V δ 2 ⁺ T cells significantly elevated among vaccinated individuals who remained uninfected during transmission season; number of memory V δ 2 ⁺ T cells associated with protection
Walk et al. 2019 ⁶⁶	CHMI following BCG vaccination	Malaria-naïve adults	All $\gamma\delta$	In half the BCG-vaccinated individuals, CD69-expressing $\gamma\delta$ T cells expanded	Trends towards increased degranulation and granzyme B production among $\gamma\delta$ T cells from BCG-vaccinated volunteers compared to unvaccinated
Viral Fenoglio et al. 2011 ⁹⁶	Influenza virus vaccination with MF59 adjuvant	HIV-positive and HIV-negative adults	V δ 1 ⁺	<i>In vivo</i> expansion of V δ 1 ⁺ $\gamma\delta$ T cells in HIV+ individuals following vaccination	Expanded population produces anti-fungal cytokines (may contribute to defence against opportunistic infections by compensating for impairment of CD4 ⁺ T cells)
Hoft et al. 2011 ⁸³	Live attenuated influenza vaccine (LAIV) and inactivated influenza vaccine (TIV)	Children	All $\gamma\delta$	$\gamma\delta$ T cells induced by LAIV, but not TIV	$\gamma\delta$ T cells induced by vaccination with LAIV develop memory responses and inhibit viral replication
Honvath et al. 2012 ⁸⁵	LAIV	Adult smokers and non-smokers	All $\gamma\delta$	$\gamma\delta$ T cells migrate to the lung following influenza infection in response to chemokines; cell population with characteristics of $\gamma\delta$ T cells increases following LAIV vaccination	
Re et al. 2006 ⁸⁴	Trivalent TIV	Elderly individuals	All $\gamma\delta$	Proliferative capacity of $\gamma\delta$ T cells decreased and number of differentiated $\gamma\delta$ T cells with effector/memory functions increased following vaccination	$\gamma\delta$ T cells showed increased production of perforins after vaccination

(Continued)

Table 1. Continued.

Author, year	Agent	Cohort	γδ T-cell subset	Impact of infection/vaccination on γδ T-cell activation	Associations between γδ T-cell features and function/clinical outcomes
Fausther-Bovendo et al. 2008 ⁸⁹	Human Immunodeficiency Virus (HIV)	HIV-1-infected adults	Vδ1 ⁺	Expansion of Vδ1 ⁺ T cells in individuals with HIV infection	Strong cytolytic capacities of Vδ1 ⁺ NKG2C ⁺ T cells against HIV-infected CD4 T cells
Garrido et al. 2018 ¹⁰⁰	HIV	ART-suppressed HIV-infected adult men	All γδ	Vδ2 ⁺ T cells expanded up to 120-fold in response to PAMML-2 <i>ex vivo</i>	γδ T cells are capable of eliminating HIV-infected targets and reduced viral replication up to 80%
He et al. 2013 ⁹⁸	HIV	HIV-positive and HIV-negative adults	Vγ9Vδ2	CD16 ⁺ and CD16 ⁺ Vδ2 ⁺ T-cell subsets performed different functions in response to various stimuli	Potential for CD16 ⁺ Vδ2 ⁺ cells to control HIV infection via antibody-dependent cell-mediated cytotoxicity
Riedel et al. 2009 ⁹¹	HIV	HIV-1-infected adults that are natural viral suppressors (NVS)	Vγ9Vδ2	Depletion of Vγ9Vδ2 T cells occurs early in HIV disease; NVS patients demonstrated an increased number of Vγ9Vδ2 T cells	
Wallace et al. 1996 ⁸⁸	HIV	Age not reported	All γδ	Increased numbers of γδ T cells in HIV-1-infected individuals	Anti-HIV responses in a large proportion of Vγ9Vδ2 T cells may help explain the phenomenon of HIV exposure without infection
Worku et al. 2001 ¹⁰¹	Canarypox ALVAC-HIV vCP205 and rgp120	Adults	All γδ	Induction of γδ T cells specific for canarypox (not HIV) antigens following vaccination	Expanded Vγ9 ⁺ γδ T cells produce IFNγ
Lafarge et al. 2001 ¹⁰⁶	Cytomegalovirus (CMV)	Renal transplant patients	All γδ		Patients with γδ T-cell expansion > 45 days after transplant had more severe symptoms than patients with early γδ T-cell expansion; CMV infection resolves following γδ T-cell expansion
Halary et al. 2005 ¹⁰⁸	CMV	Renal- and lung-transplanted patients with CMV	All γδVδ2 ⁻	Vδ2 ⁻ T cells express receptors involved in intestinal homing	Numerous Vδ1 ⁺ , Vδ3 ⁺ and Vδ5 ⁺ patient clones express TNFα, kill CMV-infected targets and limit CMV growth <i>in vitro</i> ; high frequency of these cells induce CD107a expression in the presence of CMV-infected cells
Pitard et al. 2008 ¹⁰²	CMV	Renal transplant patients with CMV and healthy adult donors (CMV seropositive/seronegative)	Vδ2 ⁻	Vδ2 ⁻ T cells expand and show effector/memory phenotype in transplanted patients and CMV+ healthy donors	Vδ2 ⁻ T cells from transplanted patients/CMV+ healthy donors show increased cytotoxicity in response to CMV <i>in vitro</i> ; secondary response to CMV associated with a faster γδ T-cell expansion and better resolution of infection compared to primary response
Knight et al. 2010 ¹⁰⁷	CMV	Allogeneic stem cell transplant patients and healthy adult donors (CMV+/-)	All γδVδ2 ⁻	Long-term expansion of Vδ2 ⁻ (not Vδ2 ⁺) T cells in transplant patients with CMV reactivation and in CMV+ healthy donors; restricted clonality	Vδ2 ⁻ T cells from CMV+ healthy donors and from a recipient of a graft from a CMV+ donor lysed CMV-infected cells <i>in vitro</i>

