Six-of-the-best: unique contributions of γδ T cells to immunology

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Abstract | γδ 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 the evidence for their unique and crucial functions. We conclude that whereas B cells and αβ T cells are commonly thought to contribute primarily to the antigen-specific effector and memory phases of immunity, γδ T cells are distinct in that they combine conventional adaptive features (inherent in their T cell receptors and pleiotropic effector functions) with rapid, innate-like responses that can place them in the initiation phase of immune reactions. This underpins a revised perspective on lymphocyte biology and the regulation of immunogenicity.

V(D)J recombination
The 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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doi:10.1038/nri3384

As their name indicates, γδ T cells develop largely in the thymus, generating their defining γδ T cell receptor (TCR) via recombination activating gene (RAG)-mediated V(D)J recombination. The resulting potential for diversity in the γδ TCR and the consequent capacity for shaping the T cell repertoire through clonal expansion appropriately assign γδ T cells to the adaptive immune compartment[1]. Furthermore, there are striking connections between γδ T cells and αβ T cells. For example, the *TCRD* locus in mice and in humans is embedded within the *TCRA* locus, and some TCR variable (V) gene segments can be used interchangeably by TCRα and TCRδ. Moreover, a common thymic progenitor may give rise to either αβ T cells or γδ T cells[2], although this does not exclude the possibility that distinct subsets of αβ and γδ T cells arise from qualitatively discrete progenitors (see below; FIG. 1). Within the adaptive compartment, it seems facile to accept the complementary value of B cells, which can secrete their antigen receptors as antibodies, and αβ T cells, which use cell-bound TCRs to induce cytolytic responses and helper functions. However, it is less easy to envision the selective pressure(s) that has sustained for over 420 million years the coexistence of two lineages of T cells (that is, αβ T cells and γδ T cells) with surface-bound TCRs. The nihilistic view is that no such selective pressure currently exists, and that γδ T cells are *en route* to extinction, having been superseded by an extraordinarily potent αβ T cell compartment. Conversely, the recent increase in the study of γδ T cells has added to the established literature in providing conspicuous cases of non-redundant γδ T cell activities. Furthermore, the lamprey, which is an extant but primitive jawless vertebrate, uses RAG-independent mechanisms to generate an adaptive immune compartment that is also characterized by three distinct receptors with diverse potential, of which one is secreted and two are cell-surface bound[3]. Hence, this type of tripartite organization may be optimal for adaptive immune function.

Here, we consider six properties that may collectively distinguish γδ T cells from αβ T cells, and thereby define their unique contributions to lymphocyte biology. One, γδ TCRs recognize qualitatively distinct antigens. Two, γδ T cells contribute to immune responses with distinct kinetics. Three, γδ T cells have unique functional potentials. Four, γδ T cells are particularly suited to the protection of defined anatomical sites. Five, γδ T cells are of primary value in young animals. And six, γδ T cells, although not invariably important, mediate crucial responses to specific pathogens, in a manner similar to natural killer (NK) cells. Because the γδ T cell population comprises heterogeneous subsets, these six properties will not apply equally to all γδ T cells. Accepting this point, we consider here the evidence for each property and its potential to explain the conservation of γδ T cells.

γδ TCRs recognize distinct antigens

Anatomical distribution of γδ T cells. The anatomical localization of lymphocytes has profound implications for their antigen specificity. Thus, the clonal selection and expansion of αβ T cells with very rare specificities relies on the fact that, following egress from the thymus, naive αβ T cells home to the lymph nodes and to the T cell zones of the spleen, where they regularly encounter vast numbers of dendritic cells (DCs) presenting diverse antigens. Although some γδ T cells home to the lymph nodes, many migrate directly to tissues such as the epidermis (in murine species), the dermis,

