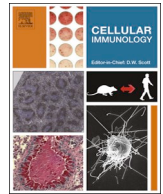




Elsevier has created a [Monkeypox Information Center](#) in response to the declared public health emergency of international concern, with free information in English on the monkeypox virus. The Monkeypox Information Center is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its monkeypox related research that is available on the Monkeypox Information Center - including this research content - immediately available in publicly funded repositories, with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the Monkeypox Information Center remains active.



## Review article

Human and murine memory  $\gamma\delta$  T cells: Evidence for acquired immune memory in bacterial and viral infections and autoimmunityKevin Comeau<sup>a</sup>, Pierre Paradis<sup>a</sup>, Ernesto L. Schiffrin<sup>a,b,\*</sup><sup>a</sup> Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, 3755 Côte-Ste-Catherine Rd., Montreal, Quebec H3T 1E2, Canada<sup>b</sup> Department of Medicine, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, 3755 Côte-Ste-Catherine Rd., Montreal, Quebec H3T 1E2, Canada

## ARTICLE INFO

## Keywords:

Gamma delta T cell  
Immune memory  
Adaptive immunity  
Infections  
Autoimmunity

## ABSTRACT

$\gamma\delta$  T cells are unconventional lymphocytes that could play a role in bridging the innate and adaptive immune system. Upon initial exposure to an antigen, some activated T cells become memory T cells that could be re-activated upon secondary immune challenge. Recently, subsets of  $\gamma\delta$  T cells with a restricted antigen repertoire and long-term persistence have been observed after clearance of viral and bacterial infections. These  $\gamma\delta$  T cells possess the hallmark ability of memory T cells to respond more strongly and proliferate to a higher extent upon secondary infection. Murine and primate models of *Listeria monocytogenes* and cytomegalovirus infection display these memory hallmarks and demonstrate  $\gamma\delta$  T cell memory responses. In addition, human and non-human primate infections with *Mycobacterium tuberculosis*, as well as non-human primate infection with monkeypox and studies on patients suffering from autoimmune disease (rheumatoid arthritis and multiple sclerosis) reveal memory-like responses corresponding with disease. Murine models of psoriatic disease (imiquimod) and parasite infections (malaria) exhibited shifts to memory phenotypes with repeated immune challenge. These studies provide strong support for the formation of immune memory in  $\gamma\delta$  T cells, and memory  $\gamma\delta$  T cells may have a widespread role in protective immunity and autoimmunity.

## 1. Introduction

Gamma delta ( $\gamma\delta$ ) T cells are a relatively small subset of unconventional lymphocytes which possess both innate and adaptive characteristics, bridging these two arms of the immune system [1]. Compared to traditional CD4+ and CD8+ alpha beta ( $\alpha\beta$ ) T cells,  $\gamma\delta$  T cells bear innate-like characteristics such as non-major histocompatibility complex (MHC) restricted antigen recognition similar to Natural Killer cells, and an ability to attack cells and microbes directly with their cytotoxic activity [2,3].  $\gamma\delta$  T cells can be found circulating in the blood and lymphoid organs, or as resident cells in peripheral tissues and barrier surfaces such as the gut ( $\gamma\delta$  intraepithelial lymphocytes, IELs)

and skin ( $\gamma\delta$  dendritic epidermal T cells, DETCs) [4].  $\gamma\delta$  T cells possess a unique ability to also act as antigen presenting cells (APCs) to  $\alpha\beta$  T cells in the context of tumour antigens and microbial peptides [5,6]. Additionally, human  $\gamma\delta$  T cells are effective at cross presentation of antigens to CD8+ T cells; surprisingly,  $\gamma\delta$  T cells can be even more effective than monocyte-derived dendritic cells (DCs) at cross-presentation *in vitro* [6]. Mouse  $\gamma\delta$  T cells have been noted to express MHC II and co-stimulatory molecules after activation *in vitro* with anti-CD3 and anti-CD28 antibodies, showing further APC capabilities [7]. Murine and human  $\gamma\delta$  T cells can act as a first defense while also influencing the behavior of their  $\alpha\beta$  T cell counterparts in infection and disease.

While  $\gamma\delta$  T cells are considered innate-like and have several innate

**Abbreviations:** APC, Antigen presenting cell; BCG, Bacille Calmette-Guerin; CCR7, C-C chemokine receptor type 7; CD, Cluster of differentiation; CMV, Cytomegalovirus; DC, Dendritic cell; DETC, Dendritic epidermal T cell; EYFP, Enhanced yellow fluorescent protein; HC, Healthy control; HLA, Human leukocyte antigen; HMBPP, Hydroxy-3-methyl-but-2-enyl pyrophosphate; HSP, Heat-shock protein; IEL, Intraepithelial lymphocyte; IFN, Interferon; IL, Interleukin; IMQ, Imiquimod; IPP, Isopentyl pyrophosphate; LM, *Listeria Monocytogenes*; MCMV, Murine cytomegalovirus; MHC, Major histocompatibility complex; MICA, MHC class I polypeptide-related sequence A; mLN, Mesenteric lymph node; MPV, Monkeypox virus; MS, Multiple sclerosis; NHP, Non-human primate; NKR, Natural killer receptor; PBMC, Peripheral blood mononuclear cell; PE, Phycocerythrin; pLN, Peripheral lymph node; RA, Rheumatoid arthritis; RRMS, Relapsing-remitting multiple sclerosis; SLO, Secondary lymphoid organ; T<sub>CM</sub>, Central memory T cell; TCR, T cell receptor; T<sub>EFF</sub>, Effector T cell; T<sub>EM</sub>, Effector memory T cell; T<sub>EMRA</sub>, T effector memory re-expressing CD45RA; TLR, Toll-like receptor; TNF, Tumor necrosis factor; T<sub>RM</sub>, Resident memory T cell

\* Corresponding author at: Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, 3755 Côte-Ste-Catherine Rd., Montreal, Quebec H3T 1E2, Canada.

E-mail address: [ernesto.schiffrin@mcgill.ca](mailto:ernesto.schiffrin@mcgill.ca) (E.L. Schiffrin).

<https://doi.org/10.1016/j.cellimm.2020.104217>

Received 16 July 2020; Received in revised form 27 August 2020; Accepted 11 September 2020

Available online 16 September 2020

0008-8749/© 2020 Published by Elsevier Inc.

characteristics, they are adaptive immune lymphocytes which have T cell receptors (TCRs) that undergo V(D)J recombination similar to their  $\alpha\beta$  counterparts. However, only a small fraction of putative  $\gamma\delta$  TCR ligands have been discovered [8]. It is generally believed that conventional and superantigens which activate  $\alpha\beta$  TCRs do not activate  $\gamma\delta$  T cells [9]. Using a combination of the  $\gamma\delta$  TCR, toll-like receptors (TLRs), and natural killer receptors (NKR),  $\gamma\delta$  T cells can recognize self-proteins such as classical and non-classical MHC molecules, like MHC class I polypeptide-related sequence A (MICA), and CD1c, as well as pyrophosphate-containing small molecules (termed phosphoantigens), heat-shock proteins (HSPs), and lipids (via CD1) [9,10]. Some examples of these small non-peptide phosphoantigens include mevalonate-derived isopentenyl pyrophosphate (IPP) associated with infected and transformed cells, as well as (*E*)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) produced by pathogenic bacteria and parasites [11]. IPP and HMBPP can stimulate the  $\gamma\delta$  TCR directly, leading to TCR-dependant activation and a rapid cytotoxic response [12]. Interestingly, enhanced diversity of  $\gamma\delta$  TCR V-J and V-D-J junctional regions has been observed in  $\gamma\delta$  T cells collected from the intestinal epithelium, as compared to  $\gamma\delta$  T cells collected from the thymus, lymph nodes, and epidermis [13]. This finding further supports the role for recognition of non-self-molecules by the  $\gamma\delta$  TCR, as the gut is continually exposed to luminal bacterial and fungal antigens, which could be a reason for this increased TCR junctional diversity. Consequently,  $\gamma\delta$  T cells can recognize a wide selection of both self (stress signals) and non-self (bacterial, viral, parasitic) antigens, and respond accordingly.

With such a diverse repertoire of recognized antigens and a lack of APC antigen processing required for recognition, it comes as no surprise that  $\gamma\delta$  T cells have been implicated in infection, immunity, and autoimmunity since their discovery. Knockout and adoptive transfer experiments with  $\gamma\delta$  T cells have revealed a substantial protective role in models of infection, including animal models of *Mycobacterium tuberculosis*, *Listeria monocytogenes*, and influenza viruses [11]. An important role for  $\gamma\delta$  T cells also occurs in autoimmunity, where they have been noted to contribute to the pathogenesis of Type 1 diabetes, rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus; although, their exact role is not yet clear in most conditions [14]. Keeping this in mind, another hallmark of the adaptive immune system is forming long-lasting immune memory T cells. Memory T cells are antigen experienced T cells residing in lymphoid organs, the circulation, and peripheral tissues, which possess a lower activation threshold and respond faster and more effectively upon re-exposure with their cognate antigen [15]. After T cells mount a response upon antigenic stimulation, some effector cells will further differentiate into long-lived memory T cells. Memory T cells can provide different signals than their naïve and effector counterparts and proliferate faster in response to antigen exposure. All of this leading to more effective host protection. Evidence also suggests that cytokines and chemokines play a crucial role in TCR activation along with cognate antigen recognition in a memory recall response [16].

Naïve, effector, and memory T cells have distinct DNA methylation patterns which likely contribute to their unique properties, including increased longevity, potent effector function, and unique memory expression programs [17]. Generally, memory T cells can be subdivided into 3 classes: central memory T cells ( $T_{CM}$ ), effector memory T cells ( $T_{EM}$ ), and resident memory T cells ( $T_{RM}$ ) [18]. Long-lived  $T_{CM}$  cells recirculate between the secondary lymphoid organs (SLOs) and blood, while  $T_{EM}$  cells can recirculate through non-lymphoid tissues, SLOs, and/or blood. Finally,  $T_{RM}$  are understood to reside permanently in peripheral non-lymphoid tissues, SLOs, and local vascular compartments without recirculating (Fig. 1).

In mice,  $T_{CM}$  cells express the lymphoid tissue homing receptors C–C chemokine receptor type 7 (CCR7) and L-selectin (CD62L) (Fig. 2), while  $T_{RM}$  cells express CD69 and integrin alpha E (CD103), known to be involved in tissue retention and recruitment, respectively [19,20]. On the other hand,  $T_{EM}$  cells fall somewhere in the middle usually not

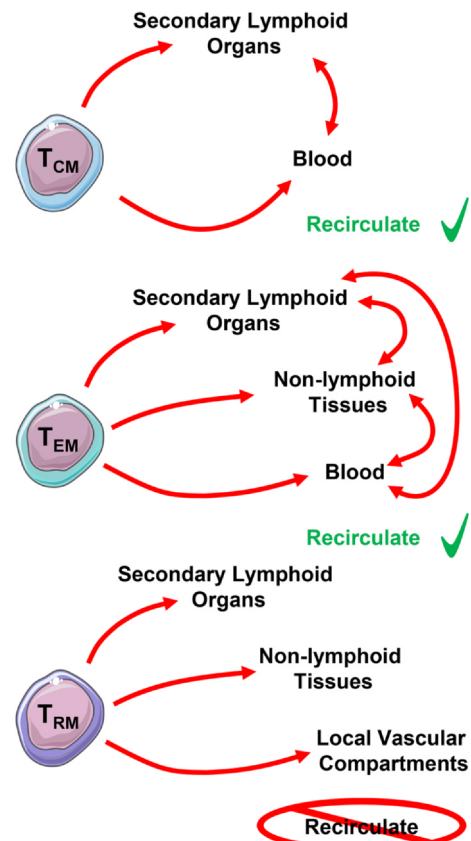


Fig. 1. Dynamics of memory T cell populations and their putative localization. Central memory ( $T_{CM}$ ) and effector memory ( $T_{EM}$ ) T cells are able to recirculate, while resident memory ( $T_{RM}$ ) T cells reside permanently in their location.

