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Six-of-the-best: unique contributions of $\gamma\delta$ T cells to immunology*

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Preface

$\gamma\delta$ T cells are a unique and conserved population of lymphocytes that have been the subject of a recent explosion of interest owing to their essential contributions to many types of immune response and immunopathology. But what does the integration of recent and long-established studies really tell us about these cells and their place in immunology? The time is ripe to consider where strong evidence exists for their unique and critical functions. We conclude that whereas $\alpha\beta$ T cells and B cells are commonly viewed as contributing primarily to antigen-specific effector and memory phases of immunity, $\gamma\delta$ T cells are distinct in that they combine conventional adaptive potentials, inherent in their T cell receptors (TCRs) and pleiotropic effector functions, with rapid, innate-like responses that place them in the initiation phase of immune reactions. This underpins a revised perspective on lymphocyte biology and the regulation of immunogenicity.

Introduction

As their name indicates, $\gamma\delta$ T lymphocytes develop largely in the thymus, generating their defining receptor via RAG-mediated V(D)J recombination. The resulting potential for diversity in the $\gamma\delta$ T cell receptor (TCR) and the consequent capacity for shaping the T cell repertoire via clonal expansion appropriately assign $\gamma\delta$ T cells to the adaptive immune compartment¹. Furthermore, there are striking connections between $\gamma\delta$ T cells and $\alpha\beta$ T cells. For example, the TCR δ locus in mice and in humans is embedded within the TCR α locus, and some TCR-V gene segments can be utilised interchangeably by TCR α or TCR δ . Moreover, a common thymic progenitor may give rise to either $\alpha\beta$ or $\gamma\delta$ T cells², although this does not exclude the possibility that distinct subsets of $\gamma\delta$ and $\alpha\beta$ T cells arise from qualitatively discrete progenitors, as indicated in Figure 1. Indeed, new findings relevant to this issue will be reviewed later in this article.

Within the adaptive compartment it seems facile to accept the complementary value of B cells, that can secrete their antigen receptors as antibodies, and $\alpha\beta$ T cells, that use cell-

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bound TCRs to induce cytolytic responses and helper functions. However, it is less easy to envision the selective pressure(s) that have over 420 million years sustained the co-existence of two lineages of T cells ($\alpha\beta$ and $\gamma\delta$) with surface-bound TCRs. The nihilistic view is that no such selective pressure currently exists, and that $\gamma\delta$ T cells are en route to extinction, having been superseded by an extraordinarily potent $\alpha\beta$ T cell compartment. Conversely, the recent increase in the study of $\gamma\delta$ T cells has added to the established literature in providing conspicuous cases of non-redundant $\gamma\delta$ T cell activities. Furthermore, the lamprey, an extant but primitive jawless vertebrate, uses RAG-independent mechanisms to generate an adaptive immune compartment that is also characterised by three distinct receptors with diverse potential, of which one is secreted and two are cell-surface bound³. Hence, this type of tripartite organization may be optimal for adaptive immune function.

In this light, we shall consider six properties that may collectively distinguish $\gamma\delta$ T cells from $\alpha\beta$ T cells, and thereby define their unique contributions to lymphocyte biology: one, that $\gamma\delta$ TCRs recognise qualitatively distinct antigens; two, that $\gamma\delta$ T cells contribute to immune responses with distinct kinetics; three, that $\gamma\delta$ T cells have unique functional potentials; four, that $\gamma\delta$ T cells are particularly suited to the protection of defined anatomical sites; five, that $\gamma\delta$ T cells are of primary value in young animals; and six, that $\gamma\delta$ T cells, although not invariably important, mediate critical responses to specific pathogens, in a manner similar to natural killer (NK) cells. Because $\gamma\delta$ T cells comprise heterogeneous subsets, these six properties will not apply equally to all $\gamma\delta$ T cells. Accepting this point, we consider here the evidence for each property, and its potential to explain the conservation of $\gamma\delta$ T cells.

$\gamma\delta$ TCRs recognise distinct antigens

Anatomical distribution of $\gamma\delta$ T cells

The anatomical localization of lymphocytes has profound implications for their antigen specificity. Thus, the clonal selection and expansion of $\alpha\beta$ T cells with very rare specificities relies on the fact that following egress from the thymus, naïve $\alpha\beta$ T cells home to the lymph nodes (LNs) and to the T cell zones of the spleen where they regularly encounter vast numbers of dendritic cells (DCs) presenting diverse antigens. While some $\gamma\delta$ T cells home to the LNs, many migrate directly to tissues such as the epidermis (in murine species), the dermis, the intestine, the lung and the uterus. Moreover, by contrast to $\alpha\beta$ T cells, splenic $\gamma\delta$ T cells are not confined to the lymphoid areas (the white pulp) but are also found throughout the red pulp⁴. Sequestration of $\gamma\delta$ T cells within tissues is incompatible with their sampling diverse antigens and the consequent clonal expansion of very rare cells. Consistent with this is the limited TCR diversity of many tissue-resident $\gamma\delta$ T cells, which in the murine epidermis or uterus are essentially monoclonal⁵. This implies that these cells recognise either pathogen-encoded antigens that are predictably encountered in specific tissues, or for self-encoded molecules reflecting a dysregulated state of that tissue.

The expression of a monoclonal, RAG-generated receptor by the majority of $\gamma\delta$ T cells in a specific compartment and its use to engage only one or few antigens was unprecedented prior to studies of murine skin $\gamma\delta$ T cells known as DETC (Dendritic Epidermal T cells)⁶. By permitting large numbers of T cells to be rapidly activated and their function mobilised without a requirement for prior clonal expansion, the mono- or oligo-clonal use of a TCR represents a profound cross-over of adaptive into innate immunity: hence the term “innate-like”. Since the seminal work on DETC, evidence has accrued for numerous other “innate-like” $\gamma\delta$ T cells including many within the predominant human peripheral blood V γ 9V δ 2⁺ compartment. In sum, anatomical considerations suggest that $\gamma\delta$ T cells are divisible into lymphoid-homing $\gamma\delta$ T cells that may be primed in the circulation and clonally expand in a

conventional adaptive fashion, and innate-like cells that respond rapidly and at relatively high frequency in many different sites.

Adaptive TCR specificities

$\gamma\delta$ TCRs are not restricted to the recognition of peptides complexed to MHC, thus distinguishing them from the great majority of $\alpha\beta$ T cells. Furthermore, the diversity in length of the CDR3, which is conferred particularly by the architecture of the *TCRD* locus, suggests that the $\gamma\delta$ TCR is not structurally constrained by the recognition of cargo presented by some specific presenting element.

Instead, an antibody-like breadth in antigen recognition by the $\gamma\delta$ TCR is suggested by the recent demonstration that some human, murine and bovine $\gamma\delta$ TCRs can bind to phycoerythrin (PE), an algal molecule readily recognised by B cells⁷. PE binding induces $\gamma\delta$ T cells to upregulate CD44, to downregulate CD62L expression, and to express cytokines, as happens when naïve $\alpha\beta$ T cells are primed. Hence, this response to nominal exogenous antigen seemingly illustrates an adaptive potential of $\gamma\delta$ T cells. However, compared to the priming of $\alpha\beta$ T cells, PE-reactive $\gamma\delta$ T cells showed conspicuously less clonal expansion, which is a defining parameter of delayed antigen-specific adaptive responses. Thus, even in this case, the functional $\gamma\delta$ T cell response was rapidly mobilised with innate-like kinetics. Moreover, the cells quickly acquired an innate-like capacity to respond to inflammatory cytokines in the absence of further antigen.

The conversion of antigen-specific naïve cells into more rapidly-responsive memory cells is a key criterion of adaptive immunity. In this regard, BCG vaccination induced mycobacteria-specific $\gamma\delta$ T cells with memory characteristics in macaques and in cattle⁸⁻¹¹. Additionally, immunoprotective murine $\gamma\delta$ cells reactive to a Herpes Simplex virus glycoprotein were obtained from infected mice¹². Nonetheless, most attempts to evoke antigen-specific $\gamma\delta$ T cells following deliberate immunization or infection of mice have conspicuously failed, even when polyclonal $\gamma\delta$ T cell responses were induced. Hence, much of $\gamma\delta$ T lymphocyte biology is not captured by the conventional concept of adaptive immunity.