(Continued)

Table 1. Continued.

Author, year	Agent	Cohort	$\gamma\delta$ T-cell subset	Impact of infection/vaccination on $\gamma\delta$ T-cell activation	Associations between $\gamma\delta$ T-cell features and function/clinical outcomes
Couzi et al. 2012 ¹⁰⁹	CMV	Kidney transplant patients and healthy donors	All $\gamma\delta/V\delta 2^-$	High expression of CD16 on $V\delta 2^-$ T cells from CMV+ individuals	$CD16^+$ $\gamma\delta$ T cells did not mediate ADCC against CMV-infected cells but produced IFN γ when incubated with IgG-opsonised virions and inhibited CMV multiplication <i>in vitro</i>
Roux et al. 2013 ¹⁰⁴	CMV	Adults from various age groups, pregnant women with primary infection, lung-transplanted patients with primary or chronic infection	All $\gamma\delta$	CMV seropositivity leads to accumulation of highly differentiated $V\delta 2^-$ (but not $V\delta 2^{+1}$) T cells; highest CD38 expression on $\gamma\delta$ T cells from individuals with primary infection compared to chronic infection or no infection	
Alejenef et al. 2014 ¹⁰³	CMV	Healthy adults and 2 immunocompromised individuals with symptomatic primary infection	$V\delta 2^-$	Highly differentiated effector memory $V\delta 2^-$ $\gamma\delta$ T cells significantly increased in CMV+ healthy individuals compared to CMV- controls in all age groups	$V\delta 2^-$ T cells from CMV+ individuals contained higher levels of intracellular perforin and granzyme than CMV- individuals; $V\delta 2^-$ T cells do not immediately produce IFN γ /TNF α /CD107a following <i>ex vivo</i> incubation with CMV-infected cells but do demonstrate effector functions after short-term culture
Kallemijn et al. 2017 ¹¹³	CMV	Healthy adults	All $\gamma\delta$	CMV associated with higher frequencies of $\gamma\delta$ T cells with effector/memory and exhausted phenotypes	
Lee et al. 2017 ¹⁰⁵	CMV	Renal transplant patients several years post-transplant and healthy donors	All $\gamma\delta/V\delta 2^-$	Percentages of $V\delta 2^-$ T cells higher in CMV+ transplant patients and correlated with CMV antibody levels; $V\delta 2^-$ T cells skewed towards terminally differentiated phenotype; many $V\delta 2^-$ T cells in CMV+ individuals express CD8	Expression of CD107a and production of IFN γ by $V\delta 2^+$ and $V\delta 2^-$ $\gamma\delta$ T cells in response to staphylococcal enterotoxin B was not altered by CMV

