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Regeneration of stalled immune responses to transformed and infected cells using $\gamma\delta$ T cells

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Abstract

Manipulation of the human immune system is becoming more of a therapeutic focus as a treatment option or complement. Prominent examples are the increasing use of monoclonal antibodies in combating malignant tumours, and the numerous adoptive immunotherapy trials underway. One important aspect of any use of the human immune system in this regard is to harness the power of professional antigen-presenting cells (pAPC), that is, dendritic cells (DC), to direct immune responses. Here, we review how recent findings regarding the biology of $\gamma\delta$ T cells have revealed that they, surprisingly, could serve as convenient tools for this purpose, in that they combine innate cytotoxic cell and pAPC functions in one cell type, with potential benefits in cancer immunotherapy and infectious disease.

The immune system evolved along two paths, the innate and the adaptive. The innate immune system originated early during animal evolution, but has retained some original principles of antigen recognition [1]. These include pathogen-recognising receptors that might or might not directly lead to phagocytosis of the recognised intruder [2]. Modern human examples of this system include receptors on endocytosing and/or phagocytosing cells, such as Fc receptors for immunoglobulins and dectin-1 [3]. The innate immune system also recognises ‘stress ligands’ on self, indicating that a cell is infected or transformed [4]. This recognition of an infected or transformed cell by an innate cytotoxic cell (e.g. a natural killer cell (NK cell)) can lead to the killing of the target cell by induction of apoptosis or outright lysis of the cell [5]. Material from the killed cell is then available for uptake by other innate members of the immune system, such as macrophages (Mf) and DC. In a second layer of recognition by the innate immune cell players, pattern-recognition receptors, such as toll-like receptors (TLR), relay ‘danger’ signals to the endocytosing and/or phagocytosing cell, determining how the cell should respond and relay information to lymphocytes [6]. The cell and/or pathogen material taken up by the endocyte and/or phagocyte is then normally degraded, which, in the case of proteins, is into short peptides.

At some point(s) during early vertebrate evolution, the high diversity of some receptors on lymphocyte-like cells developed, enabling the formation of specialisation of lymphocyte reactivity against pathogens and transformed cells, as well as the ability to form memory cells. In one lineage forming the ancestors of humans, the jawed vertebrates, this resulted in the highly diverse T cell receptors (TCR) and immunoglobulins in the form of B cell receptors as well as secreted antibodies. To enable the TCR to recognise antigens (foreign as well as self), these are presented as peptides in specialised pockets in major histocompatibility complex (MHC) molecules on the surface of highly specialised endocytosing and/or phagocytosing cells called pAPC. What distinguishes pAPC from other potentially APCs (e.g., epithelial or endothelial) is their ability to degrade protein antigens in specialised compartments, process these to peptides of suitable lengths, and load the resulting peptides on MHC I- and MHC II-type molecules for transport to the cell surface and presentation to naïve specific antigen-inexperienced TCR-positive T cells [7]. This TCR-mediated recognition of antigens also helps to instruct the formation of antigen-specific antibodies and, thus, forms the backbone of what is referred to as the 'adaptive immune system'.

Both of these arms of the current human immune system take part in the formation of normal vaccine responses, typically using antigen complexes in combination with formulated adjuvants, as well as in current cancer immunotherapeutic attempts to form a response against, and direct the eradication of, established tumours. Cancer immunotherapy is still in its infancy, but has shown already in human clinical trials to have significant therapeutic benefits for patients with established cancers.

Modern cancer immunotherapeutic protocols, as well as vaccination formulations, typically make use of DC as pAPC. In this review, we outline recent realisations about the major impact that human $\gamma\delta$ T cells might have on how molecular information associated with infections, as well transformed cells, could be relayed through $\gamma\delta$ T cells in these contexts. We also highlight how $\gamma\delta$ T cells appear, remarkably, to combine specific innate killing mechanisms, with specific endocytic and/or phagocytic uptake, antigen processing and antigen loading on MHC molecules for presentation to other T cells, including naïve T cells. These responding T cells then become activated, proliferate and, in turn kill, the specific target. This new knowledge highlights areas where future drug targeting and interventions could be attempted and developed.