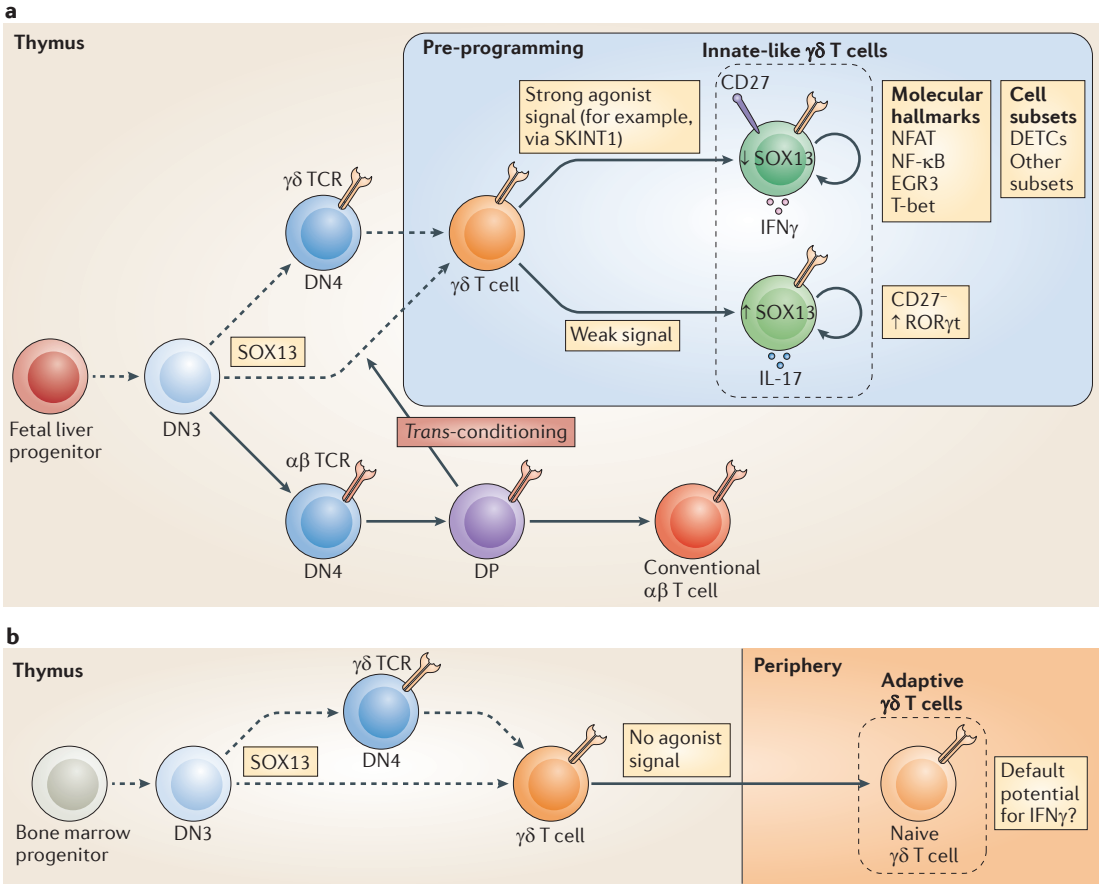

Figure 1 | **An overview of prenatal and postnatal γδ T cell development. a** | The diagram illustrates the development of mouse γδ T cells from fetal liver progenitors. These cells develop through several steps of differentiation, starting at the double-negative 1 (DN1) stage, which is characterized by a $CD44^{+}CD25^{-}$ phenotype, and then progressing into the $CD44^{+}CD25^{+}$ DN2 stage (not shown). At this point, the β-, γ- and δ-chains of the T cell receptor (TCR) are rearranged. Functional expression of the γδ TCR drives cells into the γδ T cell lineage, supported by the expression of SRY-box 13 (SOX13). The dashed line indicates the potential generation of γδ T cells from progenitors with either a DN3 or a DN4 phenotype. Cells that fail to produce a functional γδ TCR undergo β-selection (which is supported by NOTCH1) and a further rearrangement of the TCR α-chain, eventually entering the double-positive (DP) stage. These cells can support γδ T cell development via *trans*-conditioning. Unlike αβ T cells, these γδ T cells undergo functional pre-programming, which depends on TCR signals and/or related signals. It is proposed that a strong, agonist-dependent signal, for example via SKINT1, results in the loss of SOX13, the upregulation of the transcription factors nuclear factor of activated T cells (NFAT), nuclear factor-κB (NF-κB), early growth response protein 3 (EGR3) and T-bet, and a capability to produce interferon-γ (IFNγ) in addition to other effector molecules (not shown). By contrast, weaker TCR signalling permits the cells to maintain SOX13 expression, increase retinoic acid receptor-related orphan receptor-γt (RORγt) expression and adopt an 'interleukin-17 (IL-17)-default position'. The curved arrows indicate the potential for lifelong self-renewal that exists in at least two prenatally derived γδ T cell compartments. Both subsets shown are innate-like because they can effect function rapidly following stimulation without substantial clonal expansion. **b** | The diagram illustrates the postnatal development of mouse γδ T cells from bone marrow-derived progenitors. There is no evidence that either dendritic epidermal γδ T cells (DETCs) or innate-like $CD27^{-}$ IL-17-producing γδ T cells can be generated from the bone marrow, implying that different thymic progenitors give rise to postnatal γδ T cells and fetus-derived γδ T cells. Postnatal γδ T cells that engage agonists may home to the intestinal intraepithelial lymphocyte (IEL) compartment. However, many postnatal γδ T cells show no overt functional pre-programming. Thus, naive unprimed γδ T cells may emerge in the periphery, possibly with a default potential for IFNγ production. In the manner of adaptive lymphocytes, these cells may functionally differentiate following antigen encounter, and only then acquire innate-like responsiveness.

Dendritic epidermal γδ T cells (DETCs). γδ T cell receptor (TCR)-expressing cells selectively localized in the epidermis that have been described in rodents and cattle but not in humans. In mice, essentially all DETCs express precisely the same TCR, forming a prototype lymphocyte repertoire of limited diversity.

the intestine, the lungs and the uterus. Moreover, in contrast to αβ T cells, splenic γδ T cells are not confined to the lymphoid areas (the white pulp) but are also found throughout the red pulp[4]. The sequestration of γδ T cells within tissues is incompatible with their sampling of diverse antigens and the consequent clonal expansion of very rare cells. Consistent with this is the limited TCR diversity of many tissue-resident γδ T cells, which in the mouse epidermis and uterus are essentially monoclonal[5]. This implies that these cells recognize either pathogen-encoded antigens that are predictably encountered in specific tissues or self-encoded molecules that reflect a dysregulated state of that tissue.