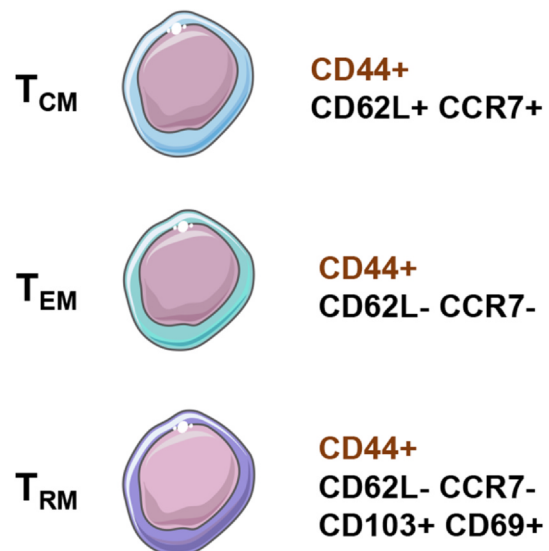
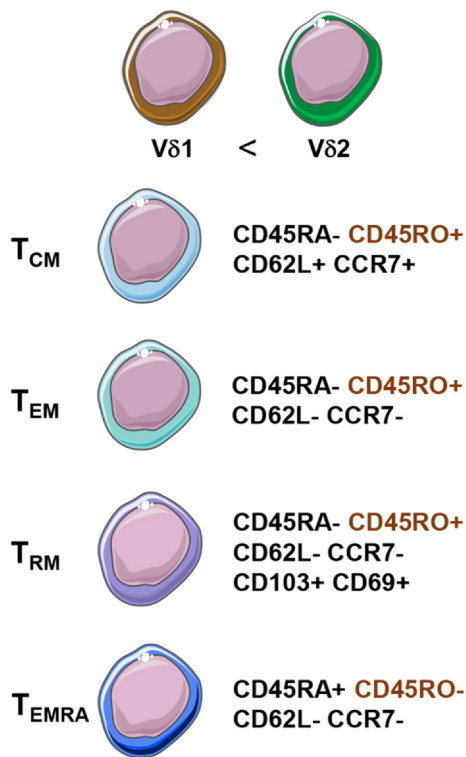


Fig. 2. Memory marker expression of murine central memory ( $T_{CM}$ ), effector memory ( $T_{EM}$ ), and resident memory ( $T_{RM}$ )  $\gamma\delta$  T cells. The expression of CD44 in murine memory  $\gamma\delta$  T cells denotes previous encounter with antigen, and CD44 stays upregulated in memory T cells.

expressing any of these markers, notably not expressing the lymphoid homing receptors CCR7 and CD62L [21]. All of these murine memory T cell subsets express the hyaluronic acid receptor and activation marker, CD44, which is upregulated after antigen encounter and stays upregulated in memory T cells [22]. Naïve murine T cells, while expressing



**Fig. 3.** Memory marker expression of human central memory ( $T_{CM}$ ), effector memory ( $T_{EM}$ ), resident memory ( $T_{RM}$ ), and effector memory re-expressing CD45RA ( $T_{EMRA}$ )  $\gamma\delta$  T cells. Vδ2  $\gamma\delta$  T cells make up the vast majority of human  $\gamma\delta$  T cells. Human memory  $\gamma\delta$  T cells are defined by their expression of CD45RO and lack of expression of CD45RA, except in the case of  $\gamma\delta T_{EMRA}$  cells, which re-express CD45RA.

CD62L and CCR7, are distinctly CD44- [23].

Human memory T cell populations can be subdivided in a similar fashion, albeit, with some different markers to the mouse. While humans share CCR7, CD62L, CD69, and CD103 with the mouse, human memory T cell subsets are further characterized using CD45RA and CD45RO, and do not express CD44 (Fig. 3) [24]. CD45RA is a marker of naïve T cells, while CD45RO denotes previous activation in a similar manner to CD44 in mice [25]. Naïve human T cells are CD45RA + CD45RO - CD62L + CCR7 +, and after activation by TCR-mediated antigen recognition the naïve T cells become effector T cells ( $T_{EFF}$ ) with potent cytotoxic and proinflammatory capabilities [15]. Following the primary immune response and contraction of  $T_{EFF}$  cell populations, some  $T_{EFF}$  become long-lived CD45RA- CD45RO + CD62L + CCR7 +  $T_{CM}$  cells [26]. These  $T_{CM}$  cells can become  $T_{EM}$  cells after re-exposure to their cognate antigen, yielding CD45RA - CD45RO + CD62L - CCR7 - effector memory T cells capable of a quick and robust proinflammatory response [27]. On the other hand, human  $T_{RM}$  are CD45RA - CD45RO + CD62L - CCR7 - CD103 + CD69 +, sharing similar markers to their murine counterparts [24].  $T_{RM}$  are thought to be derived from precursors that entered the tissues during the effector phase of the immune response, which remain positioned in the periphery [28]. Both mouse and human T cells are thought to follow a similar stepwise, progressive shift from naïve T cells, to  $T_{EFF}$  cells, then  $T_{CM}$  cells, and eventually  $T_{EM}$  cells based on antigen recognition, activation, and the presence of cytokines [21,26]. However, it is speculated that memory T cell development may not be entirely linear, as several hypothetical models exist with supporting evidence for the development pathway of memory T cells [29].

$\gamma\delta$  T cells share memory marker expression patterns with  $\alpha\beta$  T cells, and they can be subdivided into memory subsets based on the same marker expression criteria. However, how well do memory  $\gamma\delta$  T cells fill

their memory roles in comparison to  $\alpha\beta$  memory T cells? In recent years, models of infection and autoimmune disease focusing on memory  $\gamma\delta$  T cells has yielded useful knowledge about the influence of these cells in infection and immunity. In this review we will explore the evidence for adaptive immune memory in  $\gamma\delta$  T cells across humans and mice in various models of disease and infection.

## 2. Human memory $\gamma\delta$ T cells

Human  $\gamma\delta$  T cells can be divided into two general subsets following their usage of the TCR $\delta$  chain: Vδ1 + and Vδ2 +  $\gamma\delta$  T cells. Vδ1 + cells possess more diversity in their TCR $\gamma$  chain expression (V $\gamma$ 2/3/4/5/8) than their Vδ2 + counterparts (almost exclusively V $\gamma$ 9 +) [30,31].  $\gamma\delta$  T cells comprise roughly 5% of T cells in the peripheral blood of healthy adults [32]. The majority of  $\gamma\delta$  T cells in peripheral blood (roughly two-thirds) bear the V $\gamma$ 9Vδ2 TCR, while the remainder are mostly Vδ1 + with even fewer Vδ3 + and Vδ5 +  $\gamma\delta$  T cells [4]. Most research into the ability of human  $\gamma\delta$  T cells to form long-lived memory cells has focused on the more frequent Vδ2 + subset. Human V $\gamma$ 9Vδ2 T cells are clinically relevant as upon exposure to cells expressing non-peptidic antigens (ex. fibroblasts, monocytes, tumour cells), they respond with a cascade of immune reactions leading to  $\alpha\beta$  T cell activation, cytokine release, cytotoxicity, and DC and B cell activation [33]. These responses may aid in fighting pathogens, killing transformed cells, or alternatively, worsen autoimmune and inflammatory diseases. The ability of Vδ2 + and Vδ2 -  $\gamma\delta$  T cells to form memory cell populations has been investigated in recent years, with evidence pointing towards the existence of these long-lived memory  $\gamma\delta$  T cell populations.

### 2.1. *Mycobacterium tuberculosis* and *Listeria monocytogenes*: Bacterial infections in primates

Early research into the  $\gamma\delta$  T cell response to *M. tuberculosis* infection led to speculation about the formation of adaptive immune memory in  $\gamma\delta$  T cells. In 1998, Hoft *et al.* showed that Bacille Calmette-Guérin (BCG) vaccination could induce memory-like characteristics and responses in  $\gamma\delta$  T cells [34]. Peripheral blood mononuclear cells (PBMCs) from BCG vaccinated and non-vaccinated healthy adults were exposed to *M. tuberculosis* antigens *in vitro*, and then immune cell populations characterized after 7 days of antigen exposure.  $\gamma\delta$  T cells had the most dramatic expansion in response to antigen exposure, and  $\gamma\delta$  T cells from BCG-vaccinated individuals expanded more than non-vaccinated controls. This expansion was observed with interleukin (IL)-2 alone, and in co-culture with CD4 + T cells, revealing that the expansion was not solely dependent on CD4 + T cell help. However, optimal expansion required CD4 + T cells in co-culture. Proliferation and expansion to a greater extent upon re-exposure to antigen is a hallmark of immune memory and previous antigen experience. This pioneering work revealed a potential role for memory formation in  $\gamma\delta$  T cells.

More recently, the memory-like responses of  $\gamma\delta$  T cells to re-exposure to *M. tuberculosis* antigens observed *in vitro* was confirmed *in vivo* using macaques [35]. Upon both intravenous and pulmonary reinfection with BCG,  $\alpha\beta$  and  $\gamma\delta$  T cells exhibited enhanced expansion versus the initial infection, eliciting this response for > 4 weeks after the first infection. *In vitro* activation using BCG phosphoantigens and  $\gamma\delta$  T cells collected from infected macaques before the BCG infection and 4 weeks later confirmed that these  $\gamma\delta$  T cells were antigen specific and expanding *in vivo* in response to the BCG reinfection. Building on this concept, the authors wanted to elicit a Vδ2 +  $\gamma\delta$  T cell specific memory response *in vivo* [36]. Macaques were vaccinated with an attenuated strain of HMBPP producing *Listeria monocytogenes* (LM), and then infected with a moderate dose of *M. tuberculosis* 12 weeks after vaccination. Both LM and *M. tuberculosis* are known to produce HMBPP, a potent phosphoantigen activator of the  $\gamma\delta$  TCR [37]. Initial immunization caused an expansion of HMBPP-specific V $\gamma$ 2Vδ2 +  $\gamma\delta$  T cells, and upon re-challenge with *M. tuberculosis*, the vaccinated

macaques fared better losing less weight and with a lower bacterial burden in the lungs than the control groups (controls vaccinated with a non-HMBPP-producing attenuated LM strain or saline). The HMBPP-producing LM strain vaccinated macaques also had better control of the infection, with more CD4<sup>+</sup> and CD8<sup>+</sup> T cell recruitment and less *M. tuberculosis* dissemination outside of the lungs. Repeating the same protocol but re-infecting 35 days after vaccination with the attenuated HMBPP-producing LM strain instead of *M. tuberculosis* yielded similar results, with a much larger expansion of V $\gamma$ 2V $\delta$ 2 +  $\gamma\delta$  T cells after the second infection versus the first [38]. These expanded V $\gamma$ 2V $\delta$ 2 +  $\gamma\delta$  T cells were also found to be potent effector cells producing, or co-producing, proinflammatory cytokines, interferon (IFN) –  $\gamma$ , tumour necrosis factor (TNF)- $\alpha$ , IL-17, and/or perforin after LM infection.