Are $\gamma\delta$ T cells, T cells? The classic view

Phenotypic signatures of $\gamma\delta$ T cells and comparisons to $\alpha\beta$ T cells: a historic perspective

It was hypothesised during the early 1970s that T cells must have polymorphic molecules that can distinguish between different antigens when presented to them by APC and, therefore, were likely to have polymorphic receptors for this purpose. In 1985 and 1986, Susumu Tonegawa and colleagues, and Tak Mak and colleagues published the first isolation and structural determination of the genes encoding the TCR on α , β as well as γ chains [8–10]. Over the following years, the working assumption was that the function and mode of antigen recognition of the α – β and the γ – δ pairs of TCR would be the same. However, by the early 1990s, it became clear that, whereas $\alpha\beta$ T cells recognise an antigen in the form of a

peptide as presented by an MHC molecule on APC, the $\gamma\delta$ T cells appeared to recognise antigens in the absence of any MHC presentation [11]. This discrepancy notwithstanding, the view that $\gamma\delta$ T cells were 'normal' T cells prevailed.

This view was further supported by the realisation that $\gamma\delta$ T cells exist as subtypes that are phenotypically reminiscent of their $\alpha\beta$ T cell counterparts [i.e., naïve, central memory (T_{CM}), effector memory (T_{EM}) and CD45RA-positive effector memory (T_{EMRA})] [12]. In addition, they were found to take on phenotypic as well as functional differentiation pathways and develop into specialised T helper (Th)1, Th2, Th17, follicular helper T (T_{fh}) and possibly T suppressor-like cells (T_{reg}), again in parallel to their $\alpha\beta$ T cell counterparts [13]. Furthermore, these specialisations appeared to be directed by the same cytokine programming as in $\alpha\beta$ T cells (Table 1). However, most significantly and tellingly, $\gamma\delta$ T cells can kill cellular (and possibly free pathogen) targets. They establish close contact with cellular targets, after which they activate cytotoxic mechanisms, including both cell membrane-anchored Fas ligand (FasL; CD95L) and its companion Fas (CD95) on a target cell [14]. In addition, they are also able to secrete perforin and granulysins from intracellular granules when encountering a cell destined for a cytotoxic response, thus taking part in killing a target [15].

Targeting functions of $\gamma\delta$ T cells

So, what are the target molecules recognised on the target cells by cytotoxically active $\gamma\delta$ T cells? Whereas cytotoxic $\alpha\beta$ T cells kill following recognition of specific peptide antigens on a target cell in the context of MHC molecules, $\gamma\delta$ T cells appear to recognise foreign as well as 'self' molecular structures serving as 'stress' signals without help from MHC [16,17]. A clear picture of targets does not exist, either in humans or in model animals (e.g., mice). However, in humans, what is known so far about the target molecules can be divided along the lines of the TCR that are used by $\gamma\delta$ T cells. For example, $\gamma 1\delta 1$ TCR receptors appear to contact nonclassic MHC-like structures on targets, such as MHC class I polypeptide-related sequence A and B (MICA and MICB), which interestingly tend to be expressed in response to 'stress' in cells [18,19]. Humans, and other Old World primates also express a $\gamma 9\delta 2$ TCR chain pair. The $\gamma 9\delta 2$ TCR appears to have specialised in recognition of phosphoantigen molecules, such as (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP), which are a component of bacterial cell walls [20]. Similar pyrophosphates can also be produced as part of the human eukaryotic mevalonate cholesterol pathway (isoprenyl pyrophosphate (IPP)), notably when cells are under stress [21]. Recently, studies showed that such phosphoantigens can bind to a pocket on the butyrophilin 3 molecule (CD277) on a target cell, enabling presentation of the phosphoantigen to $\gamma 9\delta 2$ TCR on $\gamma\delta$ T cells [22]. How this recognition relates to the recognition of bacterial IPP is unclear. In addition, other human TCR chain combinations occur and are functionally important, but their specificities are less well understood. These TCR chain gene numbers and their usages are less numerous than $\alpha\beta$ T cell TCR genes and their products. However, this does not necessarily exclude the possibility that their individual chain specificities can lead to variation between $\gamma\delta$ T cells within each chain combination (e.g. $\gamma 1\delta 1$ and $\gamma 9\delta 2$).