The expression of a monoclonal RAG-generated receptor by the majority of γδ T cells in a specific compartment and its use to engage only one or a few antigens was unprecedented before studies of murine skin γδ T cells known as dendritic epidermal γδ T cells (DETCs)[6]. By permitting large numbers of T cells to be rapidly activated and their functions mobilized without a requirement for prior clonal expansion, the monoclonal or oligoclonal use of a TCR represents a profound crossover of adaptive and innate immunity. Hence, the term 'innate-like' has been used to describe γδ T cells. Since the seminal work on DETCs, evidence has accrued for numerous other innate-like γδ T cell subsets, including many within the predominant human peripheral blood Vγ9Vδ2$^+$ compartment. In sum, anatomical considerations suggest that the γδ T cell population is divisible into two groups: lymphoid-homing γδ T cells that may be primed in the circulation and clonally expand in a conventional 'adaptive' manner; and innate-like cells that respond rapidly and at a relatively high frequency in many tissue sites.

Table 1 | **Activating ligands for γδ T cells**

Subset	Antigen	Refs
Human		
Vδ1 (IELs)	MICA	17
Vδ2	ULBP4	18
Vδ1 (clones)	CD1c	20
Vδ1 (blood γδ T cells)	CD1d tetramers loaded with sulphatide	21
Vγ4Vδ5 (clone)	EPCR	19
Vδ1 (clones)	Lipohexapeptides	119
Various	Phycoerythrin	7
Vγ9Vδ2	Phosphoantigens	120
Vγ9Vδ2	F1-ATPase	27
Vγ1.3Vδ2	Histidyl-tRNA synthetase	30
Mouse		
Various	H2-T10, H2-T22	14,15
Vγ2Vδ5 (clone)	I-E^k	16
Vγ2Vδ8 (clone)	HSV glycoprotein I	121
Vγ1 (clones)	Cardiolipin, apolipoprotein H	39
Various	Phycoerythrin	7
Vγ1 (clones)	Insulin peptide (B:9–23)	22

EPCR, endothelial protein C receptor; HSV, herpes simplex virus; IEL, intraepithelial lymphocyte.

Adaptive TCR specificities. γδ TCRs are not restricted to the recognition of peptides bound to MHC molecules, which distinguishes them from the great majority of αβ T cells. Furthermore, the diversity in the length of the γδ TCR complementarity-determining region 3 (CDR3) loops, which is conferred particularly by the architecture of the *TCRD* locus, suggests that the γδ TCR is not structurally constrained to recognize cargo presented by some specific presenting element.

Instead, an antibody-like breadth in antigen recognition by the γδ TCR is suggested by the recent demonstration that some human, murine and bovine γδ TCRs can bind to phycoerythrin, an algal molecule readily recognized by B cells[7]. Binding to phycoerythrin induces γδ T cells to upregulate CD44, downregulate CD62L and express cytokines, as happens when naive αβ T cells are primed. Hence, this response to an exogenous antigen seemingly illustrates an adaptive potential of γδ T cells. However, compared with αβ T cell priming, the priming of phycoerythrin-reactive γδ T cells results in conspicuously less clonal expansion[7], which is a defining parameter of delayed, antigen-specific adaptive responses. Thus, even in this case, the functional γδ T cell response was rapidly mobilized with innate-like kinetics. Moreover, the cells quickly acquired an innate-like capacity to respond to pro-inflammatory cytokines in the absence of further antigens.

The conversion of antigen-specific naive lymphocytes into more rapidly responsive memory lymphocytes is a key criterion of adaptive immunity. In this regard, *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) vaccination has been shown to induce mycobacterium-specific γδ T cells with memory characteristics in macaques and in cattle[8–11]. In addition, immunoprotective γδ T cells reactive to a herpes simplex virus glycoprotein were obtained from an infected mouse[12]. Nonetheless, most attempts to evoke antigen-specific 'memory' γδ T cells following the deliberate immunization or infection of mice have conspicuously failed, even when polyclonal γδ T cell responses were induced. Hence, much of γδ T cell biology is not captured by the conventional concept of adaptive immunity.