Self-reactivity

Few $\gamma\delta$ TCR specificities have been deduced; even fewer are supported by biochemical binding data; and of these the general representativeness is not always clear (Table 1). Nonetheless, as hypothesized almost 25 years ago¹³, several $\gamma\delta$ TCRs are reactive either to self-MHC molecules independently of their cargo or to MHC-related proteins, including murine T10/T22, a non-peptide binding MHC class Ib molecule^{14, 15}; the murine MHC class II molecule I-E^k, but with the epitope lying outside the peptide-binding groove¹⁶; and human HLA (P. Fisch, *personal communication*). It has also been reported that some human $\gamma\delta$ TCRs engage members of the MHC class I-like MICA and ULBP families (see below)^{17, 18}. Recently, the TCR from a $\gamma\delta$ T cell clone derived from a CMV-infected transplant patient was shown to directly bind endothelial protein C receptor (EPCR), a lipid carrier with similar structure to CD1, although again $\gamma\delta$ TCR engagement was cargo independent¹⁹. This evoked an earlier report, albeit lacking biochemical data, that CD1c was a ligand for several human V δ 1⁺ T cell clones²⁰.

Other $\gamma\delta$ T cells are specific for antigen presented by MHC or MHC-related molecules. For example, in healthy individuals, V δ 1⁺ cells (which are more prevalent in tissues than in the peripheral blood) compose the majority of T cells reactive to CD1d tetramers loaded with sulphatide, a myelin glycosphingolipid²¹. Also, several $\gamma\delta$ T cell clones derived from diabetes-prone NOD mice respond to the insulin-derived peptide B:9–23, which is also

presented to CD4⁺ αβ T cells by disease-associated MHC class II molecule I-Ag7²². However, the nature of peptide recognition by γδ T cells was quite distinct from that of αβ T cells, in that MHC was not required. Thus, γδ TCRs and αβ TCRs have qualitatively distinct modes of antigen recognition, that in some cases may provide complementary means to detect a single target. γδ T cells therefore increase the scope of lymphocyte recognition.

Cross-reactivity

Some antigens appear to be unique targets of γδ cells. Thus, low molecular mass alkyl diphosphates termed phosphoantigens or phosphoagonists are the prototypical, naturally occurring moieties recognized by Vγ9Vδ2⁺ cells, the predominant subset of γδ T cells in peripheral blood. The most potent is hydroxymethyl-but-2-enyl-pyrophosphate (HMBPP), an intermediate in the alternative deoxyxylulose (non-mevalonate) pathway of cholesterol synthesis that is used by numerous bacterial species and by some highly significant eukaryotic pathogens, notably *Plasmodium* spp., but not by vertebrate cells²³. However, Vγ9Vδ2⁺ T cells are also activated by isopentenyl pyrophosphate (IPP)²⁴, which is an intermediate in a part of the mevalonate pathway that is conserved in prokaryotes and eukaryotes. Hence IPP is a self-antigen.

Given this cross-reactivity of human Vγ9Vδ2⁺ T cells to foreign and self-phosphoantigens, there is understandable interest in elucidating how TCR-signalling can be induced by such small molecules. Phosphoantigens can directly activate Vγ9Vδ2⁺ T cells, but such activation is greatly enhanced by monocytes²⁵. Thus, either phosphoantigens are presented as cargo to γδ TCRs, or their cellular processing somehow sensitises cells to recognition by Vγ9Vδ2 TCRs, for example by stabilising surface expression of a TCR-binding ligand. A candidate molecule involved in intracellular phosphoantigen processing is the F1-ATPase, which was reported to directly bind a Vγ9Vδ2 TCR, and which may also interact with ApppI, an adenosine derivative of IPP²⁶⁻²⁸. Furthermore, IPP–ApppI interconversion may be catalysed by an aminoacyl tRNA synthetase²⁹, which is interesting given that a conformational epitope on histidyl-tRNA synthetase is recognised by an autoreactive γδ TCR from a patient with a rare form of γδ T cell-mediated myositis³⁰. Of note, histidyl-tRNA synthetase was previously implicated in autoimmunity as a self-antigen, Jo-1, targeted by autoreactive B cells. Thus, human γδ T cells may collectively monitor multiple components of pathways regulating cholesterol biosynthesis and nucleotide metabolism that are likely to be altered by infection or other forms of stress.

In this regard, it was recently found that phosphoantigen-mediated, Vγ9Vδ2⁺ T cell activation can be mimicked and inhibited, respectively, by different antibodies against the widely-expressed immunoglobulin superfamily member, butyrophilin 3A1 (CD277)^{31, 32}. Although the involvement of CD277 in phosphoantigen recognition is not yet unresolved, it is interesting that mouse γδ T cells neither display phosphoantigen reactivity nor express a CD277 homolog. However, CD277 displays high structural similarity to *Skint1*, a murine immunoglobulin superfamily gene expressed by medullary thymic epithelial cells (mTECs) and keratinocytes and which is critical to the development of Vγ5Vδ1⁺ DETC (see below)^{33, 34}. Thus conserved molecular mechanisms may underlie the activation of disparate γδ T cell subsets in mice and in humans.

The collective TCR specificities of γδ T cells permit them to respond both to infection and to dysregulated self. HMBPP clearly qualifies as a pathogen-associated molecular pattern (PAMP), and HMBPP-specific Vγ9Vδ2⁺ T cells in the presence of monocytes respond strongly to neutrophils that have taken up HMBPP⁺ but not HMBPP⁻ clinically relevant bacterial strains³⁵. However, upregulation of endogenous IPP in human cells in response to infection or non-infectious dysregulation^{36, 37} also provokes Vγ9Vδ2⁺ T cell reactivity, albeit at a lower sensitivity.

Similarly, murine hepatic $\gamma\delta$ T cells respond to CD1d presenting cardiolipin, a major bacterial cell wall phospholipid that is also an endogenous component of mitochondria³⁸. Given the potential for diversity in adaptive immune receptors, cross-reactivity is inevitable and can be documented in B cells and $\alpha\beta$ T cells. However, the cross-reactivity reviewed here for $\gamma\delta$ T cells is an overt functional cross-reactivity that clouds whether cells are responsive primarily to foreign or to self-antigens. This is similar to the status of B-1 cells, which have been proposed to mount rapid responses to common molecular signatures of infection or dysregulation³⁹. Thus, even though few $\gamma\delta$ TCR specificities have so far been defined, it seems clear that peripheral $\gamma\delta$ T cells are distinct from their TCR $\alpha\beta$ ⁺ counterparts both in their constellation of antigens recognised and in the strong representation of self-antigens within that constellation.

$\gamma\delta$ T cell response kinetics

Lymphoid Stress-Surveillance

The capacity to recognise antigens rapidly displayed following infection or other forms of stress, and to respond in large numbers without requiring extensive clonal expansion permits $\gamma\delta$ T cells to participate in the early stages of an immune response, known as the “afferent phase”. This means that they act in synchrony with innate immune cells as sensors of dysregulation, thereby setting in motion downstream efferent immune responses mediated by conventional adaptive lymphocytes. By contrast, CD4⁺ $\alpha\beta$ T cells would not be able to participate in tissue immune surveillance, because their activation requires processed antigens to be presented by highly specialised antigen presenting cells. In this regard, afferent sensing is ordinarily attributed to myeloid cells, particularly DCs that are typically viewed as the primary orchestrators of adaptive immunity. Therefore, to highlight the capacity of $\gamma\delta$ T cells to play an equivalent role, we have applied the term lymphoid stress-surveillance⁴⁰.

We previously considered that epithelial cells express a set of gene-products that regulate immune cells. We termed this the Epimmunome, which encompasses cell-surface molecules upregulated in response to numerous forms of cell dysregulation⁴¹. These include murine Rae-1 and H60, and human MICA, MICB and ULBPs, which are all members of a large family of MHC class I-related molecules that engage the activating receptor, NKG2D, which is expressed by many $\gamma\delta$ T cell subsets, NK cells, CD8⁺ T cells and some CD4⁺ T cells.

The importance of the NKG2D pathway is attested to by the plethora of strategies employed by viruses and tumours to evade it^{42, 43}. Given the evidence that $\gamma\delta$ T cells make TCR-dependent responses to heat-shocked but not normal cells⁴⁴, it has long been hypothesized that some $\gamma\delta$ TCRs recognize moieties upregulated on the surface of stressed cells. Indeed, tetramers composed of the V γ 5V δ 1 DETC TCR bound to a keratinocyte determinant transiently expressed adjacent to wounded skin⁴⁵. Moreover, we have mentioned that MICA is reportedly a ligand for some human V δ 1⁺ TCRs¹⁷. However, because only few stress-regulated surface molecules have so far been identified, the generality of $\gamma\delta$ TCRs engaging “stress antigens” is hard to assess.