These *M. tuberculosis* and LM experiments show that V $\gamma$ 2V $\delta$ 2-specific immunization may lead to a potent adaptive memory response upon re-challenge. However, although these studies show a memory-like response in  $\gamma\delta$  T cells to *M. tuberculosis* and LM re-infection, they do not provide a profile of  $\gamma\delta$  T cells further than characterization of their V $\delta$  and V $\gamma$  chain usage. Further characterization of memory subsets and memory marker expression would be helpful in determining the role and phenotype of these cells. Nonetheless, this provides a useful framework for further investigations of human and non-human primate (NHP) memory  $\gamma\delta$  T cell responses to bacterial infection.

## 2.2. Monkeypox and CMV: Viral infections in primates

$\gamma\delta$  T cells have also shown memory-like responses to viral infections in NHP models. Cynomolgus monkeys vaccinated with vaccinia virus-derived Dryvax followed by infection with monkeypox (MPV) 2 months later led to potent memory-like expansion of  $\gamma\delta$  T cells upon re-infection [39]. Researchers co-vaccinated macaques with vaccinia virus and the antiviral drug cidofovir to produce sub-optimal anti-MPV immunity, and then challenged animals with MPV 2 months later. Despite the suboptimal immune priming due to cidofovir, 4 of 6 immunized macaques had an up to 10-fold increase in the frequency of V $\gamma$ 2V $\delta$ 2 + T cells in the blood after MPV infection. This expansion was greater than what was seen in the mock-vaccinated macaques after MPV challenge, and greater than what was observed after vaccination alone without secondary infection. Vaccinia virus/cidofovir co-vaccination alone induced little to no expansion of V $\gamma$ 2V $\delta$ 2 + T cells. The memory-like expansion of V $\gamma$ 2V $\delta$ 2 + T cells after MPV infection in vaccinated macaques represents a hallmark of adaptive immune memory and trained immunity. Interestingly, orthopoxviruses do not produce phosphoantigen, so this response may be mediated by non-TCR mechanisms or reciprocal interactions with  $\alpha\beta$  T cells. On the other hand, there may be an unknown, non-phosphoantigen TCR antigen within the viral genome that activates the V $\delta$ 2 +  $\gamma\delta$  TCR. The latter seems more likely given the antigen/TCR driven memory responses observed in  $\gamma\delta$  T cells.

Human cytomegalovirus (CMV) also induces characteristics of immune memory in  $\gamma\delta$  T cells post-infection. Analyzing PBMCs from blood samples collected from CMV<sup>+</sup> and CMV<sup>-</sup> patients by flow cytometry revealed changes in V $\delta$ 2 –  $\gamma\delta$  T cell populations, but not V $\delta$ 2 + [40]. Within the V $\delta$ 2 – compartment, CMV<sup>+</sup> donors exhibited an increased frequency of terminally differentiated  $\gamma\delta$  T<sub>EM</sub> re-expressing CD45RA (termed T<sub>EMRA</sub>)  $\gamma\delta$  T cells versus CMV<sup>-</sup> donors (68.4% vs 13.7% respectively,  $P < 0.001$ ). T<sub>EMRA</sub> cells are similar to T<sub>EM</sub> cells, however, they can be associated with a unique upregulation of cytotoxic molecules [41]. T<sub>EMRA</sub> cells re-express the CD45RA isoform after antigenic stimulation, while their T<sub>EM</sub> counterparts express CD45RO [42]. The researchers also studied the recall response of  $\gamma\delta$  T cells in renal allografts from CMV<sup>+</sup> patients into CMV<sup>-</sup> patients and vice versa. They observed quicker expansions of V $\delta$ 2 –  $\gamma\delta$  T cells in organ transplant patients experiencing CMV reactivation under immunosuppressive treatment (CMV<sup>+</sup> patients transplanted with CMV<sup>-</sup> allografts, mean of 17 days for expansion) compared with patients undergoing primary infection (CMV<sup>-</sup> patient transplanted with CMV<sup>+</sup> allograft, mean of

66 days for expansion,  $P < 0.005$ ) [40]. An increase in T<sub>EMRA</sub> cells was also seen over the course of the primary infection in D +/R- patients, while the frequency remained stable and high in the D-/R+ patients following CMV reactivation from immunosuppression. The expansion of effector-memory V $\delta$ 2 –  $\gamma\delta$  T cells in response to CMV infection, coupled with a faster expansion upon re-challenge with CMV is yet another example of memory-like responses within the  $\gamma\delta$  T cell subset. As V $\delta$ 2 – T cells can kill CMV-infected cells *in vitro*, it is likely that V $\delta$ 2 – T cells possessing a terminally differentiated T<sub>EMRA</sub> phenotype with an increased potential for cytotoxicity play an important role in the antiviral response [43]. Consequently, viral models induce a similar memory recall response upon re-infection to bacterial infection models in humans and NHPs, and together these data suggest a role for  $\gamma\delta$  T cell adaptive immune memory in protective immunity.

## 2.3. Multiple sclerosis and rheumatoid arthritis: Autoimmune disease in humans

Memory  $\gamma\delta$  T cells in human autoimmune disease has been investigated to a much lower extent. Shifts in memory T cell populations and recall-like expansions upon immune challenge have been observed in several human autoimmune disorders, including systemic lupus erythematosus, multiple sclerosis (MS), rheumatoid arthritis (RA), and Crohn's disease [15]. The memory response of  $\gamma\delta$  T cells in autoimmunity is much less understood. Recently, circulating  $\gamma\delta$  T cells were characterized in relapsing-remitting multiple sclerosis (RRMS) patients (either in remission or relapse) versus healthy controls (HC) [44]. Researchers found no differences in frequency of total  $\gamma\delta$  T cells in peripheral blood among RRMS patients and HC, but increased circulating naïve  $\gamma\delta$  T cells in the RRMS group versus HC. Interestingly, a strong decrease in  $\gamma\delta$  T<sub>CM</sub> cells was observed in remission RRMS versus relapse RRMS and HC, potentially a result of decreased antigen stimulation needed to maintain the T<sub>CM</sub> pool over time. In addition, relapse RRMS patients had fewer circulating terminally differentiated T<sub>EMRA</sub>  $\gamma\delta$  T cells. The researchers hypothesized that this was the result of migration of these cells out of the circulation into inflamed tissues in relapse RRMS patients, where T<sub>EMRA</sub>  $\gamma\delta$  T cells are highly represented. T<sub>EMRA</sub> cells express receptors for migration into inflamed tissue compartments, and express intracellular perforin and granzysin showing potent cytotoxicity; T<sub>EMRA</sub> cells are not commonly found in SLOs [45,46]. This RRMS example highlights the dynamics of memory cell populations in autoimmune disease, with increased disease severity linked to probable extravasation of T<sub>EMRA</sub>, and decreased disease severity associated with depletion of T<sub>CM</sub>  $\gamma\delta$  T cells (potentially leading to decreased circulating memory cells capable of a strong immune response). However, more research is required to support this hypothesis, as the migration of these cells and their effector functions have not been fully elucidated.

$\gamma\delta$  T<sub>EM</sub> cells have also shown that they possess the potential to worsen RA in humans. V $\gamma$ 9V $\delta$ 2 +  $\gamma\delta$  T cells were observed in high numbers in the peripheral blood and synovial fluid collected from RA patients, most bearing markers of T<sub>EM</sub>  $\gamma\delta$  T cells [47]. These V $\gamma$ 9V $\delta$ 2 + T<sub>EM</sub> cells exhibited a strong APC capability (high expression of human leukocyte antigen (HLA)-DR and CD80/86, molecules associated with professional APC function), and the ability to simultaneously secrete both IFN- $\gamma$  and IL-17 (two potent proinflammatory cytokines) upon *in vitro* stimulation with IPP. These results suggest that human V $\gamma$ 9V $\delta$ 2 +  $\gamma\delta$  T<sub>EM</sub> cells possess the ability to both activate CD4<sup>+</sup> T cells through APC function, and directly secrete proinflammatory cytokines in the context of RA, hinting to a role in the development of the disease. This example highlights the cytokine potential of an upregulated population of memory  $\gamma\delta$  T cells in RA, but it does not examine the memory response itself. While these autoimmune examples do not present direct evidence for a role of memory  $\gamma\delta$  T cells in human autoimmune disease progression, they show interesting evidence in favour of this hypothesis by characterizing memory marker expression and analyzing the responses of these cells *in vitro*. Further analysis of the recall response of

human memory  $\gamma\delta$  T cells in autoimmune disease is needed to support this evidence.

### 3. Murine memory $\gamma\delta$ T cells

Murine  $\gamma\delta$  T cells can be subdivided based on their  $V\gamma$  and  $V\delta$  chain usage. However, their isoforms differ from what is observed in humans. Mouse  $\gamma\delta$  T cells are primarily grouped based on  $V\gamma$  chain expression.  $\gamma\delta$  T cell  $V\gamma$  chains are most often characterized using either the Heilig & Tonegawa or Garman naming systems. The Garman system proposes 7  $V\gamma$  chain subsets:  $V\gamma 1.1$ ,  $V\gamma 1.2$ ,  $V\gamma 1.3$ ,  $V\gamma 2$ ,  $V\gamma 3$ ,  $V\gamma 4$ , and  $V\gamma 5$ , which coincide with Heilig & Tonegawa's  $V\gamma 1$ ,  $V\gamma 2$ ,  $V\gamma 3$ ,  $V\gamma 4$ ,  $V\gamma 5$ ,  $V\gamma 6$ , and  $V\gamma 7$  subsets, respectively [48,49]. Following Heilig & Tonegawa's naming system, some important murine  $\gamma\delta$  T cell subsets are the  $V\gamma 5V\delta 1 +$  DETCs in the epidermis,  $V\gamma 1 +$  and  $V\gamma 7 +$  IELs in the gastrointestinal tract,  $V\gamma 4 + \gamma\delta$  T cells in the dermis,  $V\gamma 6V\delta 1 + \gamma\delta$  T cells in the female reproductive tract, tongue, and peritoneal cavity, and  $V\gamma 1 +$  and  $V\gamma 4 + \gamma\delta$  T cells in the SLOs and lung [50]. Whereas murine  $\gamma\delta$  T cells do show some diversity in their  $V\delta$  chain usage, they are usually classified based on their  $V\gamma$  chain [51]. Murine  $\gamma\delta$  T cells represent roughly 3% of spleen and lymph node  $CD3 +$  T cells in rodents, and exhibit a similar capability to produce the cytokines IL-17 and/or IFN- $\gamma$  to human  $\gamma\delta$  T cells [4,52]. Knockout models of various  $V\gamma$   $\gamma\delta$  T cell subsets has revealed that the composition of murine  $\gamma\delta$  T cells influences population dynamics of memory  $CD4 +$  and  $CD8 + \alpha\beta$  T cells in non-immunized mice [53].  $\gamma\delta$  T cells display characteristics of immune memory in murine bacterial, viral, and parasitic infections, and experimental models of autoimmune disease. In mice, phycoerythrin (PE) is a putative antigen of the  $\gamma\delta$  TCR. After immunizing mice with PE, PE specific  $\gamma\delta$  T cells were shown to transition from a naïve ( $CD44 - CD62L +$ ) to a  $T_{EM}$  ( $CD44 + CD62L -$ ) phenotype [54]. It was also reported that these cells have a rapid induction of effector capabilities, producing large amounts of IL-17A after PE immunization. These data suggest a TCR dependant shift from naïve to memory  $\gamma\delta$  T cells upon encounter with cognate TCR antigen, and further examples will build on this concept.