Immunoregulatory functions of $\gamma\delta$ T cells

Other important functions of $\gamma\delta$ T cells are likely to centre on their known immunoregulatory activities, in combination with their innate and TCR-mediated cytotoxic activities. This has been assumed to be achieved partly through their cytokine production and molecular interactions with myeloid cells (e.g., DC) and neutrophils. Although present in human blood at frequencies from 1% to 10% of CD3+ cells in normal individuals, they can reach frequencies of over 20% of CD3+ cells in individuals with infectious diseases (e.g., bacterial and parasitic in nature) [23]. However, they are most numerous in different mucosal epithelia, such as in the intestine, where their number and type have been found to correlate and be associated with autoimmune conditions, such as coeliac disease [24]. In addition, other autoimmune conditions also appear to affect $\gamma\delta$ T cell numbers [23].

$\gamma\delta$ T cell targeting of tumour cells

When investigators started to look at the reactivity of $\gamma\delta$ T cells with different tumour cell lines, it soon became clear that some tumour-specific antibodies increased the cytotoxic $\gamma\delta$ T cell responses against the tumour cells. In 1992, Sandor *et al.* hypothesised that the presence of Fc receptors on $\gamma\delta$ T cells might lead to their ability, similar to NK cells, to assist antibody-dependent cellular cytotoxicity (ADCC) [25]. However, it was not until well into the new millennium before conclusive studies showed that ADCC was an integral part of $\gamma\delta$ T cell function, potentially both in autoimmune diseases [26] and in cytotoxic responses against tumour cells [27]. In the latter case, this realisation was not made until humanised monoclonal antibodies (MAb) with tumour cell specificity, such as rituximab, were tested. One reason for this might be that human CD16 does not bind some mouse antibody isotypes well, if at all. Capietto and colleagues, in 2011, then showed that this effect can also be seen *in vivo* in HER2+ breast cancer cells [28].

Cancer immunotherapy using $\gamma\delta$ T cells

Cellular immunotherapy aims to enhance or modify pre-existing subsets of immune effector cells to generate enhanced antitumour responses. The potential of many cell types, including tumour-infiltrating lymphocytes (TILs), NK cells and engineered $\alpha\beta$ T cells, has been investigated. Clinical trials of $\gamma\delta$ T cell-based cellular immunotherapy are in early phases, and capitalise on the ease of expanding the V δ 2+ subset of $\gamma\delta$ T cells using phosphoantigen stimulation in combination with interleukin 2 (IL-2). This kind of immunotherapy relies on the ability to expand $\gamma\delta$ T cells to functionally useful numbers and retain their antitumour cytotoxicity, and trials fall into two categories: those that expand a patient's $\gamma\delta$ T cells *ex vivo* and reinfuse them as part of adoptive transfer protocols [29–33], and those that use drug treatments to enrich a patient's $\gamma\delta$ T cell population *in vivo* [34–37]. *In vivo* expansion is achieved using the combination of IL-2 and an aminobisphosphonate, such as zoledronic acid. Aminobisphosphonates are inhibitors of farnesyl pyrophosphate synthase and lead to a build-up of upstream metabolites, such as IPP, in treated cells. Given the low EC₅₀ of zoledronic acid for $\gamma\delta$ T cell activation (EC₅₀ = 0.003 μ M), a concentration easily achievable using standard dose infusions, this is a feasible means of $\gamma\delta$ T cell stimulation. *In vitro* expansion is usually achieved using synthetic phosphoantigens, such as BrHPP, although *in vitro* zoledronic acid treatment also yields high numbers of V δ 2+ cells. Typically, $\gamma\delta$ T cell-

based immunotherapy has been trialled in immunogenic tumours, such as renal cell carcinoma, non-small cell lung cancer and melanoma, but other cancer types, including breast, prostate and haematological malignancies, have also been treated. Clinical responses vary; patients whose $\gamma\delta$ T cells mount a good proliferative response to stimulation *in vitro* have better outcomes than those who do not, a factor that has been considered an inclusion criteria for some trials. It is hard to draw firm conclusions about the efficacy of $\gamma\delta$ T cell-based immunotherapy because no formal comparisons have been made with established treatments, but results are promising [38].

Are $\gamma\delta$ T cells innate NK-like lymphocytes with a TCR? The alternative hypothesis

The NK cell link

Two discoveries have a potentially important impact on our understanding and ability to utilise $\gamma\delta$ T cells in drug discovery and new treatment modalities in both infectious disease and cancer. The first is the realisation that $\gamma\delta$ T cells express more activating as well as inhibiting NK cell-type receptors compared with their $\alpha\beta$ TCR+ counterparts [39]. This appears to be true for both nonclonotypic NK cell-type receptors, such as NKG2A-D, as well as for killer immunoglobulin-like receptors (KIR), which are expressed clonotypically on NK cells; by inference, it is reasonable to hypothesise that this will also be the case on $\gamma\delta$ T cells. The second discovery is that $\gamma\delta$ T cells can take up antigens from their surrounding and from cellular targets and present these on MHC molecules to other T cells. We concentrate the rest of this review on this relatively novel $\gamma\delta$ T cell APC concept and describe how this finding has now become the focus of $\gamma\delta$ T cell work in some laboratories.