Self-reactivity. Few γδ TCR specificities have been deduced; even fewer are supported by biochemical binding data; and whether these are representative of the general γδ T cell population is not always clear (TABLE 1). Nonetheless, as hypothesized almost 25 years ago[13], several mouse γδ TCRs are reactive either to self MHC molecules independently of their cargo or to MHC-related proteins, including: mouse H2-T10 and H2-T22 (which are non-peptide-binding MHC class Ib molecules)[14,15]; the mouse MHC class II molecule I-E^k, but with the epitope lying outside the peptide-binding groove[16]; and human HLA molecules (P. Fisch, personal communication). It has also been reported that

some human γδ TCRs engage members of the MHC class I-like MICA and ULBP families[17,18] (see below). Recently, the TCR from a γδ T cell clone derived from a cytomegalovirus (CMV)-infected transplant patient was shown to directly bind to endothelial protein C receptor (EPCR), which is a lipid carrier with a similar structure to CD1, although again γδ TCR engagement was cargo independent[19]. This was reminiscent of an earlier report, albeit lacking biochemical data, that CD1c is a ligand for several human Vδ1$^+$ T cell clones[20].

Other γδ T cells are specific for antigens presented by MHC or MHC-related molecules. For example, in healthy individuals, Vδ1$^+$ cells (which are more prevalent in tissues than in the peripheral blood) constitute the majority of T cells reactive to CD1d tetramers loaded with sulphatide (a myelin glycosphingolipid)[21]. In addition, several γδ T cell clones derived from non-obese diabetic mice (NOD mice) respond to the insulin-derived peptide B:9–23, which is also presented to CD4$^+$ αβ T cells by the disease-associated MHC class II molecule I-A^{g7} (REF. 22). However, the nature of peptide recognition by γδ T cells was quite distinct from that of αβ T cells, in that an MHC molecule was not required. Thus, γδ TCRs and αβ TCRs have qualitatively distinct modes of antigen recognition, which 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 T cells. For example, low-molecular-mass alkyl diphosphates termed phosphoantigens or phosphoagonists are the prototypical, naturally occurring moieties recognized by human Vγ9Vδ2$^+$ cells, the predominant subset of γδ T cells in peripheral blood. The most potent is hydroxymethyl-but-2-enyl-pyrophosphate (HMBPP)[23], which is 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 cells. 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 and is thus a self phosphoantigen.

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 monocytes[25]. Thus, either phosphoantigens are presented to γδ TCRs by monocytes as cargo in an MHC-independent context or their cellular processing by monocytes somehow promotes the recognition of these cells by Vγ9Vδ2 TCRs, for example by stabilizing 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 to a Vγ9Vδ2 TCR[26] and which may also interact with ApppI, an adenosine derivative of IPP[26–28]. Furthermore, IPP–ApppI interconversion may be catalysed by an aminoacyl-tRNA synthetase[29], which is interesting given that a conformational epitope on histidyl-tRNA synthetase is recognized by an autoreactive γδ TCR from a patient with a rare form of γδ T cell-mediated myositis[30]. Of note, histidyl-tRNA synthetase was previously implicated in autoimmunity as a self antigen (termed Jo1) that is targeted by autoreactive B cells[31]. 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 the phosphoantigen-mediated activation of human Vγ9Vδ2$^+$ T cells can be mimicked or inhibited by agonist or antagonist antibodies, respectively, specific for the widely expressed immunoglobulin superfamily member CD277 (also known as butyrophilin 3A1)[32,33]. Although the involvement of CD277 in phosphoantigen recognition is unresolved, it is interesting that mouse γδ T cells do not display phosphoantigen reactivity or express a CD277 homologue. However, CD277 has a high level of structural similarity to SKINT1, a mouse immunoglobulin superfamily member expressed by medullary thymic epithelial cells (mTECs) and keratinocytes that is crucial for the development of Vγ5Vδ1$^+$ DETCs (see below)[34,35]. Thus, conserved molecular mechanisms may underlie the activation of disparate γδ T cell subsets in mice and in humans.

In sum, the collective TCR specificities of γδ T cells permit these cells 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 clinically relevant HMBPP$^+$ bacterial strains (but not to neutrophils that have taken up HMBPP$^-$ strains)[36]. However, the upregulation of endogenous IPP in human cells in response to infection or non-infectious dysregulation[37,38] also provokes Vγ9Vδ2$^+$ T cell reactivity, albeit with a lower sensitivity. Similarly, mouse hepatic γδ T cells respond to CD1d molecules presenting cardiolipin, a major bacterial cell-wall phospholipid that is also an endogenous component of mitochondria[39].

Given the potential for diversity in adaptive immune receptors, cross-reactivity is inevitable and is well documented for B cells and αβ T cells. However, the cross-reactivity reviewed here for γδ T cells is an overt functional cross-reactivity that clouds whether cells are responsive primarily to foreign or self antigens. This in part reflects the unusual nature of the thymic selection of γδ T cell progenitors (see below). It is also similar to the status of B-1 cells, which have been proposed to mount rapid responses to common molecular signatures of infection or dysregulation[40]. Thus, even though few γδ TCR specificities have so far been defined, it seems clear that peripheral γδ T cells are distinct from their αβ T cell counterparts both in the constellation of antigens that they recognize and in the strong representation of self antigens within that constellation.

Non-obese diabetic mice
(NOD mice). An inbred strain of mice that spontaneously develop T cell-mediated autoimmune diabetes, which is dependent on their expression of the MHC class II molecule I-A^{g7}.

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 a low affinity and a broad specificity.