#### 3.1. *Listeria monocytogenes* and *Staphylococcus aureus*: Murine bacterial infections

Similar to human  $\gamma\delta$  T cells, murine  $\gamma\delta$  T cells possess memory characteristics in models of bacterial infection and reinfection. In a murine model of oral LM infection, LM elicited a population of  $CD44 + V\gamma 4V\delta 1 +$  (Garman notation)  $\gamma\delta$  T cells in the mesenteric lymph node (mLN), but not the peripheral lymph nodes (pLN) [55]. Mice were reinfected with LM 170 days after primary infection either orally or intravenously, and then rested for an additional 124 days prior to a tertiary oral LM infection. After secondary oral challenge with LM, the frequency of  $CD44 + \gamma\delta$  T cells increased to a higher extent than the primary infection within 5 days of the secondary infection (Fig. 4). The frequency of  $CD44 + \gamma\delta$  T cells was even higher after the tertiary infection, pointing to a stepwise acquisition of trained immune memory with each infection. This increase in  $CD44 + \gamma\delta$  T cells was not elicited when mice were administered LM intravenously, showing specificity to oral (mucosal) infection. Further, the researchers confirmed a role for  $\gamma\delta$  T cell mediated immunity in LM by using antibodies to deplete  $CD4 +$  and  $CD8 +$  T cells and/or internalize the  $\gamma\delta$  TCR before re-infection with LM. Antibody mediated  $\gamma\delta$  TCR internalization or  $CD4 +/CD8 +$  depletion alone caused only minimal loss of protection, while both combined resulted in the least protection among LM-experienced mice. This suggests that  $\gamma\delta$  T cells exert an important protective role in oral LM infection in conjunction with  $CD4 +$  and  $CD8 + \alpha\beta$  T cells. Using antibodies against the  $V\gamma 1.1 +$  and  $V\gamma 2 +$  TCRs (the other  $\gamma\delta$  T cells commonly found in the mucosa) along with anti- $CD4 +/CD8 +$  antibodies conferred more protection than using an antibody not specific for certain  $\gamma\delta$  TCRs, showing that it is likely the

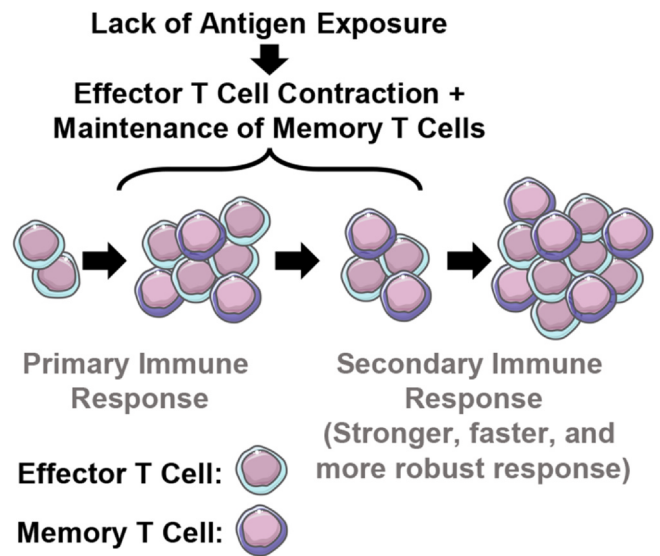


Fig. 4. The primary immune response to pathogen infection elicits effector and some memory T cells. As demonstrated by Lefrancois et al. (2013), upon resolution of infection and contraction of immune effectors coinciding with reduced antigen exposure, a population of long-lived memory T cells develops which can react more strongly to reencounter with cognate antigen (secondary, tertiary, etc. immune response).

memory  $V\gamma 4 + \gamma\delta$  T cells that play the most significant role in protection from LM among all  $\gamma\delta$  T cells. LM-experienced mice also had lower viral loads in the mLN and small intestine upon reinfection than naïve mice undergoing primary infection. These experiments demonstrate that LM-elicited  $CD44 + \gamma\delta$  T cells are maintained long term and can respond to secondary and tertiary infection with robust proliferation and effector functions (that increase host immunity). These are hallmarks of adaptive immune memory.

Further investigation into LM-elicited memory  $V\gamma 4 + \gamma\delta$  T cells in mice revealed that these cells possess characteristic markers of  $T_{RM}$  in the mLN after infection [56]. RNA sequencing demonstrated that most ( $> 70\%$ ) LM-elicited  $V\gamma 4 + CD44 + \gamma\delta$  T cells in the mLN expressed  $T_{RM}$  tissue retention marker CD69, but not  $T_{CM}$  markers CD62L and CCR7 30 days post primary infection. Furthermore, parabiosis of LM-experienced Thy1.2 mice and naïve Thy1.1 mice revealed that these  $V\gamma 4 + \gamma\delta$  T cells are indeed resident memory T cells, as frequencies did not equilibrate in the mLN between naïve and LM-experienced animals after 9–11 days. On the other hand, circulating and mLN frequencies of naïve  $CD4 + \alpha\beta$  T cells and  $V\gamma 1.1/2/3 + \gamma\delta$  T cells equilibrated fully between animals, which showed the tissue-resident properties of these  $V\gamma 4 + \gamma\delta$  T cells.  $\gamma\delta T_{RM}$  cells were more IL-17A positive than any other cells in the mLN upon re-challenge with LM (analyzed via flow cytometry staining), and blockade of IL-17A with antibodies resulted in increased bacterial burden and delayed clearance of LM. Therefore, as these  $\gamma\delta T_{RM}$  cells are a primary source of IL-17A in the mLN upon LM re-challenge, and IL-17A production correlates with bacterial clearance, it seems probable that  $T_{RM} \gamma\delta$  T cells play an important role in controlling LM infection in mice. Together, these experiments demonstrate that memory  $V\gamma 4 + \gamma\delta$  T cells may help in resisting LM infection: LM-elicited  $V\gamma 4 + \gamma\delta$  T cells are primarily of the  $T_{RM}$  subtype,  $V\gamma 4 + \gamma\delta$  T cells undergo progressively increased proliferation upon each re-challenge with LM (a hallmark of immune memory), and these  $\gamma\delta T_{RM}$  are a crucial source of IL-17A upon reinfection with LM (which correlates with increased protection and decreased bacterial burden).

Research on memory  $\gamma\delta$  T cells in bacterial infections using a model of *Staphylococcus aureus* revealed a similar role for  $V\gamma 4 + \gamma\delta$  T cells *in vivo* in mice [57].  $\gamma\delta$  T cells were the predominant source of IL-17 during infection, and prior immune challenge with *Staphylococcus aureus* expanded a population of  $V\gamma 4 +$  T cells capable of upregulated

IL-17 production during reinfection 21 days after initial exposure. Mice previously exposed to *S. aureus* infection had lower bacterial loads, increased bacterial clearance, and increased IL-17 production post-infection than mice with no prior exposure, demonstrating a memory response. The frequency of CD44+ and IL-17+  $\gamma\delta$  T cells was also higher in mice with prior exposure versus mice not previously exposed 21 days after infection, showing the formation and longevity of memory  $\gamma\delta$  T cells. This increased cytokine response is a characteristic of  $T_{EM}$  cells. However, the authors did not phenotype the cells for  $T_{EM}$  memory marker expression further than CD27 and CD44. Consequently, it seems murine and human V $\gamma$ 4+ and V $\delta$ 2+  $\gamma\delta$  T cells share many common aspects in their memory response to LM infection. The murine immune response to *Staphylococcus aureus* infection has many similarities to the memory V $\gamma$ 4+  $\gamma\delta$  T cell response to LM, notably in cytokine production.

### 3.2. Murine Cytomegalovirus: Viral infection model in mice

Memory  $\gamma\delta$  T cell responses have also been observed in viral infections of the mouse. Murine CMV (MCMV) reproduces a similar T cell and natural killer cell response as human CMV, and therefore serves as a useful model to study immune responses to CMV [58]. Recently,  $\gamma\delta$  T cells have been shown to have an important influence in resistance to MCMV. Mice lacking either TCR $\alpha$  or TCR $\delta$  were able to survive MCMV infection, while mice lacking CD3 $\epsilon$  (which have no T cells) died at low doses of MCMV [59]. These authors then determined the frequency and marker expression patterns of  $\gamma\delta$  T cells in response to MCMV infection. They found increased numbers of V $\gamma$ 1+, and to a lesser extent, V $\gamma$ 4+  $\gamma\delta$  T cells in the liver and lungs 3 days after infection, and an increase in only V $\gamma$ 1+  $\gamma\delta$  T cells in the spleen (Heilig & Tonegawa notation). Notably, the proportion of  $T_{EM}$  (CD44+ CD62L-) cells among V $\gamma$ 1+ and V $\gamma$ 4+  $\gamma\delta$  T cells increased after 3 days post infection in the liver, lungs, and spleen, eventually representing up to ~80% of each V $\gamma$   $\gamma\delta$  T cell subset by day 56. This increase in  $T_{EM}$  was concomitant with a decrease in  $T_{CM}$  (CD44+ CD62L+) V $\gamma$ 1+ and V $\gamma$ 4+  $\gamma\delta$  T cells, potentially showing the dynamics of memory T cell differentiation in infection. To reveal the capability of  $\gamma\delta$  T cells to mount a memory response upon re-challenge with MCMV, the researchers transferred  $\gamma\delta$  T cells from MCMV-experienced and MCMV-negative TCR $\alpha$  knockout mouse donors into CD3 $\epsilon$  knockout mice and infected the recipient mice with MCMV after transfer. The CD3 $\epsilon$  knockout mice that received  $\gamma\delta$  T cells from MCMV positive donors had a much higher survival (> 60% survival) than the mice that received  $\gamma\delta$  T cells from MCMV negative donors (0% survival) after 40 days post-infection. The marked increase in  $T_{EM}$  cells after both human and mouse CMV infection, which is associated with increased pathogen control and clearance, presents strong evidence for a role of  $\gamma\delta$  T cell memory in this context. The ability of MCMV-induced  $\gamma\delta$  T cells, but not MCMV-inexperienced  $\gamma\delta$  T cells, to confer increased protection from MCMV in mice lacking all T cells further supports this hypothesis. However, it is not yet known if murine  $\gamma\delta$  T cells recognize stressed-induced antigens on CMV-infected host cells like their human counterparts [60].