A different perspective is that the $\gamma\delta$ TCR constitutively engages self-ligand, thereby predisposing $\gamma\delta$ T cells to respond to stress via co-stimulatory receptors such as NKG2D and/or receptors for cytokines such as IL-1 and IL-15 which are upregulated by tissue dysregulation. Consistent with this, DETCs can be activated *in vivo* simply by acute, keratinocyte-specific upregulation of Rae-1⁴⁶. Furthermore, recently published images show the V γ 5V δ 1 DETC TCR at steady state constitutively signalling at specific sites of interaction with keratinocytes⁴⁷. The steady-state ligand recognised by the V γ 5V δ 1 TCR

was not identified, but Skint1 (see above) is a candidate for contributing to this interaction since it is expressed by keratinocytes at steady-state. This study also confirmed the observation that following activation DETCs make overt contacts with Langerhans cells (LC)⁴⁶, supporting the possibility that LC also express a DETC TCR ligand. For DETC to respond rapidly to changes in epithelial cells and to then communicate with LC reinforces the assignment of $\gamma\delta$ T cell function to the afferent sensing phase of an immune response, consistent with lymphoid stress-surveillance, and distinct from the biology of most $\alpha\beta$ T cells. However, this does not imply that $\gamma\delta$ T cells do not also contribute to downstream effector and regulatory phases of immunity.

Co-stimulatory and inhibitory receptors

Conventional $\alpha\beta$ T cells are regulated by the coincident provision of Signals 1, 2 and 3, and if any one of these is lacking, the cells may anergise, thereby ensuring that they are activated only in cases of genuine infection and dysregulation. As was just considered, $\gamma\delta$ T cells may respond to these signals in sequence rather than coincidentally: for example, the DETC response to NKG2D-ligands by cells with pre-engaged TCRs. Similarly, PE-primed $\gamma\delta$ T cells acquired the capacity to respond to inflammatory cytokines, IL-1 and IL-23, alone⁷.

Such acquired responsiveness to Signals 2 or 3 alone raises the issue of what ordinarily limits the activation of $\gamma\delta$ T cells so as to prevent chronic inflammation. While this issue is not resolved, it is notable that $\gamma\delta$ T cells can express many receptors regulating their responsiveness to their environment. Among these are inhibitory Ly49 receptors, whose expression on $\alpha\beta$ TCR CD8 $\alpha\alpha^+$ intraepithelial lymphocytes (IELs) is associated with hyporesponsiveness⁴⁸. Innate-like $\gamma\delta$ T cell activation may additionally require input from unconventional co-stimulators. Thus, DETCs express junctional adhesion molecule-like (JAML), which upon engagement of the coxsackie adenovirus receptor (CAR) expressed by keratinocytes activates phosphoinositide 3-kinase (PI3K) which is likewise mediates co-stimulation via CD28 or NKG2D⁴⁹. DETCs also express semaphorin 4D (CD100), which has been implicated in cell migration and morphology. When plexin B2, expressed by keratinocytes, engages CD100 on DETC, it induces Erk activation and promotes DETC activation-dependent rounding and cytokine production⁵⁰, possibly explaining why CD100-deficient mice show defects in wound healing. The plexin–semaphorin axis operates in many systems, and engagement of CD100 on conventional T cells by plexin B2 expressed by plasmacytoid dendritic cells affects IL-12 production and T cell priming⁵¹.

Aryl Hydrocarbon Receptor (AhR)

The AhR is highly expressed by DETCs and $\gamma\delta$ IELs as well as by Th17 cells, where it most conspicuously regulates IL-22 production^{52, 53}. DETCs and $\gamma\delta$ IELs make neither IL-17 nor IL-22 (see below), but they are strongly influenced by AhR in that AhR-deficient mice fail to sustain either DETC or $\gamma\delta$ IEL numbers over time⁵⁴. AhR is broadly expressed, including by epithelial cells and Langerhans cells. However, its effects on DETCs and $\gamma\delta$ IELs are dependent on its expression by RAG-dependent lymphoid cells, and seem to reflect the cells' intrinsic responses to aryl hydrocarbons found in food and other components of the environment. However, the effects of AhR are not specific to either $\gamma\delta$ T cells or IELs, with earlier studies of AhR-deficient mice reporting defects in systemic T cell compartments⁵⁵. Nonetheless, AhR regulation of $\gamma\delta$ T cells within tissues emphasises the diversity of receptor-mediated interactions that transmit to $\gamma\delta$ T cells the status of their environment and that regulate them both positively and negatively. For example, IFN- γ production by human V γ 9V δ 2⁺ T cells is strongly attenuated by co-engagement of the TCR and CD46, a complement receptor for which ligands would certainly be available during infection or tissue damage⁵⁶. The need now is to learn under which environmental conditions specific

molecular sensors operate; which sensors are integrated with others and which function independently; and how the activities of specific sensors relate to $\gamma\delta$ cell biology.

$\gamma\delta$ T cell functions

Activated murine systemic $\gamma\delta$ T cells and human peripheral blood $\gamma\delta$ T cells can express high levels of IFN- γ , TNF, and granzymes; IL-17 is produced by distinct subsets of systemic $\gamma\delta$ T cells and by those in the dermis and intestinal lamina propria; and CD4⁺ IL-4-producing $\gamma\delta$ T cell clones have been described⁵⁷. Collectively these effector functions permit $\gamma\delta$ T cells to participate in the later, “efferent phase” of immune responses.

However, $\gamma\delta$ T cells can also display a pleiotropy that contrasts with the functional limitations of conventional Th1, Th2, and Th17 $\alpha\beta$ T cell subsets. For example, while DETCs can produce IFN- γ and express high levels of granzymes, they also express IL-13⁵⁸ that can regulate B cells; growth factors such as IGF1 that may regulate neighbouring stromal cells⁵⁹; and numerous chemokines that will recruit other leukocytes. This combination of CTL-, Th1-, and Th2-type phenotypes evokes the finding that the transcription factor NFIL3 promotes IL-13 production by chronically stimulated Th1 and NK cells⁶⁰. It may enable rapidly-responsive $\gamma\delta$ cells to contribute to the afferent phase of immune responses via pivotal interactions with other cells (Figure 2), as will now be considered.

Interactions with B cells

In interacting with other cells, a key function of $\gamma\delta$ T cells may be their expression of chemokines (Table 2). Thus, activated V γ 9V δ 2⁺ T cells can produce large amounts of CXCL13⁶¹ that regulates the organization of B cells within follicles of lymphoid tissues⁶². Indeed, there is clear evidence for an impact of $\gamma\delta$ T cells on B cells from both mice and humans. Thus, germinal centres (albeit small) and the production of high levels of T cell-dependent immunoglobulins, notably IgE, can be detected in $\alpha\beta$ T cell-deficient mice, particularly following infection⁶³. The immunoglobulins in these mice are mostly not specific for challenging antigens, reiterating that immunization seldom evokes pathogen-specific $\gamma\delta$ T cells in a conventional sense, but rather promotes rapid $\gamma\delta$ T cell responses to a dysregulated state, the details of which differ according to the challenge. Consistent with this, when mild physical perturbation of the skin coincides with epicutaneous exposure to antigen, high levels of IgE result that are at least partially dependent on a normal DETC compartment and upon NKG2D, to which DETC respond by making IL-13, a “Th2-cytokine” that upregulates IgG1 and IgE⁵⁸.

In humans, a hypomorphic *RAG1* mutation was recently reported that results in a predominance of $\gamma\delta$ T cells⁶⁴. Despite their strong $\alpha\beta$ T cell deficiency, patients with this mutation had normal immunoglobulin levels and responses to infectious agents and vaccination. One patient displayed a hyper-IgE syndrome concomitant with elevated numbers of circulating eosinophils, while another with very high absolute numbers of circulating $\gamma\delta$ T cells also presented high titres of circulating IgM, IgG and IgA. Likewise, of two patients with a novel leaky CD3 δ mutation that mostly affects the $\alpha\beta$ T cell compartment⁶⁵, the patient with the most responsive $\gamma\delta$ T cells also presented with hyper-IgE syndrome and eosinophilia, further demonstrating a role for $\gamma\delta$ T cells in antibody production. Indeed, healthy individuals harbour a subset of V γ 9V δ 2⁺ T cells that expresses CXCR5, has a T_{FH}-like phenotype and can provide B cell help⁶⁶, particularly in the presence of IL-21, the prototypic T_{FH} cell-inducing cytokine⁶⁷.