$\gamma\delta$ T cells are endocytosing and/or phagocytosing professional APCs

In 1998, Collins *et al.* showed that cattle $\gamma\delta$ T cells can present soluble antigens to CD4+ T cells via MHCII [40]. They noted that this was concomitant with the expression of co-stimulatory molecules and, therefore, hypothesised that they operate as a 'proper' APC. Following similar findings in porcine $\gamma\delta$ T cells [41], Brandes *et al.* demonstrated that human $\gamma\delta$ 2T cells can take up protein antigens, process and present peptides via MHCII to naïve CD4+ $\alpha\beta$ T cells, while upregulating co-stimulatory molecules [42]. What appeared to control the increased expression of MHCII, as well as co-stimulatory molecules was the short-term co-culture of the $\gamma\delta$ 2T cells with IPP. This finding was followed by evidence from the same laboratory that $\gamma\delta$ 2T cells were also able to cross-present exogenous soluble protein antigens to CD8+ cytotoxic T cells via MHCI [43,44]. Endosomal acidification, following antigen uptake, was shown to be significantly delayed compared with DC uptake, suggesting that $\gamma\delta$ 2T cells are particularly suited to cross-present peptides on MHCI. In addition, mouse $\gamma\delta$ T cells have also been shown to take up, process and present soluble antigens on MHCII [45]. However, it is unclear to what extent and how easily mouse $\gamma\delta$ T cells are able to upregulate MHCII and co-stimulatory molecules.

Given that human $\gamma\delta$ T cells express differing levels of the Fc γ RIII (CD16) receptor for immunoglobulin (Ig)G immune complexes, and that CD16 is involved in receptor-mediated endocytic (<0.5 μ m complex size) and phagocytic (>0.5 μ m complex size) uptake on some

myeloid cells, we investigated whether large opsonised particulate matter could also be taken up by $\gamma\delta$ T cells. By using fluorescently labelled *Escherichia coli* bacteria, as well as fluorescently labelled synthetic latex beads (1 μ m size), in combination with confocal microscopy, we showed that both are taken up by small numbers of freshly purified (from peripheral blood) and short-term cultured TCR+ $\gamma\delta$ 2T cells [46]. Transmission electron microscopy studies confirmed that $\gamma\delta$ TCR-positive cells had taken up whole *E. coli* bacteria. We then showed that protein antigens (influenza M1 protein) attached to 1- μ m latex beads could be processed and presented via MHCII to M1-specific T cell hybridoma cells, following opsonisation and uptake by the purified $\gamma\delta$ 2T cells. Using cytochalasin D and chloroquine inhibitors during the uptake, we concluded that the uptake was dependent on actin reorganisation compatible with phagocytosis, and on pH-dependent degradation and processing of the M1 for MH II loading. In addition, blocking experiments showed that CD16 molecules appeared to be crucial for the uptake.

$\gamma\delta$ T cells take up cellular target material and process and present associated antigens on MHC molecules

Inspired by the novel finding that $\gamma\delta$ T cells take up bacteria and large immune complexes, and process associated protein antigens for presentation on MHC to other T cells, we then turned to cells as targets for the $\gamma\delta$ T cell uptake mechanism, because they are the classic target for $\gamma\delta$ T cell cytotoxicity. B lymphoma cell lines, such as Daudi and Raji, are known to become apoptotic after opsonisation with a MAb in clinical use, Rituximab (Rxb), directed against CD20 on B cells. In addition, these MAb are known to support ADCC by $\gamma\delta$ T cells [27]. We stained Daudi lymphoma cells fluorescently, using different labels, and observed $\gamma\delta$ T cell uptake of the fluorescing Daudi material following Rxb opsonisation [47]. In one experiment, depicted in Fig. 1, we used red fluorescence to stain the Daudi cells, and then observed uptake into the purified $\gamma\delta$ 2T cells together with nuclear DAPI and green phalloidin actin staining. It is noteworthy how part of the large typical $\gamma\delta$ T lymphocyte nucleus is indented during the formation of a vacuole as a result of the uptake. We also wanted to ask whether, following the antibody-mediated lysis by $\gamma\delta$ T cells of the tumour cell targets, tumour-associated antigens (TAA) could be taken up by the $\gamma\delta$ T cells, processed and cross-presented to CD8+ cytotoxic $\alpha\beta$ T cells by MHCI on the $\gamma\delta$ T cells. We showed that this was indeed the case and that this cross-presentation was dependent on, or at the very least strongly enhanced by, the presence of IgG antibodies interacting with CD16 receptors on the $\gamma\delta$ T cells. We proposed that this ‘licensing’ by antibody is a mechanism whereby the adaptive pAPC properties of $\gamma\delta$ T cells are induced by their innate killing and uptake functions in a spatially and temporally controlled manner [48].