### 3.3. Malaria: Murine and human similarities

$\gamma\delta$  T cells also possess distinctive anti-parasitic properties like the  $\alpha\beta$  T cells. Recently, studies using mouse models of malaria (*Plasmodium chabaudi*) have detailed the memory response of  $\gamma\delta$  T cells to parasitic reinfection. C57BL/6 mice were infected with *P. chabaudi* and then treated on day 14 post-infection [61]. Twelve weeks after drug treatment, splenocytes from *P. chabaudi*-experienced and naïve mice were harvested and stimulated *in vitro* with *P. chabaudi*-infected red blood cells. The splenocytes from previously infected mice had a significantly higher amount of CD107a+ (a marker for cytotoxicity) expressing and IFN- $\gamma$  producing  $\gamma\delta$  T cells after re-exposure than splenocytes from naïve mice [61,62]. Repeating this experiment followed by

flow cytometric characterization of memory markers revealed that after *P. chabaudi* infected red blood cell re-exposure,  $\gamma\delta$  T cells from previously infected mice had a significantly higher proportion of cytotoxic  $T_{EM}$  (CD44+ CD62L- CD107a+ IFN- $\gamma$ +)  $\gamma\delta$  T cells than naïve mice. This shows that the  $\gamma\delta$  T cell memory response to malaria is largely confined to the cytotoxic  $T_{EM}$  compartment. RNA-sequencing revealed that the  $T_{EM}$   $\gamma\delta$  T cells from malaria-experienced mice had enhanced IFN- $\gamma$  production, cytotoxicity, and chemokine expression. They also observed an increase in genes related to antigen presentation, and generally, *P. chabaudi*-experienced  $\gamma\delta$  T cells displayed more activation characteristics than naïve  $\gamma\delta$  T cells.

Interestingly, experiments in humans have demonstrated a similar role for  $T_{EM}$   $\gamma\delta$  T cells in the primate immune response to *Plasmodium falciparum* [63]. These CD45RO+ CD62L-  $\gamma\delta$   $T_{EM}$  cells showed a comparable ability to their murine counterparts to produce IFN- $\gamma$  in response to *in vitro* infection by *Plasmodium falciparum*, and the majority of cytokine producing T lymphocytes had a  $T_{EM}$  phenotype (including  $\gamma\delta$  T cells). The memory response was observed for up to 140 days post-infection *in vitro* by collecting PBMCs from immunized patients and challenging them with *Plasmodium falciparum* infection at various time points. The percentage of IFN- $\gamma$  producing  $T_{EM}$  lymphocytes in previously immunized individuals increased more dramatically after infection of PBMCs compared to malaria-inexperienced individuals, demonstrating a trained response. In addition, adding  $\gamma\delta$  T cells isolated from PBMCs pre-exposed to *Plasmodium falciparum* to PBMCs that had not previously encountered *Plasmodium falciparum* resulted in a higher proportion of IFN- $\gamma$  producing lymphocytes after *in vitro* rechallenge than adding naïve PBMCs to naïve  $\gamma\delta$  T cells. The proportion of IFN- $\gamma$  producing cells also increased when pre-exposed PBMCs were added to pre-exposed  $\gamma\delta$  T cells and stimulated *in vitro* with *Plasmodium falciparum*, however, IFN- $\gamma$  producing cells reached a similar level to that when only the  $\gamma\delta$  T cells had been pre-exposed. This suggests that the memory response is heavily dependent on the  $\gamma\delta$  T cell subset. Additionally, the combined IFN- $\gamma$  recall response by all immune subsets remained undiminished for at least 14 months after a single infection. Together, these experiments demonstrate a role for both human and murine memory  $\gamma\delta$  T cells in protective immunity from malaria. However, further research is required to unravel the role and specific  $\gamma\delta$  T cell subset responsible for the memory response and protective effects observed in malaria and other parasitic infections. It would be useful to perform adoptive transfer experiments with malaria exposed and not exposed  $\gamma\delta$  T cells into TCR $\delta$  knockout animals to see the level of protection conferred by the malaria-experienced  $\gamma\delta$  T cells upon reinfection *in vivo*.

### 3.4. Imiquimod: A model of murine psoriatic autoimmune disease

Similar to their human counterparts, memory  $\gamma\delta$  T cells may play a role in mouse models of autoimmunity. Treatment with the topical TLR-7/8 ligand and immune activator, imiquimod (IMQ), can induce an inflammatory psoriasis-like skin condition in humans and mice [64]. IMQ treatment is considered a valid mouse model of psoriatic disease [65]. Researchers found that mice exposed to IMQ had elevated numbers of V $\gamma$ 4+ (Heilig & Tonegawa notation)  $\gamma\delta$  T cells in noninflamed skin and pLNs after treatment, and they stayed elevated post-treatment [66]. After treating the skin of the back of animals with IMQ, researchers took biopsies from unaffected ear skin and analyzed  $\gamma\delta$  T cell frequencies. Surprisingly, they observed a 20-fold increase in V $\gamma$ 4+  $\gamma\delta$  T cells in the skin of the ear 30 days after IMQ treatment, which persisted for 7 months. This may explain the ability of IMQ to induce skin inflammation at sites distant from application. Interestingly, V $\gamma$ 4+  $\gamma\delta$  T cells from the ear tissue of animals sensitized to IMQ, which was initially applied to the skin of the back, had greater proliferation and IL-17 production (quantified via intracellular flow cytometry staining) upon re-exposure to IMQ (applied to the ear for secondary exposure) than naïve animals. This coincided with a more rapid increase in

inflammation of the ear skin in IMQ-experienced animals, showing a marked memory-like response upon secondary exposure to IMQ. IMQ-sensitized mice had a more rapid increase in the thickness of the ear (quantifying inflammation) and in neutrophil accumulation than naïve mice upon secondary exposure to IMQ. The accelerated worsening of inflammation in sensitized wild-type mice was not observed in *Sox13*-mutant mice (which lack  $V\gamma4+$   $\gamma\delta$  T cells), revealing that it is indeed the  $V\gamma4+$   $\gamma\delta$  T cell subset responsible for this memory response.

To further investigate this model, a reporter mouse was generated using Cre-lox technology in which IL-17A/F co-producing cells express the reporter enhanced yellow fluorescent protein (EYFP) [67]. Sixty days after initial treatment with IMQ, macro and microscopic inflammation was totally resolved. However, a higher proportion of EYFP+ cells was observed in the skin of the ear than in unexposed mice, and most of these EYFP+ cells were  $V\gamma4+$   $\gamma\delta$  T cells. In the skin draining lymph nodes, most EYFP+  $\gamma\delta$  T cells were CD44+, CD62L-, and CD103+ after primary IMQ exposure, revealing a resident memory phenotype. Secondary challenge with IMQ after 60 days of recovery induced stronger and more robust inflammation and resulted in a significant increase in the number of EYFP+  $V\gamma4+$   $\gamma\delta$  T cells in the skin of the ear compared to primary exposure. To ensure that it was the  $\gamma\delta$  T cells that were responsible for the memory response to secondary IMQ exposure, TCR $\alpha$  knockout mice and wild-type mice were challenged and re-challenged with IMQ. There was a similar increase in ear skin inflammation in the TCR $\alpha$  knockout mice to that observed in the wild-type mice after repeated IMQ exposure. The inflammation observed in response to the secondary exposure to IMQ was the same when IMQ was applied to the either the same ear as the primary exposure, or the other ear, in both wild-type and TCR $\alpha$  knockout mice. Taking into account the prolonged persistence of  $V\gamma4+$   $\gamma\delta$  T cells observed in the skin after primary IMQ treatment, these  $\gamma\delta$  T cells display hallmarks of  $T_{RM}$  cells. Administration of a TCR internalizing anti- $V\gamma4+$  antibody before and during IMQ treatment resulted in loss of exacerbated disease and resulted in identical inflammation to both the primary and secondary IMQ exposure, which was lower than in animals not treated with the anti- $V\gamma4+$  antibody. Together, these data suggest that: the  $V\gamma4+$   $\gamma\delta$  T cell subset is sensitive to IMQ treatment,  $V\gamma4+$   $\gamma\delta$  T cells respond more strongly to secondary IMQ challenge than to primary challenge by enhanced production of proinflammatory cytokines (IL-17),  $V\gamma4+$   $\gamma\delta$  T cells have the characteristics of long-lived  $T_{RM}$  in the skin, and depletion of  $V\gamma4+$   $\gamma\delta$  T cells blunts the inflammatory response to primary and secondary exposure to IMQ. However, the putative TCR antigen that the  $\gamma\delta$  TCR recognizes in response to IMQ-induced inflammation remains unknown. These experiments suggest that memory  $\gamma\delta$  T cells may play a role in murine autoimmune disease progression and sterile inflammation, specifically in the context of models of IMQ-induced psoriatic disease.

#### 4. Conclusion

The ability to knockout, deplete, and transfer  $\gamma\delta$  T cell subsets and control infection parameters in mice has been extremely useful in showing a role for memory  $\gamma\delta$  T cells in infection and immunity. In addition, models using NHPs provide another animal model which can be manipulated experimentally, while more closely resembling humans. At the moment, experiments in humans investigating  $\gamma\delta$  T cell memory have only been able to characterize the memory  $\gamma\delta$  T cells rather than manipulate them directly, except in the context of *in vitro* experiments. Nonetheless, human and NHP experiments using bacterial and viral infections/immunizations have revealed characteristics distinct to adaptive immune memory in  $\gamma\delta$  T cells. Shifts in memory  $\gamma\delta$  T cell populations have been seen in human models of relapsing autoimmune disease, and  $\gamma\delta$  T cells bearing memory markers have been shown to increase in human autoimmunity. The evidence for memory  $\gamma\delta$  T cells in murine autoimmunity is slightly stronger (as in the case of psoriasis), but more work needs to be done using other animal models of

autoimmune disease.