Interactions with Dendritic Cells

Given that tissue-associated DC can respond to numerous molecular sentinels of infection and stress; can migrate to the LNs, and can there prime efferent adaptive responses, one might reasonably question the importance of an afferent role for $\gamma\delta$ T cells. One explanation is that $\gamma\delta$ T cells collaborate with DC, refining the quality of information that DC receive about the status of a tissue, and thereby improving the criteria for whether or not an immunogenic or tolerogenic response ensues. For example, DC lack NKG2D and hence may only be able to sense epithelial cell stress if this status is communicated to them by responding $\gamma\delta$ T cells. Indeed, the visualisation of DETC–Langerhans cell contacts in the skin^{46, 47} encourages the view that activated $\gamma\delta$ T cells directly regulate DC function, in contrast to the conventional view that DCs always function upstream of T cells.

Aminobisphosphonates (NBP) include clinically approved entities such as zoledronate that inhibit a downstream enzyme in the mevalonate pathway thereby causing the accumulation of IPP and sensitising cells to $V\gamma9V\delta2^+$ T cells. Thus, NBP-treated human DCs mimic DC infected with specific types of bacteria, protozoa, or viruses (see above). $V\gamma9V\delta2^+$ T cells potentiate the maturation of NBP-treated DC via their production of IFN- γ and TNF in a cell-cell contact⁶⁸. Additionally, immature DCs are reciprocally efficient at promoting $V\gamma9V\delta2^+$ T cell activation⁶⁹. The significance of such interactions is suggested by the finding that activated $V\gamma9V\delta2^+$ T cells can relieve a block to DC maturation imposed by *M. tuberculosis* infection⁷⁰.

Interactions with $\alpha\beta$ T cells

A second explanation for the importance of $\gamma\delta$ T cells in the afferent phase is that they may mimic the functions of DC, thereby amplifying or even substituting for them. Perhaps the signatory function of DC is their presentation to T cells of antigenic peptide-MHC complexes. B cells can also present antigen⁷¹, and whereas the case for conventional human T cells is less compelling, it is clear that human $V\gamma9V\delta2^+$ T cells can also present antigen to $CD4^+$ T cells and cross-present antigen to $CD8^+$ T cells⁷². Activated $V\gamma9V\delta2^+$ T cells efficiently take up soluble antigens and can opsonize and phagocytose target cells^{73, 74}. Moreover, whereas steady-state $V\gamma9V\delta2^+$ T cells localise mostly to the peripheral blood or to tissues, their activation rapidly induces CCR7, driving their migration to LNs, and upregulates MHC class I and class II and the co-stimulators CD80 and CD86 to levels equivalent to those on mature DCs⁷². This combination of properties is well-suited to antigen presentation to LN T cells, underlining the innate-like potential of $\gamma\delta$ T cells to initiate antigen-specific adaptive responses against virus-infected cells and tumours.

$\gamma\delta$ T cells may also regulate the $\alpha\beta$ T cell repertoire. Thus, the interaction of fetal $V\gamma5V\delta1^+$ DETC progenitors with *Skint1*-expressing mTECs (see above) induces those epithelial cells to express AIRE which regulates the promiscuous gene expression that in turn purges the $\alpha\beta$ T cell repertoire of strongly autoreactive cells⁷⁵. Conversely, there is little evidence for either a specialised peripheral regulatory $\gamma\delta$ T subset or one that is Foxp3, although intestinal $\gamma\delta$ IELs produce high levels of TGF β , that may downregulate local T cell responses⁷⁶. Indeed, hyperactivity of systemic $\alpha\beta$ T cells is a common phenotype of $\gamma\delta$ T cell-deficient mice^{77, 78} (see below). In sum, $\gamma\delta$ T cells do not have unique functional capabilities, but may be unique in their capacity to orchestrate a diversity of functions appropriate to their participation in each of the afferent, efferent, and regulatory phases of immunity.

Tissue-Associated $\gamma\delta$ T cells

Selective homing and retention

What processes lead to the formation of tissue-localised $\gamma\delta$ cell compartments well-suited to the afferent phase of immune responses? In mice, the answer relates in part to the schedule of $\gamma\delta$ T cell development, with the first wave of cells homing to the epidermis; the second wave to the genital tract; and subsequent waves to the lung and to the gut, *etc.* Cells in successive waves developmentally acquire specific chemokine receptors: thus, upon interaction with Skint1⁺ mTEC in the foetal thymus, V γ 5V δ 1⁺ DETC progenitor thymocytes acquire CC-chemokine receptor 10 (CCR10) which in part directs them to the epidermis⁷⁹. Additionally, $\gamma\delta$ cells seemingly engage in steady-state interactions that stabilise their retention within particular tissues^{47, 80}, although the molecules responsible for this important process are largely unelucidated.

Micro-anatomical specialisation and Pre-programming

Within tissues, $\gamma\delta$ T cells display overt microanatomical localisation. Thus, the murine skin juxtaposes IFN- γ -producing intraepithelial T cells (DETC) with IL-17-producing dermal $\gamma\delta$ T cells, while the intestine juxtaposes IFN- γ -producing IEL and IL-17-producing $\gamma\delta$ T cells in the lamina propria. It is not known why these specific effector functions are best suited to the outer and inner layers of the tissue, respectively. Nonetheless, hard-wired commitment of $\gamma\delta$ T cell compartments to particular functional programmes is important because it permits the rapid responsiveness that is essential for the afferent response. As will now be outlined, this is achieved by developmental pre-programming.

By monitoring T10/T22-specific $\gamma\delta$ T cells (see above) developing in mice that do or do not express T10/T22, it was possible to show that agonist encounter during thymic development committed cells to a differentiation pathway characterized by IFN- γ production, in place of IL-17⁸¹. Similarly, V γ 5V δ 1⁺ DETC progenitors that engage Skint1⁺ mTECs express IFN- γ , whereas V γ 5V δ 1⁺ thymocytes in FVB mice from Taconic farms, which lack the *Skint1* gene, continue to express IL-17. The selective impact of Skint1 is at least in part achieved by the upregulation of *Nfat*, *Nfkb*, and *Egr3*, which collectively promote T-bet expression and suppress transcription of *Rorgc* and the “ $\gamma\delta$ -marker” gene, *Sox13*⁸².

This induced gene regulatory network is not limited to Skint1-selected DETC progenitors, being detected in adult $\gamma\delta$ thymocytes identified by their phenotypic similarity to Skint1-selected V γ 5V δ 1⁺ foetal thymocytes. However, the network in these cells is induced by molecules other than Skint1 that remain to be identified. This is relevant to the observations that murine peripheral $\gamma\delta$ T cells can be sub-divided into CD27⁺ $\gamma\delta$ T cells that produce IFN- γ , and CD27⁻ $\gamma\delta$ T cells that produce IL-17, and that many such cells are also developmentally pre-programmed⁸³. Provocatively, the Skint1-induced gene regulatory network can be induced in adult thymocytes by direct TCR stimulation⁸², consistent with the prospect that TCR agonists are primary agents of functional pre-programming. This is in striking contrast to the developmental impact of agonists on $\alpha\beta$ T cell progenitors, which is usually to promote apoptosis or the upregulation of FoxP3 that suppresses effector function.

According to these studies, IL-17-producing $\gamma\delta$ cell progenitors may not have encountered TCR-binding agonists. However, they may still have received critical differentiation signals, possibly via ligand-independent TCR signalling. Thus, these cells are CD44^{hi}CD62L^{low}CD127^{hi}TCR^{hi}, a phenotype consistent with pre-activation. Moreover, peripheral IL-17-producing CD27⁻ cells can be activated simply by exposure to IL-1 and/or IL-23⁷, that is also consistent with prior TCR signalling. However, the possibility that innate-like IL-17-producing $\gamma\delta$ T cells develop without direct TCR-ligand engagement

contradicts the common view that innate-like T cells emerge primarily as a result of positive agonist selection.

There is also a murine $\gamma\delta$ T cell subset that is pre-programmed toward IL-4 production following interactions between signalling lymphocytic activation molecule (SLAM) on $\gamma\delta$ T cell progenitors and SLAM-associated protein (SAP) on immature CD4⁺CD8⁺ (“DP”) $\alpha\beta$ T cell progenitors⁸⁴. Interestingly, DP cells also regulate the differentiation of IFN γ - and IL-17-producing $\gamma\delta$ thymocytes in a process termed *trans*-conditioning that is in part mediated by lymphotoxin^{85, 86}. This and other mechanisms regulating $\gamma\delta$ T cell development have been extensively discussed elsewhere⁸⁷.