$\gamma\delta$ T cells are combined innate killer cells and pAPCs

Consequently, as outlined in Fig. 2, this suggests that, in an *in vivo* situation, $\gamma\delta$ T cells might be able to: (i) kill a tumour or infected cell target; (ii) take up lysed or fragmented material from the target cell by mechanisms resembling receptor-mediated (i.e., CD16) endocytosis and/or phagocytosis; (iii) shuttle the antigenic cargo to endosomal, lysosomal and proteasomal locations where the protein antigens are degraded, processed and loaded on both MHCI and II; and (iv) present at the cell surface, by respective restricting of the MHC molecule to CD8+ cytotoxic $\alpha\beta$ T cells, as well as CD4+ helper $\alpha\beta$ T cells. Although not yet

conclusive, given that several groups have shown that both MHCI and II are expressed and able to present antigens that $\gamma\delta$ T cells have taken up, it is reasonable to suggest that $\gamma\delta$ T cells, similar to DC, organise and possibly mediate CD4+ $\alpha\beta$ T cell help by way of MHCII presentation to the CD8+ cytotoxic $\alpha\beta$ T cells that receive cross-presentation from the $\gamma\delta$ T cells. It is also clear that this cross-presentation might operate as specialised cross-priming of truly naïve $\alpha\beta$ T cells, as suggested by Brandes and colleagues [49]. Furthermore, although speculative at this stage, it is also reasonable to suggest that the $\gamma\delta$ T cell orchestration of the TAA presentation to CD8+ cytotoxic T cells, assisted by CD4+ helper T cells and initiated by the $\gamma\delta$ T cell killing of specific tumour cells, results in increased specific killing of tumour cells by the resulting activated cytotoxic $\alpha\beta$ T cells. If so, $\gamma\delta$ T cells would constitute ideal tools in novel forms of immunotherapies.

Use of $\gamma\delta$ T cells in regenerative immunotherapies: promise, potential and uncertainty

We suggest that future regenerative immunotherapies could be devised using $\gamma\delta$ T cells against both infected cells, tumour cells and, conceivably, as tools for tolerance induction in autoimmunity as well as transplantation. Previous clinical studies have shown that *in vivo* expansion and activation of $\gamma\delta$ T cells appear to be well tolerated. In addition, *ex vivo* expansion and adoptive transfer serves as a viable alternative modality for such therapies. However, we need to understand what governs the $\gamma\delta$ T cells ability to direct activation or tolerisation of the $\alpha\beta$ T cells that they interact with and present specific antigens to. Here, they are again likely to operate in similar ways to DC, but the opportunity to intervene and manipulate $\gamma\delta$ T cells is greater than with DC for several reasons: first, they can be greatly expanded *ex vivo*. Second, their ability specifically to kill by a combination of NK cell-type receptors, antibody bound to their Fc receptors and their TCR, might allow for greater specificity and molecular manipulation, followed by expansion. Such gene manipulation could include altering the expression of co-stimulatory molecules, thereby differentially directing responding $\alpha\beta$ T cells towards activation and cytotoxicity or alternatively inhibition and/or suppression. Finally, the seemingly restricted and demanding means of uptake of antigens by the $\gamma\delta$ T cell, following antibody opsonisation and TCR engagement, might enable TAA and other antigens to be administered to the $\gamma\delta$ T cells in a relatively specific way, which might be possible to replicate in an *in vivo* setting.