γδ T cell response kinetics

Lymphoid stress-surveillance. The capacity to recognize antigens that are rapidly displayed following infection or other forms of stress, and to respond in large numbers without requiring extensive clonal expansion, permits γδ 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, $CD4^+$ αβ T cells would not be able to participate in the immunosurveillance of tissues, because their activation requires processed antigens to be presented by highly specialized antigen-presenting cells. In this regard, afferent sensing is ordinarily attributed to myeloid cells, particularly DCs, which are typically viewed as the primary orchestrators of adaptive immunity. Therefore, to highlight the capacity of γδ T cells to perform an equivalent function to myeloid cells during the afferent phase, we have used the term 'lymphoid stress-surveillance'[41].

As we have discussed previously[42], epithelial cells express a set of gene products that regulate immune cells. We termed this set the 'epimmunome', which encompasses cell-surface molecules that are upregulated in response to numerous forms of cell dysregulation[42]. These include mouse RAE1 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 γδ 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 used by viruses and tumours to evade it[43,44]. Given the evidence that γδ T cells mediate TCR-dependent responses to heat-shocked cells but not to normal cells[45], it has long been hypothesized that some γδ TCRs recognize moieties that are upregulated on the surface of stressed cells. Indeed, tetramers composed of the DETC-expressed Vγ5Vδ1 TCR were shown to bind to a keratinocyte determinant that is transiently expressed adjacent to wounded skin[46]. Moreover, as mentioned above, MICA is reportedly a ligand for some human Vδ1 TCRs[17]. However, because only a few stress-regulated surface molecules have so far been identified, the generality of γδ TCRs engaging 'stress antigens' is hard to assess.

A different perspective is that γδ TCRs constitutively engage self ligands, thereby predisposing γδ T cells to respond to stress via co-stimulatory receptors such as NKG2D and/or receptors for cytokines such as interleukin-1 (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 RAE1 (REF. 47). Furthermore, recently published images show that constitutive signalling by the Vγ5Vδ1 TCR of DETCs occurs at specific sites of interaction with keratinocytes in the steady state[48]. The steady-state ligand recognized by the Vγ5Vδ1 TCR was not identified, but SKINT1 is a candidate for contributing to this interaction because it is expressed by keratinocytes in the steady state. This study also confirmed the observation that, following activation, DETCs make overt contacts with Langerhans cells[47], supporting the possibility that Langerhans cells also express a ligand for the DETC TCR. That DETCs respond rapidly to changes in epithelial cells and then communicate with Langerhans cells reinforces the assignment of γδ T cell function to the afferent sensing phase of an immune response, consistent with lymphoid stress-surveillance and distinct from the biology of most αβ T cells. Note, however, that γδ T cells also contribute to downstream effector and regulatory phases of immunity.

Co-stimulatory and inhibitory receptors. Conventional αβ 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 become anergic. This ensures that they are activated only in cases of genuine infection and dysregulation. As considered above, γδ T cells may respond to these signals in sequence rather than coincidentally; for example, pre-engagement of the TCR on DETCs is required for their response to NKG2D ligands. Similarly, phycoerythrin-specific γδ T cells acquire the capacity to respond to the pro-inflammatory cytokines IL-1 and IL-23 in the absence of other signals only after priming[7].

Such acquired responsiveness to signal 2 (co-stimulation) or signal 3 (cytokines) alone raises the issue of what ordinarily limits the activation of γδ T cells so as to prevent chronic inflammation. Although this issue is not resolved, it is notable that γδ T cells can express many receptors that regulate their responsiveness to their environment. Among these are inhibitory LY49 family receptors, the expression of which by αβ $TCR^+CD8\alpha\alpha^+$ intraepithelial lymphocytes (IELs) is associated with hyporesponsiveness[49].

Innate-like γδ T cell activation may additionally require input from unconventional co-stimulators. Indeed, DETCs express junctional adhesion molecule-like (JAML), which engages the coxsackievirus and adenovirus receptor (CAR) expressed by keratinocytes. This interaction activates phosphoinositide 3-kinase (PI3K), a signalling molecule that also mediates co-stimulatory signals from CD28 and NKG2D[50]. DETCs also express CD100 (also known as semaphorin 4D), which has been implicated in cell migration and morphology. Engagement of CD100 on DETCs by plexin B2, expressed by keratinocytes, induces the activation of extracellular-signal-regulated kinase (ERK) and promotes DETC activation-dependent rounding and cytokine production[51], possibly explaining why CD100-deficient mice show defects in wound healing. The plexin–semaphorin axis operates in many systems, and the engagement of CD100 on conventional T cells by plexin B2 expressed by plasmacytoid DCs affects IL-12 production and T cell priming[52].