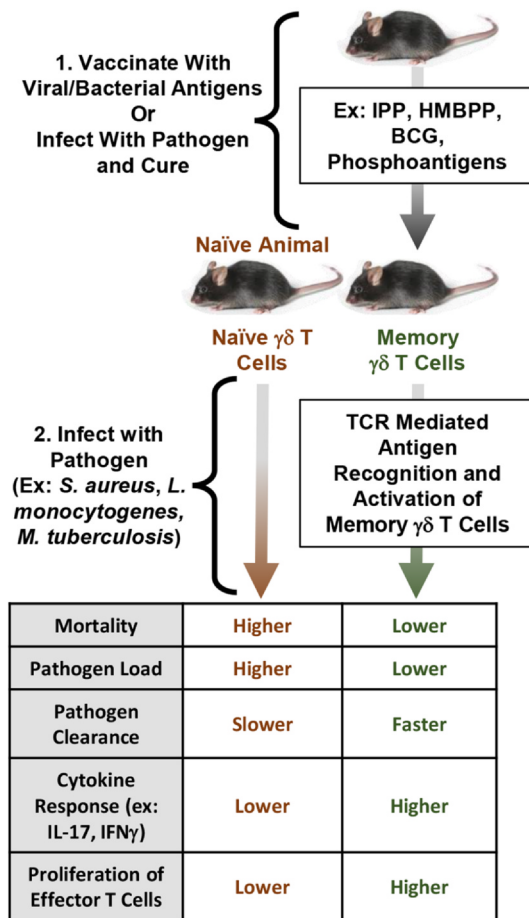
While countless experiments have shown a prominent role for  $\gamma\delta$  T cells in infection and immunity, only recently has there been an interest in adaptive immune memory development in these innate-like cells [68]. Memory  $\gamma\delta$  T cells share many common aspects between mice and primates, and most importantly, they share the hallmark ability of memory cells to respond more strongly to secondary and tertiary exposure to the pathogenic agent. Given that  $\gamma\delta$  T cells are potent sources of proinflammatory mediators across species (notably IL-17 and IFN- $\gamma$ ), it makes sense that  $\gamma\delta$  T cells have been found to have a significant role in inflammation and the response to infection and cellular stress [69]. In addition, their ability to be activated in a non-MHC restricted manner and act as professional APCs allows them to also act as first-line innate-like sensors able to manipulate the immune response [1].  $\gamma\delta$  T cells perform important reciprocal interactions with  $\alpha\beta$  T cells, and can influence the heterogeneity of  $\alpha\beta$  T cell populations in response to inflammation and stress [53]. These characteristics along with the ability of  $\gamma\delta$  T cells to express NKR and TLRs yields a very potent, yet small population of immune cells capable of mounting a rapid proinflammatory response to immune challenge [12]. The ability of  $\gamma\delta$  T cells to respond quickly to immune challenge and orchestrate the subsequent immune response uniquely bridges innate and adaptive immunity.

On the other hand, the ability of  $\gamma\delta$  T cells to undergo TCR-mediated activation and V(D)J TCR recombination is a distinct feature of the adaptive immune system [70]. Hallmarks of adaptive immunity include specificity, immunological memory, and self/non-self-recognition, and  $\gamma\delta$  T cells display all of these characteristics in various contexts of infection and disease [71]. Further research will be required to determine the antigen repertoire of the  $\gamma\delta$  TCR, as putative  $\gamma\delta$  TCR antigens remain mostly unknown in mice and primates. Even with this limited knowledge of  $\gamma\delta$  TCR diversity, it is almost undoubtable that through TCR-dependent mechanisms  $\gamma\delta$  T cells have a capacity to become long-lived memory cells capable of a more robust secondary response. Prominent roles for  $T_{EM}$  and  $T_{RM}$   $\gamma\delta$  T cell subsets have been shown in experimental models of disease, but the role of  $\gamma\delta$   $T_{CM}$  cells is less well-developed. Further investigations using immune challenge/re-challenge models of infections and autoimmunity will help shed light on the role of the  $\gamma\delta$   $T_{CM}$  subset in comparison to the better characterized  $\gamma\delta$   $T_{EM}$  and  $T_{RM}$  cells.

In many ways memory  $\gamma\delta$  T cells resemble their memory  $\alpha\beta$  T cell counterparts, notably in both phenotype and recall-like response [72]. It is understood that antigen-specific naïve T cells can become immune memory cells after cognate antigen recognition [73]. Where  $\gamma\delta$  T cells differ from traditional  $\alpha\beta$  T cells is that  $\gamma\delta$  T cells do not require MHC presentation for antigen recognition; therefore, the initial mechanics of memory  $\gamma\delta$  T cell generation post antigen-recognition may be slightly different than in  $\alpha\beta$  T cells [3]. It is probable that  $\gamma\delta$  T cell immune memory generation is primarily antigen driven, and enhanced TCR signal strength combined with local cytokine milieu likely drives the differentiation from effector  $\gamma\delta$  T cells to memory  $\gamma\delta$  T cells in a similar manner to  $\alpha\beta$  T cell memory generation [74]. As many of the pathogens mentioned above produce potent activators of the  $\gamma\delta$  TCR (for example: HMBPP and IPP [11]), it is likely that TCR antigens are directly responsible for the initial activation and subsequent immune memory response observed in  $\gamma\delta$  T cells. This response may be tissue localization-dependent as the  $\gamma\delta$  TCR has varying degrees of TCR junctional diversity across tissue compartments, which could coincide with the level of diversity of local pathogens [13]. The effector functions and cytokine-producing capabilities of  $\gamma\delta$  T cells also differ depending on tissue localization. Accordingly, tissue compartment localization and frequency of local pathogens in number and recurrence may have an effect on the ability of  $\gamma\delta$  T cells to form long-lived memory cell populations [73].

Clonal selection and expansion of various functionally useful  $\gamma\delta$  TCRs accompanied by phenotypic differentiation and acquisition of distinct functional biology has been observed in  $\gamma\delta$  T cells, which





**Fig. 5.** Memory  $\gamma\delta$  T cells primed via previous exposure to pathogens expedite the immune response to infection. Memory T cells proliferate faster and respond more strongly via increased cytokine production and enhanced effector functions in the memory response. Vaccination using pathogen-specific  $\gamma\delta$  TCR antigens (ex: HMBPP, IPP, phosphoantigens) may result in higher survival and enhanced pathogen clearance upon infection.

suggests acquisition of trained immune memory based on effectiveness of TCR recognition [75]. As increases in certain antigen-specific V $\gamma$  and V $\delta$  subsets of memory  $\gamma\delta$  T cells were observed across several models of infection, it seems that memory  $\gamma\delta$  T cells exhibit restricted clonality and a strong ability to clonally expand antigen-specific cells post-reinfection. Evidence from human CMV infection supports this restricted clonality hypothesis, as most CMV<sup>+</sup> allogeneic stem cell donors had a primarily oligoclonal V $\delta$ 1, polyclonal V $\delta$ 2, and oligoclonal V $\delta$ 3 profile, showing a primarily oligoclonal V $\delta$ 2– $\gamma\delta$  T cell population in CMV<sup>+</sup> individuals [76]. Targeting various antigen-specific  $\gamma\delta$  T cell clonotypes for vaccination may be a useful strategy to induce protective immunity, as these experiments point to an important role for antigen-specific memory  $\gamma\delta$  T cells in resisting bacterial and viral infections. In general, development of  $\gamma\delta$  T cell memory after exposure to a pathogen yields more resilient immunity, allowing for faster pathogen clearance, a higher frequency of cytokine producing cells, and lower individual mortality (Fig. 5). The longevity of  $\gamma\delta$  T cells with immune memory post infection remains unknown, but the experiments mentioned above reveal that they can persist for months to years. There is no current evidence in the literature to suggest that memory  $\gamma\delta$  T cells are any more long-lived or, on the contrary, more short-lived, than their  $\alpha\beta$  T cell counterparts. Consequently, memory cell longevity may be primarily influenced by the inflammatory environment of the T cell itself [77].

In conclusion,  $\gamma\delta$  T cells play a part in both innate and adaptive

immunity, and recent research has revealed characteristics of adaptive immune memory in  $\gamma\delta$  T cells in both mice and primates. Research on infection and immunity that has found a prominent role for  $\gamma\delta$  T cells would benefit from further exploring the possibility of long-lived memory  $\gamma\delta$  T cells that could worsen disease or promote immunity.

#### Source of funding

The work of the authors was supported by Canadian Institutes of Health Research (CIHR) First Pilot Foundation Grant 143348, a Canada Research Chair (CRC) on Hypertension and Vascular Research by the CRC Government of Canada/CIHR Program, and by the Canada Fund for Innovation, all to ELS.

#### CRediT authorship contribution statement

**Kevin Comeau:** Conceptualization, Investigation, Writing - original draft, Visualization. **Pierre Paradis:** Writing - review & editing, Visualization. **Ernesto L. Schiffrin:** Writing - review & editing, Visualization, Supervision, Funding acquisition.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### References

- [1] M. Lawand, J. Déchanet-Merville, M.-C. Dieu-Nosjean, Key features of gamma-delta t-cell subsets in human diseases and their immunotherapeutic implications, *Front. Immunol.* 8 (2017), <https://doi.org/10.3389/fimmu.2017.00761>.
- [2] Y. Zhao, C. Niu, J. Cui, Gamma-delta ( $\gamma\delta$ ) T cells: friend or foe in cancer development? *J. Transl. Med.* 16 (2018) 3, <https://doi.org/10.1186/s12967-017-1378-2>.
- [3] R.M. Welsh, M.Y. Lin, B.L. Lohman, S.M. Varga, C.C. Zarozinski, L.K. Selin, Alpha beta and gamma delta T-cell networks and their roles in natural resistance to viral infections, *Immunol. Rev.* 159 (1997) 79–93, <https://doi.org/10.1111/j.1600-065x.1997.tb01008.x>.
- [4] C. Khairallah, T.H. Chu, B.S. Sheridan, Tissue adaptations of memory and tissue-resident gamma delta t cells, *Front. Immunol.* 9 (2018), <https://www.frontiersin.org/article/10.3389/fimmu.2018.02636>.
- [5] M. Brandes, K. Willmann, B. Moser, Professional antigen-presentation function by human gammadelta T Cells, *Science.* 309 (2005) 264–268.
- [6] M. Brandes, K. Willmann, G. Bioley, N. Lévy, M. Eberl, M. Luo, R. Tampé, F. Lévy, P. Romero, B. Moser, Cross-presenting human  $\gamma\delta$  T cells induce robust CD8+  $\alpha\beta$  T cell responses, *Proc. Natl. Acad. Sci.* 106 (2009) 2307–2312, <https://doi.org/10.1073/pnas.0810059106>.
- [7] L. Cheng, Y. Cui, H. Shao, G. Han, L. Zhu, Y. Huang, R.L. O'Brien, W.K. Born, H.J. Kaplan, D. Sun, Mouse gammadelta T cells are capable of expressing MHC class II molecules, and of functioning as antigen-presenting cells, *J. Neuroimmunol.* 203 (2008) 3–11, <https://doi.org/10.1016/j.jneuroim.2008.06.007>.
- [8] X. Xi, Y. Guo, H. Chen, C. Xu, H. Zhang, H. Hu, L. Cui, D. Ba, W. He, Antigen specificity of gammadelta T cells depends primarily on the flanking sequences of CDR3delta, *J. Biol. Chem.* 284 (2009) 27449–27455, <https://doi.org/10.1074/jbc.M109.011684>.
- [9] M.K. Heng, M.C.Y. Heng, Antigen Recognition by  $\gamma\delta$  T-Cells, *Landes Bioscience*, 2013. <https://www.ncbi.nlm.nih.gov/books/NBK6165/> (accessed May 12, 2020).
- [10] A.R. Kazen, E.J. Adams, Evolution of the V, D, and J gene segments used in the primate  $\gamma\delta$  T-cell receptor reveals a dichotomy of conservation and diversity, *Proc. Natl. Acad. Sci.* 108 (2011) E332–E340, <https://doi.org/10.1073/pnas.1105105108>.
- [11] Y. Zhao, L. Lin, Z. Xiao, M. Li, X. Wu, W. Li, X. Li, Q. Zhao, Y. Wu, H. Zhang, J. Yin, L. Zhang, C.H. Cho, J. Shen, Protective role of  $\gamma\delta$  T cells in different pathogen infections and its potential clinical application, *J. Immunol. Res.* 2018 (2018) e5081634, <https://doi.org/10.1155/2018/5081634>.
- [12] D. Wu, P. Wu, F. Qiu, Q. Wei, J. Huang, Human  $\gamma\delta$ T-cell subsets and their involvement in tumor immunity, *Cell. Mol. Immunol.* 14 (2017) 245–253, <https://doi.org/10.1038/cmi.2016.55>.
- [13] R.L. Mosley, M. Whetsell, D. Stickney, L. Whetsell, F.V. Schaefer, K.S. Miller, J.R. Klein, Phenotype and TCR gamma delta variable gene repertoire of intestinal intraepithelial lymphocytes in wild mice (*Mus musculus domesticus*): abundance of V gamma 1 transcripts and extensive delta gene diversity, *Int. Immunol.* 6 (1994) 231–238, <https://doi.org/10.1093/intimm/6.2.231>.
- [14] S. Paul, G. Shilpi, Lal, Role of gamma-delta ( $\gamma\delta$ ) T cells in autoimmunity, *J. Leukoc. Biol.* 97 (2015) 259–271, <https://doi.org/10.1189/jlb.3RU0914-443R>.
- [15] P. Devarajan, Z. Chen, Autoimmune effector memory T cells: the bad and the good,