Two origins of $\gamma\delta$ T cells

As stated, the biological significance of pre-programming is not fully understood: what, for example, is the benefit of extinguishing IL-17 potential in agonist-selected $\gamma\delta$ T cells? Moreover, many naïve peripheral $\gamma\delta$ T cells do not display the molecular hallmarks of pre-programming, and retain the potential for IL-17 production *in vivo* even if they emerge from among CD27⁺ cells, more typically associated with IFN- γ production. Collectively, such non-pre-programmed cells may compose the adaptive $\gamma\delta$ T cell compartment, with specificity for diverse antigens, including PE (see above). They may develop in the thymus with no selective educational input from the TCR.

The contrast of the “IL-17-default position” of distinct subsets of TCR $\gamma\delta$ ⁺ thymocytes that fail to engage agonists, with the “non-committed” state of many other post-natal TCR $\gamma\delta$ ⁺ thymocytes strongly implies distinct developmental origins of different $\gamma\delta$ T cell subsets. Possibly the “IL-17-default position” marks $\gamma\delta$ T cell progenitors that will form the bulk of innate-like cells, whereas other murine $\gamma\delta$ T cells will form the predominant reservoir of lymphoid-homing, adaptive $\gamma\delta$ T cells (Figure 1). Indeed, evidence was recently presented for the divergence of innate and adaptive T cell precursors prior to commitment to the $\alpha\beta$ and $\gamma\delta$ T cell lineages⁸⁸. More specifically, those $\gamma\delta$ T cells that are rapidly-responsive via innate co-stimulatory or cytokine receptors (see above) readily accommodate co-expression of TCR β , consistent with the view that signalling via their own $\gamma\delta$ TCR is the dominant force in their development. Conversely, TCR β expression is selected against in those $\gamma\delta$ T cells with more adaptive properties, such as PE-reactive $\gamma\delta$ T cells, since in the absence of pre-commitment, such cells might readily be diverted toward an $\alpha\beta$ T cell fate by TCR β chain expression and the consequent formation of a preTCR. The presence of discrete progenitors for innate and adaptive $\gamma\delta$ cells would readily explain why different gene regulatory networks are found in discrete subsets of $\gamma\delta$ TCR⁺ thymocytes⁸⁹. Moreover, most innate-like $\gamma\delta$ T cells may derive from the foetus and may not be reconstituted from the bone marrow. This is certainly true for DETCs and for IL-17-producing CD27⁻ $\gamma\delta$ cells, and may extend to a substantial numbers of human peripheral blood $\gamma\delta$ T cells. This has profound clinical implications in terms of bone marrow transplantation, and it leads to the importance of ontogeny in $\gamma\delta$ cell biology.

$\gamma\delta$ T cells and ontogeny

$\gamma\delta$ T cells are the first T cells to develop in every vertebrate in which T cell ontogeny has been examined. In cattle, from which some of the best data derive for adaptive $\gamma\delta$ cell responses, the T cell compartment throughout the first year of life can be dominated by $\gamma\delta$ cells. As was just considered, murine DETCs and many IL-17-producing $\gamma\delta$ T cells are exclusively generated from foetal progenitors. Indeed, the development of mouse and human IL-17-producing $\gamma\delta$ T cells is selectively promoted by IL-7, whose expression is highest in neonates⁹⁰. This may explain why human IL-17-producing $\gamma\delta$ T cells are readily evoked from cord blood but are very difficult to evoke from the peripheral blood of healthy adults⁹¹.

Moreover, although DETCs and IL-17-producing $\gamma\delta$ T cells are functionally distinct, they clearly share a capacity for life-long self-renewal. The same may be true of many human peripheral blood, IFN- γ -producing V γ 9V δ 2⁺ cells that also derive from foetal progenitors and that undergo substantial expansion in early life⁹². Understanding such self renewal of differentiated cells may inform the biology of Langerhans cells and microglia that were also recently found to derive exclusively from foetal progenitors⁹³.

This striking ontogeny has suggested that the primary contribution of $\gamma\delta$ T cells is to neonatal protection, when conventional $\alpha\beta$ T cell responses are severely functionally impaired and DCs are immature. This reasoning complies with the growing belief that the neonatal immune compartment is not simply an immature version of that which arises in adults, but is qualitatively distinct. In support of this hypothesis, human $\gamma\delta$ T cells are functionally precocious relative to $\alpha\beta$ T cells^{94, 95}. Moreover, in independent cases of cytomegalovirus (CMV) transmission *in utero*, the dramatic expansion of human V δ 1⁺ T cells with highly related TCRs has been reported, suggesting a common response to a single epitope⁹⁶.

In two instances of parasite infection in mice, $\gamma\delta$ T cells were required for the protection of young mice but not adults^{97, 98}. However, even as adults, the combined deficiency of $\alpha\beta$ and $\gamma\delta$ T cells increased susceptibility to parasite infection relative to TCR β -deficiency alone, demonstrating that responsive $\gamma\delta$ T cells persist in adults⁹⁹. Additionally, the frequent association of $\gamma\delta$ T cells with IgE induction may reflect their role in early life, since B cells that directly switch to IgE production rather than via an IgG1⁺ intermediate are most abundant in very young mice¹⁰⁰. In sum, the greatest dependence of cell-mediated immunity on $\gamma\delta$ cells may exist in newborns and may have been largely overlooked because of the scarcity of immunological studies in young animals. However, given their capacity to self-renew, $\gamma\delta$ cells of fetal origin may variably persist in adults, and combine with those derived from post-natal progenitors to make additional, key contributions to immunoprotection and, conversely, to immunopathology.

$\gamma\delta$ T cell responses to specific challenges

$\gamma\delta$ T cells are present in adult animals at considerably lower numbers than $\alpha\beta$ T cells, and are seemingly irrelevant to immunity against certain well-studied infections such as lymphocytic choriomeningitis virus (LCMV). Thus, it is easy to understand why little attention may have been paid to $\gamma\delta$ T cells. However, it is now clear that these cells are essential to myriad host processes.

For example, mice infected intraperitoneally with vaccinia virus show increased numbers of IFN γ -secreting splenic $\gamma\delta$ T cells by 2 days post infection¹⁰¹. Such cells show increased reactivity toward vaccinia virus-infected cells, but there is no evidence of virus specificity. Nonetheless, $\gamma\delta$ T cell-deficient mice show substantial increases in virus titres immediately post-infection as well as increased mortality compared with control mice. However, in surviving vaccinia virus-infected *Tcrd*^{-/-} mice, immunity develops normally¹⁰¹, consistent with $\gamma\delta$ T cells making innate-like contributions to the primary response, but failing to mediate adaptive memory.

By contrast, $\gamma\delta$ T cell deficiency in mice infected with West Nile virus (an emerging mosquito-borne pathogen) impairs responses to both primary and secondary infection, not because $\gamma\delta$ T cells form memory cells in these animals, but because they critically contribute to the quality of recall CD8⁺ cells that are generated during primary infection¹⁰². An analogous influence of $\gamma\delta$ T cells exists over CD4⁺ T cell memory generated during intra-vaginal infection by herpes simplex virus 2¹⁰³. In humans, $\gamma\delta$ T cell population expansion is

strongly associated with CMV infection^{104, 105}, and $\gamma\delta$ T cell clones derived from CMV-infected individuals secrete cytokines in response to both infected cells and tumour cells¹⁹.

Likewise, $\gamma\delta$ T cells are a critical source of rapid IL-17 production in response to diverse bacterial infections, and *Tcrd*^{-/-} mice show substantially increased susceptibility to infections by *Nocardia*, *Klebsiella*, *Listeria*, *E. coli*, *Salmonella*, *Mycobacterium*, and *Pseudomonas*, for example¹⁰⁶. Additionally, *Tcrd*^{-/-} mice show impaired responses to infection by certain parasites, including *Plasmodium*, where IFN γ -production by $\gamma\delta$ T cells may be more important than IFN- γ production by NK cells or $\alpha\beta$ T cells^{107, 108}. Beyond infection, $\gamma\delta$ T cells confer resistance to particular regimens of chemical carcinogenesis and to certain spontaneously arising tumours in transgenic mice, by mechanisms that have not been clarified in depth¹⁰⁹.