Aryl hydrocarbon receptor. The aryl hydrocarbon receptor (AHR) is highly expressed by DETCs and γδ IELs, as well as by T helper 17 (T_H17) cells, where it most conspicuously regulates IL-22 production[53,54]. DETCs and γδ IELs do not make IL-17 or IL-22 (see below), but they are strongly influenced by AHR, in that AHR-deficient mice fail to sustain DETC and γδ IEL numbers

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 expressed by most NK cells and NKT cells, by many γδ T cells and by antigen-experienced cytolytic $CD8^+$ αβ T cells. NKG2D in humans recognizes MHC class I polypeptide-related sequence A (MICA) and MICB, and at least four related ULBP family proteins, and in mice recognizes multiple members of the structurally related retinoic acid early transcript 1 (RAE1) and H60 families and MULT1. Such ligands are generally expressed at the surface of infected, stressed or transformed cells.

Stress antigens
Molecules, such as MICA and RAE1, that are upregulated by cellular dysregulation and are recognized by lymphocytes as part of a process of immune surveillance.

Signals 1, 2 and 3
Cell signalling pathways that are activated by the engagement of the antigen receptor (signal 1), co-stimulatory receptors such as CD28 (signal 2) and cytokine receptors such as the interleukin-2 receptor (signal 3).

Anergic
A state in which a T cell is almost completely non-responsive to TCR engagement. This may occur when a peripheral T cell is exposed to an antigen in the absence of co-stimulation, and it is interpreted as a means to suppress potentially autoreactive T cell responses in the absence of infection.

Intraepithelial lymphocytes (IELs). T cells that reside in the basolateral side of an epithelium, above the basement membrane. They express either an αβ TCR or a γδ TCR, and in the murine gut they frequently express the CD8αα homodimer rather than the CD8αβ heterodimer that is expressed by conventional $CD8^+$ T cells in the lymph nodes and by another subset of IELs. It has been proposed that $CD8\alpha\alpha^+$ IELs are self-reactive, TCR agonist-selected cells that have regulatory properties.

over time[55]. AHR is broadly expressed, including by epithelial cells and Langerhans cells. However, its effects on DETCs and γδ IELs are dependent on its expression by RAG-dependent lymphoid cells and seem to reflect the intrinsic responses of lymphoid cells to aryl hydrocarbons found in food and other components of the environment. However, the effects of AHR are not specific to either γδ T cells or IELs, as earlier studies of AHR-deficient mice reported defects in systemic T cell compartments[56]. Nonetheless, AHR-mediated regulation of γδ T cells within tissues emphasizes the diversity of receptor-mediated interactions that transmit the status of the microenvironment to γδ T cells and that regulate these cells both positively and negatively. For example, interferon-γ (IFNγ) production by human $V\gamma9V\delta2^+$ 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 damage[57]. The need now is to elucidate the environmental conditions under which specific molecular sensors operate; which sensors are integrated with others and which function independently; and how the activities of specific sensors relate to γδ T cell biology.

γδ T cell functions

Activated mouse systemic γδ T cells and human peripheral blood γδ T cells can express high levels of IFNγ, tumour necrosis factor (TNF) and granzymes. In addition, IL-17 is produced by distinct subsets of systemic γδ T cells and by those in the dermis and intestinal lamina propria, and $CD4^+$ IL-4-producing γδ T cell clones have been described[58]. Collectively, these effector functions permit γδ T cells to participate in the later, efferent phase of immune responses.

However, γδ T cells can also display a broad functional phenotype that contrasts with the functional limitations of the conventional T_H1, T_H2 and T_H17 αβ T cell subsets. For example, DETCs can produce IFNγ and express high levels of granzymes, but they also express IL-13 (REF. 59), which can regulate B cells; growth factors such as insulin-like growth factor 1 (IGF1) that may regulate neighbouring stromal cells[60]; and numerous chemokines that recruit other leukocytes. This combination of cytotoxic T lymphocyte-, T_H1- and T_H2-type phenotypes brings to mind the finding that the transcription factor NFIL3 (also known as E4BP4) promotes the production of the T_H2-type cytokine IL-13 by chronically stimulated T_H1 and NK cells[61]. The broad functional phenotypes of rapidly responsive γδ T cells may enable them to contribute to the afferent phase of immune responses via pivotal interactions with other cells (FIG. 2), which we consider below.

Interactions with B cells. In interacting with other cells, a key function of γδ T cells may be chemokine production (TABLE 2). For example, activated human $V\gamma9V\delta2^+$ T cells can produce large amounts of CXC-chemokine ligand 13 (CXCL13)[62], which regulates the