- Immunol Res. 57 (2013) 12–22.
- [16] G. Lauvau, S.M. Soudja, Mechanisms of memory T cell activation and effective immunity, *Adv. Exp. Med. Biol.* 850 (2015) 73–80, [https://doi.org/10.1007/978-3-319-15774-0\\_6](https://doi.org/10.1007/978-3-319-15774-0_6).
- [17] B. Youngblood, J.S. Hale, H.T. Kissick, E. Ahn, X. Xu, A. Wieland, K. Araki, E.E. West, H.E. Ghoneim, Y. Fan, P. Dogra, C.W. Davis, B.T. Konieczny, R. Antia, X. Cheng, R. Ahmed, Effector CD8 T cells dedifferentiate into long-lived memory cells, *Nature*. 552 (2017) 404–409, <https://doi.org/10.1038/nature25144>.
- [18] S.C. Jameson, D. Masopust, Understanding subset diversity in T cell memory, *Immunity*. 48 (2018) 214–226, <https://doi.org/10.1016/j.immuni.2018.02.010>.
- [19] D. Cibrián Danay, CD69: from activation marker to metabolic gatekeeper, *Eur. J. Immunol.* 47 (2017) 946–953.
- [20] V.S. Fear, S.P. Lai, G.R. Zosky, K.L. Perks, S. Gorman, F. Blank, C. von Garnier, P.A. Stumbles, D.H. Strickland, A pathogenic role for the integrin CD103 in experimental allergic airways disease, *Physiol. Rep.* 4 (2016), <https://doi.org/10.14814/phy2.13021>.
- [21] V. Golubovskaya, L. Wu, Different subsets of T cells, memory, effector functions, and CAR-T immunotherapy, *Cancers*. 8 (2016), <https://doi.org/10.3390/cancers8030036>.
- [22] R.C. Budd, J.C. Cerottini, C. Horvath, C. Bron, T. Pedrazzini, R.C. Howe, H.R. MacDonald, Distinction of virgin and memory T lymphocytes. Stable acquisition of the Pgp-1 glycoprotein concomitant with antigenic stimulation, *J. Immunol. Baltim. Md 1950* (138) (1987) 3120–3129.
- [23] M.K. Jenkins, J.J. Moon, The role of naive T cell precursor frequency and recruitment in dictating immune response magnitude, *J. Immunol. Baltim. Md 1950* (188) (2012) 4135–4140, <https://doi.org/10.4049/jimmunol.1102661>.
- [24] G. Benichou, B. Gonzalez, J. Marino, K. Ayasoufi, A. Valujskikh, Role of memory T cells in allograft rejection and tolerance, *Front Immunol.* 8 (2017) 170.
- [25] N.J. Booth, A.J. McQuaid, T. Sobande, S. Kissane, E. Agius, S.E. Jackson, M. Salmon, F. Falciani, K. Yong, M.H. Rustin, A.N. Akbar, M. Vukmanovic-Stejic, Different proliferative potential and migratory characteristics of human CD4<sup>+</sup> regulatory T cells that express either CD45RA or CD45RO, *J. Immunol.* 184 (2010) 4317–4326, <https://doi.org/10.4049/jimmunol.0903781>.
- [26] Y.D. Mahnke, T.M. Brodie, F. Sallusto, M. Roederer, E. Lugli, The who's who of T-cell differentiation: human memory T-cell subsets, *Eur. J. Immunol.* 43 (2013) 2797–2809, <https://doi.org/10.1002/eji.201343751>.
- [27] M.M. Opatka, R. Stephens, Early decision: effector and effector memory T cell differentiation in chronic infection, *Curr. Immunol. Rev.* 9 (2013) 190–206, <https://doi.org/10.2174/1573395509666131126231209>.
- [28] J.M. Schenkel, D. Masopust, Tissue-resident memory T cells, *Immunity* 41 (2014) 886–897, <https://doi.org/10.1016/j.immuni.2014.12.007>.
- [29] D.J. Gasper, M.M. Tejera, M. Suresh, CD4 T-cell memory generation and maintenance, *Crit. Rev. Immunol.* 34 (2014) 121–146.
- [30] D. Wesch, T. Hinz, D. Kabelitz, Analysis of the TCR Vgamma repertoire in healthy donors and HIV-1-infected individuals, *Int. Immunol.* 10 (1998) 1067–1075, <https://doi.org/10.1093/intimm/10.8.1067>.
- [31] C. Bottino, G. Tambussi, S. Ferrini, E. Ciccone, P. Varese, M.C. Mingari, L. Moretta, A. Moretta, Two subsets of human T lymphocytes expressing gamma/delta antigen receptor are identifiable by monoclonal antibodies directed to two distinct molecular forms of the receptor, *J. Exp. Med.* 168 (1988) 491–505, <https://doi.org/10.1084/jem.168.2.491>.
- [32] S. Fonseca, V. Pereira, C. Lau, M. dos A. Teixeira, M. Bini-Antunes, M. Lima, Human Peripheral Blood Gamma Delta T Cells: Report on a Series of Healthy Caucasian Portuguese Adults and Comprehensive Review of the Literature, *Cells*. 9 (2020). <https://doi.org/10.3390/cells9030729>.
- [33] S. Nedellec, M. Bonneville, E. Scotet, Human Vγ9Vδ2 T cells: from signals to functions, *Semin. Immunol.* 22 (2010) 199–206, <https://doi.org/10.1016/j.smim.2010.04.004>.
- [34] D.F. Hoft, R.M. Brown, S.T. Roodman, Bacille Calmette-Guérin vaccination enhances human gamma delta T cell responsiveness to mycobacteria suggestive of a memory-like phenotype, *J. Immunol. Baltim. Md 1950* (161) (1998) 1045–1054.
- [35] X. Lai, Y. Shen, D. Zhou, P. Sehgal, L. Shen, M. Simon, L. Qiu, N.L. Letvin, Z.W. Chen, Immune biology of macaque lymphocyte populations during mycobacterial infection, *Clin. Exp. Immunol.* 133 (2003) 182–192, <https://doi.org/10.1046/j.1365-2249.2003.02209.x>.
- [36] L. Shen, J. Frencher, D. Huang, W. Wang, E. Yang, C.Y. Chen, Z. Zhang, R. Wang, A. Qaqish, M.H. Larsen, H. Shen, S.A. Porcelli, W.R. Jacobs, Z.W. Chen, Immunization of Vγ2Vδ2 T cells programs sustained effector memory responses that control tuberculosis in nonhuman primates, *Proc. Natl. Acad. Sci. U.S.A.* 116 (2019) 6371–6378, <https://doi.org/10.1073/pnas.1811380116>.
- [37] C. Belmant, E. Espinosa, R. Poupot, M.-A. Peyrat, M. Guiraud, Y. Poquet, M. Bonneville, J.-J. Fournié, 3-Formyl-1-butyl pyrophosphate A novel mycobacterial metabolite-activating human γδ T Cells, *J. Biol. Chem.* 274 (1999) 32079–32084, <https://doi.org/10.1074/jbc.274.45.32079>.
- [38] B. Ryan-Payseur, J. Frencher, L. Shen, C.Y. Chen, D. Huang, Z.W. Chen, Multifactorial-functional immune responses of HMBPP-specific Vγ2Vδ2 T cells in nonhuman primates inoculated with *Listeria monocytogenes* ΔactA prfA\*, *J. Immunol. Baltim. Md 1950* (189) (2012) 1285–1293, <https://doi.org/10.4049/jimmunol.1200641>.
- [39] L. Shao, D. Huang, H. Wei, R.C. Wang, C.Y. Chen, L. Shen, W. Zhang, J. Jin, Z.W. Chen, Expansion, reexpansion, and recall-like expansion of Vγ2Vδ2 T cells in smallpox vaccination and monkeypox virus infection, *J. Virol.* 83 (2009) 11959–11965, <https://doi.org/10.1128/JVI.00689-09>.
- [40] V. Pitard, D. Roumanes, X. Lafarge, L. Couzi, I. Garrigue, M.-E. Lafon, P. Merville, J.-F. Moreau, J. Déchanet-Merville, Long-term expansion of effector/memory Vδ2–γδ T cells is a specific blood signature of CMV infection, *Blood* 112 (2008) 1317–1324, <https://doi.org/10.1182/blood-2008-01-136713>.
- [41] Y. Tian, M. Babor, J. Lane, V. Schulten, V.S. Patil, G. Seumois, S.L. Rosales, Z. Fu, G. Picarda, J. Burel, J. Zapardiel-Gonzalo, R.N. Tennekoon, A.D. De Silva, S. Premawansa, G. Premawansa, A. Wijewickrama, J.A. Greenbaum, P. Vijayanand, D. Weiskopf, A. Sette, B. Peters, Unique phenotypes and clonal expansions of human CD4 effector memory T cells re-expressing CD45RA, *Nat. Commun.* 8 (2017) 1473, <https://doi.org/10.1038/s41467-017-01728-5>.
- [42] F. Sallusto, J. Geginat, A. Lanzavecchia, Central memory and effector memory T cell subsets: function, generation, and maintenance, *Annu. Rev. Immunol.* 22 (2004) 745–763, <https://doi.org/10.1146/annurev.immunol.22.012703.104702>.
- [43] F. Halary, V. Pitard, D. Dlubek, R. Krzysiek, H. de la Salle, P. Merville, C. Dromer, D. Emilie, J.-F. Moreau, J. Déchanet-Merville, Shared reactivity of Vδ2(neg) gamma}delta T cells against cytomegalovirus-infected cells and tumor intestinal epithelial cells, *J. Exp. Med.* 201 (2005) 1567–1578, <https://doi.org/10.1084/jem.20041851>.
- [44] A. Monteiro, C. Cruto, P. Rosado, A. Martinho, L. Rosado, M. Fonseca, A. Paiva, Characterization of circulating gamma-delta T cells in relapsing vs remission multiple sclerosis, *J. Neuroimmunol.* 318 (2018) 65–71, <https://doi.org/10.1016/j.jneuroim.2018.02.009>.
- [45] F. Dieli, F. Poccia, M. Lipp, G. Sireci, N. Caccamo, C. Di Sano, A. Salerno, Differentiation of effector/memory Vdelta2 T cells and migratory routes in lymph nodes or inflammatory sites, *J. Exp. Med.* 198 (2003) 391–397, <https://doi.org/10.1084/jem.20030235>.
- [46] N. Caccamo, S. Meraviglia, V. Ferlazzo, D. Angelini, G. Borsellino, F. Poccia, L. Battistini, F. Dieli, A. Salerno, Differential requirements for antigen or homeostatic cytokines for proliferation and differentiation of human Vγ9Vδ2 naive, memory and effector T cell subsets, *Eur. J. Immunol.* 35 (2005) 1764–1772, <https://doi.org/10.1002/eji.200525983>.
- [47] C. Hu, L. Qian, Y. Miao, Q. Huang, P. Miao, P. Wang, Q. Yu, H. Nie, J. Zhang, D. He, R. Xu, X. Chen, B. Liu, D. Zhang, Antigen-presenting effects of effector memory Vγ9Vδ2 T cells in rheumatoid arthritis, *Cell. Mol. Immunol.* 9 (2012) 245–254, <https://doi.org/10.1038/cmi.2011.50>.
- [48] R.D. Garman, P.J. Doherty, D.H. Raulat, Diversity, rearrangement, and expression of murine T cell gamma genes, *Cell* 45 (1986) 733–742, [https://doi.org/10.1016/0092-8674\(86\)90787-7](https://doi.org/10.1016/0092-8674(86)90787-7).
- [49] J.S. Heilig, S. Tonegawa, Diversity of murine gamma genes and expression in fetal and adult T lymphocytes, *Nature* 322 (1986) 836–840, <https://doi.org/10.1038/322836a0>.
- [50] D.J. Pang, J.F. Neves, N. Sumaria, D.J. Pennington, Understanding the complexity of γδ T-cell subsets in mouse and human, *Immunology* 136 (2012) 283–290.
- [51] A. Ezquerro, R.Q. Cron, T.J. McConnell, R.B. Valas, J.A. Bluestone, J.E. Coligan, T cell receptor delta-gene expression and diversity in the mouse spleen, *J. Immunol. Baltim. Md. 1950* (145) (1990) 1311–1317.
- [52] J. Gertner, E. Scotet, M. Poupot, M. Bonneville, J.-J. Fournié, Lymphocytes: Gamma Delta, in: John Wiley & Sons, Ltd (Ed.), *Encycl. Life Sci.*, John Wiley & Sons, Ltd, Chichester, UK, 2007: p. a0001195.pub2. <https://doi.org/10.1002/9780470015902.a0001195.pub2>.
- [53] S.P. Phalke, Y. Huang, K. Rubtsova, A. Getahun, D. Sun, R.L. Reinhardt, R.L. O'Brien, W.K. Born, γδ T cells shape memory-phenotype αβ T cell populations in non-immunized mice, *PLoS ONE* 14 (2019) e0218827, <https://doi.org/10.1371/journal.pone.0218827>.
- [54] X. Zeng, Y. Wei, J. Huang, E.W. Newell, H. Yu, B.A. Kidd, M.S. Kuhns, R.W. Waters, M.M. Davis, C.T. Weaver, Y. Chien, γδ T cells recognize a microbial encoded B cell antigen to initiate a rapid antigen specific Interleukin 17 response, *Immunity* 37 (2012) 524–534, <https://doi.org/10.1016/j.immuni.2012.06.011>.
- [55] B.S. Sheridan, P.A. Romagnoli, Q.M. Pham, H.H. Fu, F. Alonzo, W.D. Schubert, N.E. Freitag, L. Lefrancois, Gammadelta T cells exhibit multifunctional and protective memory in intestinal tissues, *Immunity* 39 (2013) 184–195.
- [56] P.A. Romagnoli, B.S. Sheridan, Q.M. Pham, L. Lefrancois, K.M. Khanna, IL-17A-producing resident memory gammadelta T cells orchestrate the innate immune response to secondary oral *Listeria monocytogenes* infection, *Proc Natl Acad Sci USA* 113 (2016) 8502–8507.
- [57] A.G. Murphy, K.M. O'Keefe, S.J. Lalor, B.M. Maher, K.H. Mills, R.M. McLoughlin, *Staphylococcus aureus* infection of mice expands a population of memory gammadelta T cells that are protective against subsequent infection, *J. Immunol.* 192 (2014) 3697–3708.
- [58] A. Krmpotic, I. Bubic, B. Polic, P. Lucin, S. Jonjic, Pathogenesis of murine cytomegalovirus infection, *Microbes Infect.* 5 (2003) 1263–1277, <https://doi.org/10.1016/j.micinf.2003.09.007>.
- [59] C. Khairallah, S. Netzer, A. Villacreces, M. Juzan, B. Rousseau, S. Dulanto, A. Giese, P. Costet, V. Praloran, J.-F. Moreau, P. Dubus, D. Vermijlen, J. Déchanet-Merville, M. Capone, γδ T cells confer protection against murine cytomegalovirus (MCMV), *PLoS Pathog.* 11 (2015) e1004702, <https://doi.org/10.1371/journal.ppat.1004702>.
- [60] C.R. Willcox, V. Pitard, S. Netzer, L. Couzi, M. Salim, T. Silberzahn, J.-F. Moreau, A.C. Hayday, B.E. Willcox, J. Déchanet-Merville, Cytomegalovirus and tumor stress surveillance by binding of a human γδ T cell antigen receptor to endothelial protein C receptor, *Nat. Immunol.* 13 (2012) 872–879, <https://doi.org/10.1038/ni.2394>.
- [61] R. Kumarasingha, L.J. Ioannidis, W. Abeysekera, S. Studniberg, D. Wijesurendra, R. Mazhari, D.P. Poole, I. Mueller, L. Schofield, D.S. Hansen, E.M. Eriksson, Transcriptional memory-like imprints and enhanced functional activity in γδ T cells following resolution of malaria infection, *Immunology* (2020), <https://doi.org/10.1101/2020.05.05.078717>.
- [62] E. Aktas, U.C. Kucuksezer, S. Bilgic, G. Erten, G. Deniz, Relationship between CD107a expression and cytotoxic activity, *Cell. Immunol.* 254 (2009) 149–154, <https://doi.org/10.1016/j.cellimm.2008.08.007>.