As mentioned above, the primary manifestation of $\gamma\delta$ T cell deficiency in mice is often an inflammatory pathology reflecting exaggerated $\alpha\beta$ T cell responses. Understandably, this has been taken as evidence that $\gamma\delta$ T cells exert a regulatory effect upon conventional T cells, possibly consistent with their production of TGF β in the gut. However, it can also be explained by the rapid response to and limitation of infection by $\gamma\delta$ T cells that thereby limits $\alpha\beta$ T cell activation. In general terms, this may not be a unique means of regulation since some human $\alpha\beta$ TCR⁺ Treg cells exert their effects by killing APCs in a phenomenon of linked suppression¹¹⁰. Importantly, this would be consistent with the sustained contribution of $\gamma\delta$ T cells to protective immunosurveillance in adults. Naturally, aggregate functional pleiotropy of $\gamma\delta$ T cells has clinical implications, in the cells' potential to cause and to regulate particular immunoopathologies, and in their utility as targets in mediating immunotherapy of cancer and of chronic infection. These are active areas of investigation, as summarised in Box 1.

Conclusions: six good reasons for $\gamma\delta$ T cells

This review has considered data, much of it recently published, in the context of six explanations for the unique contributions of $\gamma\delta$ T cells in the immune system. While much remains to be learned, there is a sufficient basis to draw some conclusions. First, $\gamma\delta$ TCRs engage a distinct constellation of antigens, thereby widening the scope of immune responsiveness. This may reflect a particularly significant contribution of adaptive $\gamma\delta$ T cells. At the same time, the overt inclusion of auto-antigens in their specificities illustrates a “beneficial auto-immunogenicity” that may be based in part on a novel perspective; namely constitutive TCR engagement facilitating rapid responses to stress or infection. Such afferent actions distinguish $\gamma\delta$ T cells functionally and kinetically from most conventional T cells, and relies in large part on the capacity of the $\gamma\delta$ TCR to see self-surface moieties expressed on cells within tissues. Moreover, the utilisation of adaptive TCRs in the afferent phase potentially offers greater breadth and selectivity over the type of stimuli to respond to, by comparison to the use of DCs or NK cells. Thus, $\gamma\delta$ T cells may be better than TLR- or NLR-dependent sensing at distinguishing between pathogenic and benign challenges and therefore at determining whether an immunogenic or tolerogenic response ensues. Consistent with this, several adaptive responses are severely impaired in the absence of $\gamma\delta$ T cells. Perhaps $\gamma\delta$ T cells are well placed to eventually substitute for DC in the role of afferent sensing.

Strictly speaking $\gamma\delta$ T cells do not express unique functions, but they can offer distinct combinations of functional potentials, such as cytolysis, IgE induction, antigen presentation and the production of growth factors (Figure 3). The disposition of distinct functions to particular subsets, particularly within tissues, permits different complexions of the $\gamma\delta$ T cell response. Thus, TCR $\gamma\delta$ ⁺ IEL may promote the exclusion of infectious agents or toxins by

eradicating targeted cells, employing IgE-mediated expulsion mechanisms, recruiting other cells, and promoting tissue re-growth. Conversely, sub-epithelial responses may promote an intergrative microbicidal innate and adaptive response to counter agents that have penetrated the basement membrane. Such capacity to form functional compartments in particular anatomical niches may reflect a further critical contribution of $\gamma\delta$ T cells to immunity. However, it is possible that such roles may be increasingly subsumed by unconventional $\alpha\beta$ T cells at human body surfaces. By contrast, $\alpha\beta$ T cells may not be able to substitute for the functional potency of $\gamma\delta$ T cells in newborns or even in the foetus where intrinsic as opposed to maternal mechanisms of immunoprotection are increasingly considered important.

Even in the neonate, however, the growing data-sets suggest that $\gamma\delta$ T cells are disproportionately responsive to particular challenges, notably CMV, tuberculosis and malaria. Moreover, although we have emphasised that the expansion of rare, antigen-specific clones is not the hallmark of $\gamma\delta$ T cell biology, both tuberculosis and CMV seemingly induce the selective expansion of clones that share reactivity for relevant PAMPs or molecular markers of infected cells. Thus, events early in life drive the expansion of specific $\gamma\delta$ T cell populations that, albeit polyclonal, represent an alteration to the starting repertoire. This is by definition a form of adaptive immunity and it reminds us of the difficulty inherent in applying strict teleological terms to immunological processes. Perhaps the simplest reconciliation is to consider that all functional $\gamma\delta$ T cells must at some point have received a signal through the TCR. This would clearly distinguish them from emerging cohorts of innate lymphoid cells. Those $\gamma\delta$ T cells that develop without pre-programming and that do not receive the requisite TCR signal until peripheral exposure are most obviously adaptive; those that receive the signal in the thymus are most obviously innate-like; and those that receive it in the periphery but during very early life may be the products of adaptive processes which rapidly convert them into innate-like cells. Given this, a particular emphasis should be placed on clarifying $\gamma\delta$ T cell biology in CMV, tuberculosis and malaria, since these agents are of great clinical significance and seem so effective at driving early $\gamma\delta$ expansion and conversion into innate-like cells. These studies may test the utility of the mouse as a model for detailed aspects of $\gamma\delta$ T cell biology. Indeed, like NK cells, $\gamma\delta$ T cells show species-specific variation that is in part reflected in highly variable gene structures¹.

It is evident that some immunology college courses still pay little attention to $\gamma\delta$ T cells. This is completely unjustified given the six signatory roles of $\gamma\delta$ T cells reviewed here. We look forward to greater consideration, more extensive investigation, and improved clinical manipulation of $\gamma\delta$ T cells, which so clearly combine effector functions with a powerful afferent potential, and which tell us so much about lymphocyte biology as well as about the cells themselves.

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Glossary terms

AIRE

The autoimmune regulator gene whose product expressed in medullary thymic epithelial cells (mTECs) promotes the promiscuous expression of genes that are otherwise specific to specific peripheral tissues, e.g. endocrine glands or the nervous

	system. Peptides derived from these tissue-specific antigens are presented by mTECs to developing ab T cells, such that any with high affinity reactive TCRs may be clonally deleted as means of central tolerance
Anergise	The conversion of a T cell to a state in which it is almost completely non-responsive to TCR engagement. This may occur when a peripheral T cell is exposed to antigen in the absence of co-stimulation and is interpreted as a means to suppress potentially auto-reactive T cell responses in the absence of infection
Dendritic epidermal $\gamma\delta$ T cells	(DETCs). $\gamma\delta$ T cell receptor (TCR) ⁺ cells localized purely in the epidermis that have been described in rodents and cattle but not humans. In mice, essentially all DETCs express precisely the same TCR, forming a prototype lymphocyte repertoire of limited diversity.
Intraepithelial lymphocytes	(IELs). Intraepithelial lymphocytes are T cells that reside in the basolateral side of the intestinal epithelium, above the basement membrane. They express either an ab TCR (T cell receptor) or a $\gamma\delta$ TCR, and in mice they frequently express the CD8 $\alpha\alpha$ homodimer.
NOD mice	An inbred strain of mice that spontaneously develop T cell-mediated autoimmune diabetes, dependent on their expression of a particular MHC class 2, molecule, I-Ag ⁷ .
B-1 cells	IgM ^{hi} IgD ^{low} MAC1 ⁺ B220 ^{low} CD23 ⁻ cells that are dominant in the peritoneal and pleural cavities. Their precursors develop in the fetal liver and omentum, and in adult mice, the size of the B-1 cell population is kept constant owing to the self-renewing capacity of these cells. B-1 cells recognize self components, as well as common bacterial antigens, and they secrete antibodies that tend to have low affinity and broad specificity.
Lymphoid stress-surveillance	The capacity of lymphocytes, as opposed to myelomonocytic cells, to sense infection or tissue dysregulation and to respond rapidly, in synchrony with innate responses.
NKG2D	(Natural-killer group 2, member D). A lectin-type activating receptor encoded by the NK complex and expressed at the surface of most NK cells and NKT cells; many $\gamma\delta$ T cells; and antigen-experienced cytolytic CD8 ⁺ ab T cells. The ligands for NKG2D are MHC class I polypeptide-related sequence A (MICA) and MICB, and at least four related ULBP proteins, and multiple members of the structurally related retinoic acid early transcript 1 (RAE1) and H60 families, and Mult-1 and in mice. Such ligands are generally expressed at the surface of infected, stressed or transformed cells.
Signals 1, 2 and 3	Cell signalling pathways activated by engagement of the antigen receptor (Signal 1); co-stimulatory receptors, e.g. CD28 (Signal 2); and cytokine receptors, e.g. IL-2 (Signal 3).
CD8$\alpha\alpha$⁺ IELs	A type of T cell found mostly in the murine intestinal epithelium. The CD8 molecule that they express is a homodimer of CD8 α , rather than the CD8 $\alpha\beta$ heterodimer that is expressed by