Concluding remarks

In both cancer and chronic infections, current treatment options centre on helping to eradicate tumour cells and pathogens by the administration of chemotherapy, radiotherapy, antibiotics and antiviral drugs. Although these means directly target the cause of the disease, some have adverse effects. Given that the human immune system can potentially perform this function in some cases where a tumour or an infectious disease is successfully defeated, the idea with immunotherapy becomes to help the immune system to restart a 'stalled' or inadequate immune response against the tumour or pathogen. We and others are hopeful that $\gamma\delta$ T cells can have a prominent part in such efforts. We have argued here that their remarkable ability to kill infected and transformed cells in combination with the uptake of associated TAA or pathogen antigens and the presentation of these in an activating, or indeed tolerising, fashion to other T cells means that they constitute an exciting and

promising new tool in immunotherapies. The fact that they can also be expanded *ex vivo* in large numbers means that they have potential advantages over DC, and this serves as an opportunity also to manipulate them *ex vivo* for dedicated purposes. Thus, we suggest that immunotherapies of different types could be revolutionised and would result in the regeneration of ‘stalled’ immune responses in cancer and infectious disease, as an adjunct therapy or as a stand-alone immunomodulating approach.

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FIGURE 1.

Confocal microscopy images depicting $\gamma\delta$ T cell uptake of B lymphoma target material. The cartoon illustrates the experimental set-up, using rituximab antibodies against CD20 on the Daudi lymphoma cell surface also binding CD16 Fc receptors on the $\gamma\delta$ T cells in conjunction with $\gamma\delta$ TCR receptors binding their targets on the Daudi cells. The co-incubation then appears to result in red Daudi material being taken up into the green $\gamma\delta$ T cells. The confocal images show staining of the same $\gamma\delta$ T cells with (a) green phalloidin staining actin filaments, (b) blue DAPI staining nuclei, (c) magenta staining $\gamma\delta$ TCR (in characteristic lipid raft-associated nanodomains) and (d) red Daudi material taken up into vacuolar structures in the $\gamma\delta$ T cells. White lines indicate 1.5 μ m scale.

FIGURE 2.

Cartoon illustrating current hypotheses about the highly plastic $\gamma\delta$ T cell function and behaviour. **(a)** $\gamma\delta$ T cells kill an infected or transformed target cell (green), and efficiently so only if specific antibodies, suitable natural killer (NK) cell receptor pairings and $\gamma\delta$ TCR targets are present. **(b)** Specific $\gamma\delta$ T cells (pink) from this interaction can then form memory cells that can be recalled for future encounters. **(c)** The target cell encounter, if efficient and in the presence of ‘licencing’ antibodies, then results in $\gamma\delta$ T cell uptake (endocytic, micropinocytotic or phagocytic) of target cell material into endosomes and/or phagosomes in the $\gamma\delta$ T cells, resulting in degradation and loading of target cell peptides onto $\gamma\delta$ T cell major histocompatibility complex (MHC) class I and MHC class II molecules. **(d)** The MHCII presentation is then able to ‘help’ the activation and cross-priming and/or presentation of MHCI-bound target cell peptides to CD8+ cytotoxic T cells. **(e)** The cross-priming and/or presentation of target cell peptides to the CD8+ cytotoxic T cells is assisted by co-stimulatory molecule upregulation and expression. **(f)** We then postulate that this

leads to increased specific recognition and killing ability of the cytotoxic T cells of the specific target cells.

TABLE 1Phenotypic and functional differences between ‘classic’ $\gamma\delta$ T cells and $\gamma\delta$ T pAPC

‘Classic’ $\gamma\delta$ T cells	$\gamma\delta$ T pAPC	Refs
Phenotype		
Naïve: CD27+, CD62L+ and CD45RA+	MHCI and II high, and CD16+	[12,13]
Central memory: CD27+, CD62L+ and CD45RA–	CD80, CD86 and CD40 high	[16,41]
Effector memory: CD27–, CD62L– and CD45RA–		[45,46]
Effector memory RA+: CD27–, CD62L–, CD45RA+ and CD16+		
Cytokine and/or other programming		
Th1–, Th2–, Th17–, Tfh–, Treg– (?) type $\gamma\delta$ T cells	$\gamma\delta$ T pAPC	
IL-2, IL-4, IL-17, IL-21, TGF β , IL-10 or IL-15 (?)	TCR and/or FcR ligation	[13]
Function		
Cytotoxicity, immunoregulation and cytokine production	Antigen uptake processing, presentation and immunoregulation, and cytokine production	[11,16,41,42,45,46]