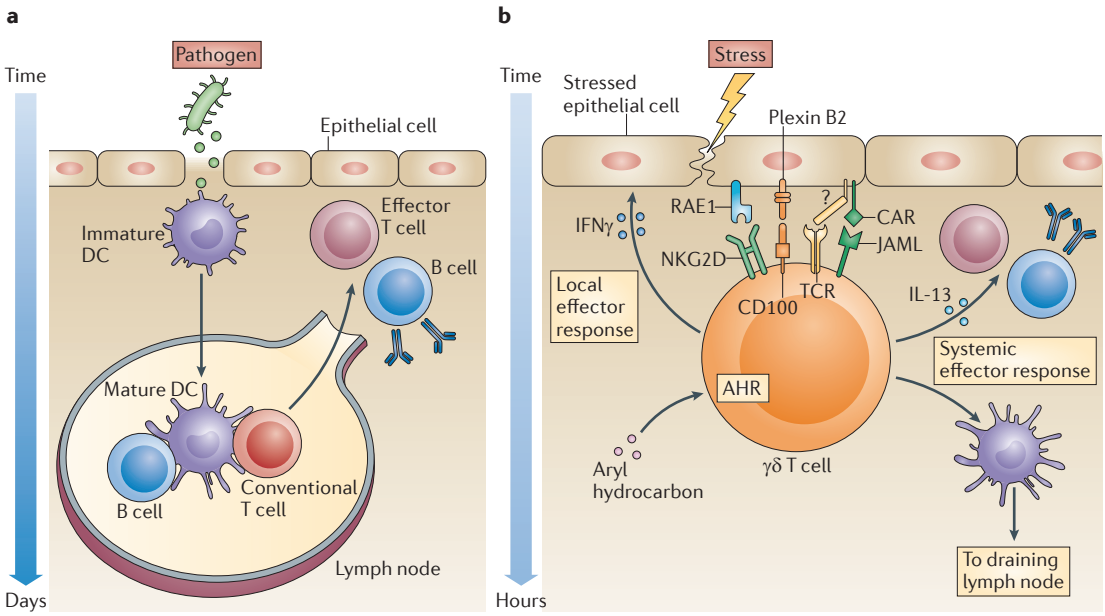

Figure 2 | **An alternative way to achieve broad systemic immune responses. a** | The textbook view of the activation of an adaptive immune response is shown. Immature dendritic cells (DCs) capture pathogens and then mature while migrating to the lymph nodes. Once there, they prime B cells and conventional (αβ) T cells, which can migrate back to the infected tissue and produce antibodies and mount effector responses, respectively. **b** | This very specific, albeit slow, response is complemented by γδ T cells, which, in response to various sources of stress (as depicted by the upregulation of RAE1 expression and the altered expression of other molecules on the stressed epithelium) mount local effector responses in addition to triggering the adaptive immune system. In contrast to antigen-specific αβ T cell responses, many γδ T cell responses are achieved within hours, following their sensing of local change and perturbation. This is termed 'lymphoid stress-surveillance'. AHR, aryl hydrocarbon receptor; CAR, coxsackievirus and adenovirus receptor; IFNγ, interferon-γ; IL, interleukin; JAML, junctional adhesion molecule-like; TCR, T cell receptor.

Table 2 | **Production of chemokines by γδ T cells**

Subset	Chemokine (alternative name)	Cell types expressing the receptor	Refs
Human			
Vδ2$^+$ cells (IPP primed)	CCL3 (MIP1α)	Macrophages	62
	CCL4 (MIP1β)	Macrophages, NK cells, other cell types	
	CXCL10 (IP10)	Macrophages, T cells, NK cells, other cell types	
	CXCL13 (BLC)	B cells	
Vδ1$^+$ cells (NKp30$^+$)	CCL3 (MIP1α)	Macrophages	122
	CCL4 (MIP1β)	Macrophages, NK cells, other cell types	
	CCL5 (RANTES)	T cells, eosinophils, basophils	
Skin Vδ2$^+$ cells	CCL1	Monocytes, NK cells, immature B cells	123
Mouse			
Lung-resident γδ T cells	CXCL1 (KC)	Neutrophils	124
	CXCL10 (IP10)	Macrophages, T cells, NK cells, other cell types	
Peritoneal Vδ1$^+$ cells	CCL3 (MIP1α)	Macrophages	125
	CCL5 (RANTES)	T cells, eosinophils, basophils	
DETCs	CCL3 (MIP1α)	Macrophages	126,127
	CCL4 (MIP1β)	Macrophages, NK cells, other cell types	
	CCL5 (RANTES)	T cells, eosinophils, basophils	
	XCL1 (lymphotactin)	CD8$^+$ cross-presenting DCs	

CCL, CC-chemokine ligand; CXCL, CXC-chemokine ligand; DC, dendritic cell; DETC, dendritic epidermal γδ T cell; IPP, isopentenyl pyrophosphate; NK, natural killer; XCL, XC-chemokine ligand.

organization of B cells within the follicles of lymphoid tissues[63]. Indeed, there is clear evidence for an impact of γδ T cells on B cells from both mice and humans. Germinal centres (albeit small ones) and the production of high levels of T cell-dependent immunoglobulins (notably IgE) can be detected in αβ T cell-deficient mice, particularly following infection[64], suggesting a compensatory role for γδ T cells in these responses. However, the immunoglobulins in these mice are mostly not specific for the challenging antigens, reiterating that immunization seldom evokes pathogen-specific γδ T cells in a conventional sense, but rather promotes rapid γδ T cell responses to a dysregulated state, the details of which differ according to the challenge. Consistent with these findings, when mild physical perturbation of the skin coincides with epicutaneous exposure to antigens, high levels of IgE result that are at least partially dependent on a normal DETC compartment and on NKG2D expression. The DETCs respond to NKG2D by making IL-13, which promotes class switching to IgG1 and IgE and the production of the corresponding immunoglobulins[58].