faster, suggesting a memory-like phenotype.¹⁰² Several studies in mice have shown that (1) $\gamma\delta$ T cells are capable of protecting $\alpha\beta$ T-cell-deficient mice against CMV-induced pathology and (2) adoptive transfer of CMV-induced $\gamma\delta$ T cells provides long-term protection in immunodeficient mice.^{111,112} These results suggest that $\gamma\delta$ T cells are important mediators of protection against CMV and support approaches using adoptive transfer of effector/memory $\gamma\delta$ T cells or targeting $\gamma\delta$ T cells in future CMV vaccine trials. The possibility of inducing exhausted $\gamma\delta$ T cells would need to be considered, however, as CMV infection has both been shown to result in higher numbers of these cells.¹¹³

In sum, results from *in vitro* and natural infection studies suggest an important role for $\gamma\delta$ T cells in controlling influenza, HIV and CMV viral replication. Targeting $\gamma\delta$ T cells through stimulation could provide an important adjuvant-type role in vaccination and/or cure-related strategies for viral infections.

CONCLUSIONS

Across the different bacterial, protozoan and viral infections examined (summarised in Table 1), there are clear patterns of $\gamma\delta$ T-cell expansion, particularly of the $V\delta 2^+$ subset, in response to both infection and vaccination. In several contexts, including infection with *Mtb*, malaria, influenza and HIV and vaccination with BCG, PfSPZ and live attenuated influenza, $\gamma\delta$ T cells are associated with protection. Further, evidence so far supports a role for $\gamma\delta$ T cells in mediating protection via direct killing and other mechanisms. Studies in animal models, such as BCG vaccination in macaques and PfSPZ vaccination in mice, are beginning to shed light on direct mechanisms of protection vs. stimulation of other immune cells that mediate protection. Clearly, future work is needed to further elucidate these mechanisms, as well as the host and infection-mediated factors that influence responsiveness of $\gamma\delta$ T cells and the relevant differences between responses to natural infection compared to response to vaccination. As new vaccine formulations targeting these diseases progress through development, the question of whether to induce $\gamma\delta$ T cells or $\gamma\delta$ T-cell subsets will become an important consideration. In fact, this approach is already being implemented in cancer, whether via administration of $V\gamma 9V\delta 2$ T-cell agonists¹¹⁴ or using BCG to stimulate $V\gamma 9V\delta 2$ T

cells as treatment for bladder cancer.^{115,116} Approaches incorporating $\gamma\delta$ T cells into strategies targeting B- or T-cell responses have also been promising so far. For example, as previously mentioned, a study testing a subunit tuberculosis vaccine combined with phosphoantigen observed a robust $\gamma\delta$ T-cell response, including expression of effector memory markers, following primary vaccination.²⁵ Finally, another intriguing approach is to expand functional $\gamma\delta$ T cells *ex vivo*, as has been tested with effector cells capable of inhibiting HIV replication¹⁰⁰ and *Mtb* infection.²³

To maximise functional responses in future similar studies, it will be important to improve our understanding of the timing of $\gamma\delta$ T-cell vs. $\alpha\beta$ T-cell responses following vaccination, as well as any potential negative effects of overstimulation of $\gamma\delta$ T cells. As specific subsets of $\gamma\delta$ T cells that correlate with protection in different contexts are identified, optimisation of methods to specifically target these subsets will be beneficial. Especially given the hypothetical possibility of $\gamma\delta$ T-cell anergy/exhaustion, it will be essential to define responses that optimally stimulate and antigens/agonists that best elicit that response. Altogether, as development of vaccines targeting infectious diseases that have long proved elusive becomes more of a reality, it will be important to broaden our perspective beyond targeting antibody-driven or T-cell responses and to intentionally target innate cells, such as $\gamma\delta$ T cells.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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