- [63] A.C. Teirlinck, M.B.B. McCall, M. Roestenberg, A. Scholzen, R. Woestenenk, Q. de Mast, A.J.A.M. van der Ven, C.C. Hermesen, A.J.F. Luty, R.W. Sauerwein, Longevity and composition of cellular immune responses following experimental *Plasmodium falciparum* malaria infection in humans, *PLoS Pathog.* 7 (2011) e1002389, <https://doi.org/10.1371/journal.ppat.1002389>.
- [64] L. van der Fits, S. Mourits, J.S.A. Voerman, M. Kant, L. Boon, J.D. Laman, F. Cornelissen, A.-M. Mus, E. Florencia, E.P. Prens, E. Lubberts, Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis, *J. Immunol. Baltim. Md. 1950 (182)* (2009) 5836–5845, <https://doi.org/10.4049/jimmunol.0802999>.
- [65] K. Sakai, K.M. Sanders, M.R. Youssef, K.M. Yanushefski, L. Jensen, G. Yosipovitch, T. Akiyama, Mouse model of imiquimod-induced psoriatic itch, *Pain* 157 (2016) 2536–2543, <https://doi.org/10.1097/j.pain.0000000000000674>.
- [66] F. Ramirez-Valle, E.E. Gray, J.G. Cyster, Inflammation induces dermal Vgamma4+ gammadeltaT17 memory-like cells that travel to distant skin and accelerate secondary IL-17-driven responses, *Proc Natl Acad Sci USA* 112 (2015) 8046–8051.
- [67] T. Hartwig, S. Pantelyushin, A.L. Croxford, P. Kulig, B. Becher, Dermal IL-17-producing  $\gamma\delta$  T cells establish long-lived memory in the skin, *Eur. J. Immunol.* 45 (2015) 3022–3033, <https://doi.org/10.1002/eji.201545883>.
- [68] S.J. Lalor, R.M. McLoughlin, Memory gammadelta T cells—newly appreciated protagonists in infection and immunity, *Trends Immunol.* 37 (2016) 690–702.
- [69] J.D. Haas, F.H.M. González, S. Schmitz, V. Chennupati, L. Föhse, E. Kremmer, R. Förster, I. Prinz, CCR6 and NK1.1 distinguish between IL-17A and IFN-gamma-producing gammadelta effector T cells, *Eur. J. Immunol.* 39 (2009) 3488–3497, <https://doi.org/10.1002/eji.200939922>.
- [70] M. Legut, D.K. Cole, A.K. Sewell, The promise of  $\gamma\delta$  T cells and the  $\gamma\delta$  T cell receptor for cancer immunotherapy, *Cell. Mol. Immunol.* 12 (2015) 656–668, <https://doi.org/10.1038/cmi.2015.28>.
- [71] J. Charles A. Janeway, P. Travers, M. Walport, M.J. Shlomchik, Principles of innate and adaptive immunity, *Immunobiol. Immune Syst. Health Dis.* 5th Ed. (2001). <https://www.ncbi.nlm.nih.gov/books/NBK27090/> (accessed May 19, 2020).
- [72] A. Lombes Amélie, Adaptive immune-like g/ T lymphocytes share many common features with their a/b T cell counterparts, *J. Immunol.* 195 (2015) 1449–1458.
- [73] P. Vantourout, A. Hayday, Six-of-the-best: unique contributions of gammadelta T cells to immunology, *Nat. Rev. Immunol.* 13 (2013) 88–100.
- [74] M.A. Williams, E.V. Ravkov, M.J. Bevan, Rapid culling of the CD4+ T Cell repertoire in the transition from effector to memory, *Immunity* 28 (2008) 533–545, <https://doi.org/10.1016/j.immuni.2008.02.014>.
- [75] M.S. Davey, C.R. Willcox, S.P. Joyce, K. Ladell, S.A. Kasatskaya, J.E. McLaren, S. Hunter, M. Salim, F. Mohammed, D.A. Price, D.M. Chudakov, B.E. Willcox, Clonal selection in the human V $\delta$ 1 T cell repertoire indicates  $\gamma\delta$  TCR-dependent adaptive immune surveillance, *Nat. Commun.* 8 (2017) 14760, <https://doi.org/10.1038/ncomms14760>.
- [76] A. Knight, A.J. Madrigal, S. Grace, J. Sivakumaran, P. Kottaridis, S. Mackinnon, P.J. Travers, M.W. Lowdell, The role of V $\delta$ 2-negative  $\gamma\delta$  T cells during cytomegalovirus reactivation in recipients of allogeneic stem cell transplantation, *Blood* 116 (2010) 2164–2172, <https://doi.org/10.1182/blood-2010-01-255166>.
- [77] N.D. Pennock, J.T. White, E.W. Cross, E.E. Cheney, B.A. Tamburini, R.M. Kedl, T cell responses: naïve to memory and everything in between, *Adv. Physiol. Educ.* 37 (2013) 273–283, <https://doi.org/10.1152/advan.00066.2013>.