	conventional CD8 ⁺ T cells in the lymph nodes and by another distinct subset of IEL. CD8aa is a ligand of the non-classical MHC class I molecule thymus leukemia antigen (TL), which is expressed by the intestinal epithelium. It has been proposed that CD8aa ⁺ IEL are self-reactive, TCR-agonist-selected cells that have regulatory properties.
Germinal centres	Highly specialized and dynamic microenvironments that give rise to secondary B cell follicles during an immune response. They are the main site of B cell maturation, leading to the generation of memory B cells and plasma cells that produce high-affinity antibody.
MRL–lpr mouse	A mouse strain that spontaneously develops glomerulonephritis and other symptoms of systemic lupus erythematosus (SLE). The lpr mutation causes a defect in CD95 (also known as FAS), preventing apoptosis of activated lymphocytes. The MRL strain contributes disease-associated mutations that have yet to be identified.
Stress Antigens	Molecules, such as MICA or Rae-1 whose expression is upregulated by cellular dysregulation and that are functionally recognised by lymphocytes as part of a process of immune surveillance
Imiquimod	An imidazoquinolene-based compound that is sensed by TLR7, is currently used for the treatment of basal cell carcinoma, but that has also been implicated in iatrogenic induction of psoriasis-like symptoms.
Autoinflammatory disease	A disease characterized by seemingly unprovoked pathological activation of the innate immune system in the absence of overt autoantibodies or autoreactive T cells.
V(D)J recombination	Somatic rearrangement of variable (V), diversity (D) and joining (J) regions of the genes that encode antigen receptors, leading to repertoire diversity of both B cell and T cell receptors.

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Box 1 **$\gamma\delta$ cells in disease and therapy**

Inevitably, functionally pleiotropic $\gamma\delta$ T cells can contribute to and ameliorate disease and are attractive targets for clinical manipulation. The capacity of some $\gamma\delta$ T cell activities to limit $\alpha\beta$ T cell functions is manifest in the increased incidence of $\alpha\beta$ T cell-dependent glomerular nephritis and lupus in MRL-*lpr* mice crossed with *Tcrd*^{-/-} mice¹¹¹. Conversely, the rapid responsiveness of IL-17- and IL-22-producing CD27⁻ $\gamma\delta$ T cells in response to infection is mirrored by a rapid response of dermal IL-17-producing cells to the epicutaneous application of imiquimod, thereby promoting a psoriasiform pathology^{112, 113}. IL-17-producing $\gamma\delta$ T cells have also been identified in psoriatic lesions but not unaffected human skin¹¹⁴. There are at least two interesting implications of these findings. First, disease may reflect violation of the anatomical functional micro-segregation that ordinarily excludes IL-17-producing $\gamma\delta$ T cells from epithelia (see above). Second, immunopathology in adults may be provoked by cells that arose in the foetus. Hence, disease might be predisposed by dysregulated persistence of foetal-derived cells.

IL-17-producing $\gamma\delta$ T cells are also implicated in experimental autoimmune encephalomyelitis (EAE), the murine model for multiple sclerosis^{115, 116}, and in the prototypical adaptive immunopathology, type 1 diabetes (unpublished). Moreover, by promoting inflammation, IL-17-producing $\gamma\delta$ T cells may also exacerbate tumour progression in cases where transformed cells were not eradicated by prior lymphoid stress surveillance¹¹⁷.

Such considerations invoke two distinct clinical approaches. On the one hand, the clear capacity of human $\gamma\delta$ T cells to detect transformed cells via NKG2D and/or other pathways, to promote cytolysis, to present antigen, and to mobilise other components of the immune systems provides an anti-tumour potential that can be readily invoked by clinically approved aminobisphosphonates that upregulate $\gamma\delta$ T cell-activating phosphoantigens. This approach has been pursued at multiple centres, is largely safe and has implied efficacy. However, $\gamma\delta$ T cells become irreversibly exhausted after chronic aminobisphosphonate treatment, which will impinge upon the application of adoptive $\gamma\delta$ T cell immunotherapy. The second approach would be to limit the activities of $\gamma\delta$ T cells in autoimmune and autoinflammatory diseases. Whereas it is common-place yet challenging to treat such diseases with agents that suppress effector function, targeting $\gamma\delta$ T cells offers the opportunity to target the afferent response to whatever is the chronic stimulus. Moreover, the attractiveness of these cells as a target would be considerable if, in some scenarios, the cells persist from the foetus and are not absolutely required for adult immune function.

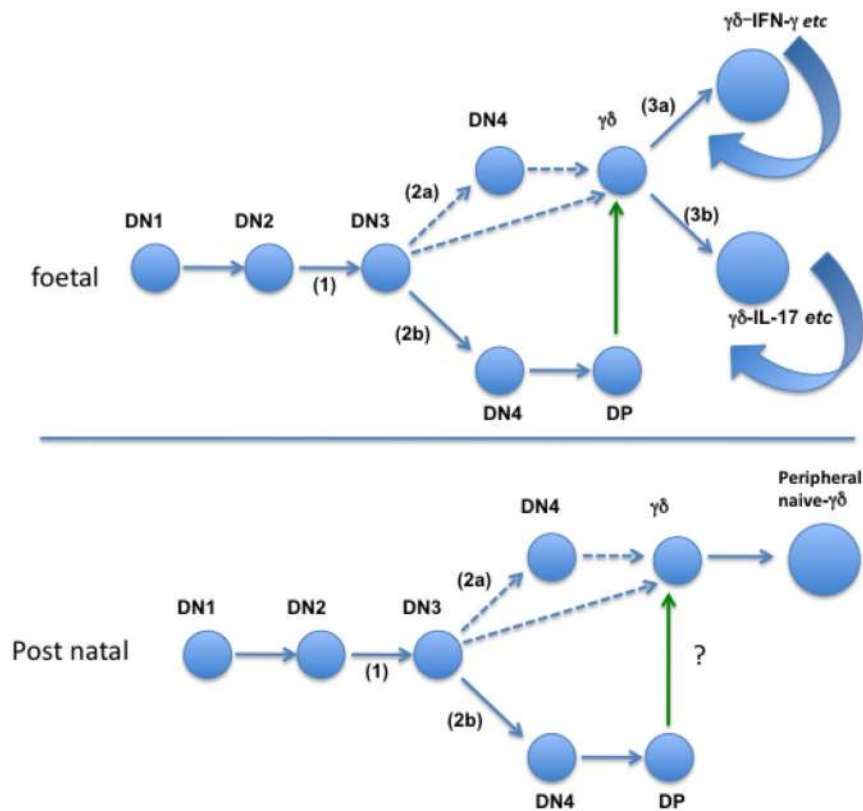


Figure 1. Overview of pre- and post-natal $\gamma\delta$ T cell development

Top: Mouse $\gamma\delta$ T cell development from foetal liver progenitors. Cells undergo development through several steps of differentiation, starting at the double negative 1 (DN1) stage characterized by a CD44⁺ CD25⁻ phenotype, followed by the CD44⁺CD25⁺ DN2 stage. At this point, the β , γ and δ chains of the TCR are rearranged. A functional $\gamma\delta$ TCR expression will drive cells into the $\gamma\delta$ lineage, supported by the expression of Sox13. Cells failing to produce a functional $\gamma\delta$ TCR will undergo β selection supported by Notch 1, with a further rearrangement of the TCR α chain, eventually entering the Double Positive (DP) stage. These cells can support $\gamma\delta$ T cell development via *trans*-conditioning (green arrow). Unlike $\alpha\beta$ T cells, $\gamma\delta$ T cells are functionally pre-programmed, depending on TCR and/or related signalling. It is proposed that a strong, agonist-dependent signal will result in the loss of Sox13 and expression of NFAT, NF κ B, Egr3 and Tbet, and a capability to produce IFN- γ among other effector molecules while weaker TCR signalling permits the cells to maintain Sox13, increase Ror γ c expression, and adopt an IL-17 “default position”. The dashed line indicates the potential origin of $\gamma\delta$ cells from progenitors with either a DN3 or DN4 phenotype. The curved arrows indicate the potential for lifelong self-renewal that exists in at least two pre-natally derived $\gamma\delta$ cell compartments.

Bottom: Mouse post-natal development from bone marrow-derived progenitors. There is no evidence that innate-like CD27⁻ IL-17-producing $\gamma\delta$ T cells can be generated from the bone marrow, implying that a different thymic progenitor gives rise to post-natal $\gamma\delta$ T cells, by comparison to foetus-derived $\gamma\delta$ cells. With no evidence for IL-17-competent progenitors, the evidence for pre-programming is less, and naïve unprimed cells may emerge in the periphery, possibly with a “default potential” for IFN- γ production.

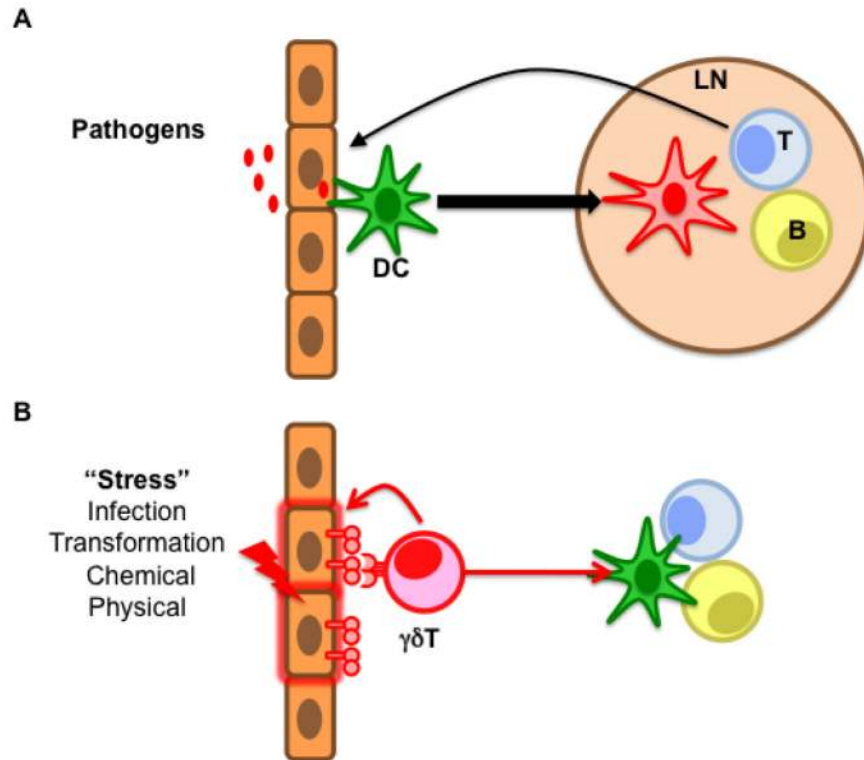


Figure 2. An alternative way to achieve broad systemic immune responses

(A) The text-book view of the activation of an adaptive immune response. Dendritic cells (DC) capture pathogens and mature while migrating to the lymph nodes, where they prime $\alpha\beta$ T and B cells which will migrate back to the infected tissue and mount effector responses or produce antibodies, respectively. This very specific albeit slow response is complemented by $\gamma\delta$ T cells which, in response to various sources of stress, not only mount immediate, local effector responses but also trigger the other arms of the adaptive immune system (B).

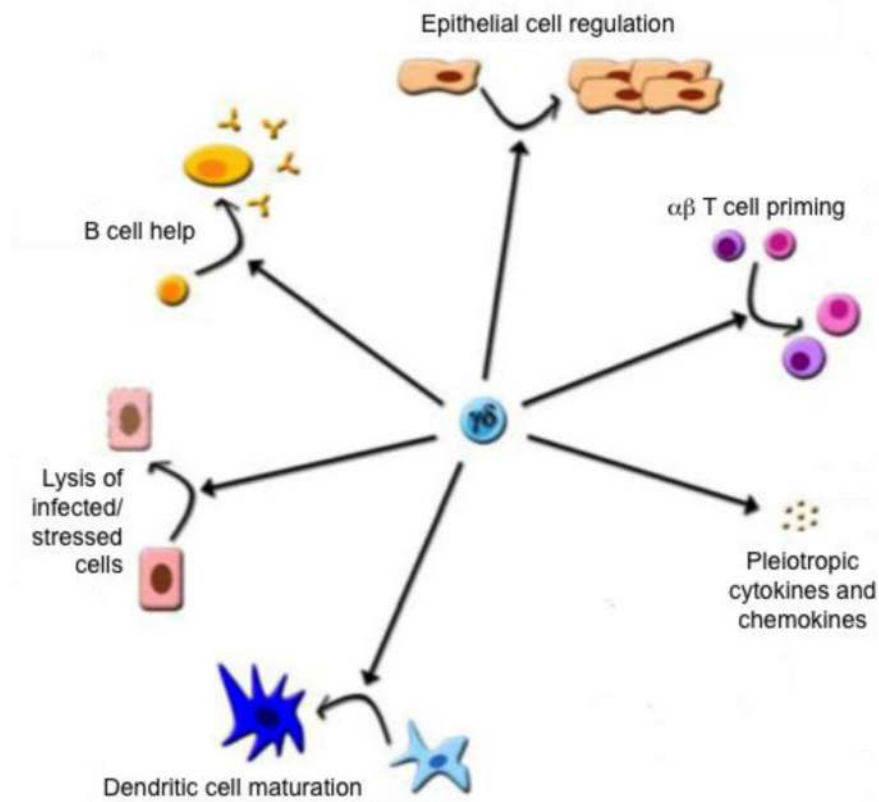


Figure 3. Six of the best $\gamma\delta$ T cell functions

An increasing body of literature now demonstrates that $\gamma\delta$ T cells can play an important central role in defending the organism against a broad range of infectious and sterile stresses by directly eliminating infected or stressed cells; by producing a diversified set of cytokines and chemokines to regulate other immune and non-immune cells; by directly promoting immune cell maturation and activation by triggering B cell help, DC maturation and $\alpha\beta$ T cell priming *via* antigen presentation; and finally by regulating stromal cell function.

Table 1Activating ligands for $\gamma\delta$ T cells.

HUMAN		
Subset	Antigen	Reference
V δ 1 (IEL)	MICA/B	17
V δ 2	ULBP4	18
V δ 1 (clones)	CD1c	20
V δ 1 (Blood)	CD1d + Sulfatides	21
V γ 4V δ 5 (clone)	EPCR	19
V δ 1 (clones)	Lipohexapeptides	118
Various	Phycoerythrin	7
V γ 9V δ 2	Phosphoantigens	119
V γ 9V δ 2	F1-ATPase	27
V γ 1.3V δ 2	Aminoacyl tRNA synthetases	30
MOUSE		
Various	T10/T22	14, 15
V γ 2/V δ 5 (clone)	I-E ^k	16
V γ 2/V δ 8 (clone)	HSV-gI	120
V γ 1 (clones)	Cardiolipin, Apolipoprotein H	38
Various	Phycoerythrin	7
V γ 1 (clones)	Insulin peptide	22

Table 2Production of chemokines by $\gamma\delta$ T cells.

HUMAN			
Subset	Chemokine (alternative name)	Receptor expressed by	Reference
V δ 2 (IPP)	CCL3 (MIP1 α)	Macrophages	61
	CCL4 (MIP1 β)	Macrophages, NK cells, other cell types	
	CXCL10	Macrophages, T cells, NK cells, other cell types	
	CXCL13	B cells	
V δ 1 (NKp30)	CCL3 (MIP1 α)	Macrophages	121
	CCL4 (MIP1 β)	Macrophages, NK cells, other cell types	
	CCL5 (RANTES)	T cells, eosinophils, basophils	
Skin V δ 2	CCL1	Monocytes, NK cells, immature B cells	122
MOUSE			
Lung-resident	CXCL1	Neutrophils	123
	CXCL10	Macrophages, T cells, NK cells, other cell types	
Peritoneal V δ 1	CCL3 (MIP1 α)	Macrophages	124
	CCL5 (RANTES)	T cells, eosinophils, basophils	
DETC	CCL3 (MIP1 α)	Macrophages	125, 126
	CCL4 (MIP1 β)	Macrophages, NK cells, other cell types	
	CCL5 (RANTES)	T cells, eosinophils, basophils	
	XCL1	CD8 ⁺ cross-presenting DCs	