In humans, a hypomorphic *RAG1* mutation was recently reported that results in a predominance of γδ T cells[65]. Despite their substantial αβ 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, and another patient with very high absolute numbers of circulating γδ T cells also had high titres of circulating IgM, IgG and IgA. Likewise, of two patients with a recently identified leaky mutation in *CD3D* that mainly affects the αβ T cell compartment[66], the patient with the most responsive γδ T cells also presented with hyper-IgE syndrome and eosinophilia, further demonstrating a role for γδ T cells in the production of antibodies, particularly IgE. Indeed, healthy individuals harbour a subset of Vγ9Vδ2$^+$ T cells that expresses CXC-chemokine receptor 5 (CXCR5), has a T follicular helper (T_{FH}) cell-like phenotype and can provide B cell help[67], particularly in the presence of IL-21, the prototypical T_{FH} cell-inducing cytokine[68].

Interactions with DCs. Given that tissue-associated DCs can respond to numerous molecular indicators of infection and stress, can migrate to lymph nodes and, once there, can prime adaptive immune responses, one might reasonably question the importance of an afferent role for γδ T cells. One explanation is that γδ T cells collaborate with DCs, refining the quality of information that DCs receive about the status of a tissue, and thereby improving the criteria for whether or not an immunogenic or tolerogenic response ensues. For example, DCs lack NKG2D and, hence, may only be able to sense epithelial cell stress if it is communicated to them by responding γδ T cells. Indeed, the visualization of DETC–Langerhans cell contacts in the skin[47,48] encourages the view that activated γδ T cells directly regulate DC function, in contrast with the conventional view that DCs always function upstream of T cells.

Aminobisphosphonates (NBPs) include clinically approved entities (such as zoledronate) that inhibit a downstream enzyme in the mevalonate pathway of cholesterol synthesis, thereby causing the accumulation of IPP and sensitizing cells to recognition by Vγ9Vδ2$^+$ T cells. Thus, NBP-treated human DCs mimic DCs that are infected with specific types of bacteria, protozoa or viruses. Vγ9Vδ2$^+$ T cells potentiate the maturation of NBP-treated DCs via their production of IFNγ and TNF in a cell–cell contact-dependent manner[69]. In addition, immature DCs are reciprocally efficient at promoting Vγ9Vδ2$^+$ T cell activation[70]. The significance of such interactions is suggested by the finding that activated Vγ9Vδ2$^+$ T cells can relieve a block on DC maturation imposed by *Mycobacterium tuberculosis* infection[71].

Interactions with αβ T cells. A second explanation for the importance of γδ T cells in the afferent phase is that they may mimic the functions of DCs, thereby amplifying their functions or even substituting for them. Perhaps the signatory function of DCs is their presentation of antigenic peptide–MHC complexes to

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 antibodies.

Leaky mutation
A mutation that results in partial rather than complete inactivation of the wild-type function.

T cells. B cells can also present antigens[72], and although the case for conventional human T cells is less compelling, it is clear that human Vγ9Vδ2$^+$ T cells can also present antigens to CD4$^+$ T cells and cross-present antigens to CD8$^+$ T cells[73]. Activated Vγ9Vδ2$^+$ T cells take up soluble antigens very efficiently and can phagocytose opsonized target cells[74,75]. Moreover, steady-state Vγ9Vδ2$^+$ T cells localize mainly to the peripheral blood or to tissues, but following activation they rapidly express CC-chemokine receptor 7 (CCR7), which drives their migration to lymph nodes, and upregulate their expression of MHC class I and class II molecules and the co-stimulators CD80 and CD86 to levels equivalent to those on mature DCs[73]. This combination of properties is well-suited for antigen presentation to T cells in lymph nodes, underlining the innate-like potential of γδ T cells to initiate antigen-specific adaptive responses against virus-infected cells and tumours. Antigen presentation by mouse γδ T cells has not been demonstrated, but activated DETCs produce large amounts of the XC-chemokine ligand 1 (XCL1), which attracts cross-presenting DCs (TABLE 2).

γδ T cells may also regulate the αβ T cell repertoire. The interaction of fetal Vγ5Vδ1$^+$ DETC progenitors with SKINT1-expressing mTECs induces the epithelial cells to express autoimmune regulator (AIRE), which regulates the promiscuous gene expression that in turn purges the αβ T cell repertoire of strongly autoreactive cells[76]. However, there is little evidence for either a specialized peripheral regulatory γδ T cell subset or one that expresses the regulatory T (T_{Reg}) cell-associated transcription factor forkhead box P3 (FOXP3), although intestinal γδ IELs produce high levels of transforming growth factor-β (TGFβ) and LAG3, which singly or jointly may downregulate local T cell responses[77]. Indeed, hyperactivity of systemic αβ T cells is a common phenotype of γδ T cell-deficient mice[78,79] (see below). In sum, γδ T cells do not have unique functional capabilities, but they may be unique in their capacity to orchestrate a diverse range of functions appropriate to their participation in the afferent, efferent and regulatory phases of an immune response.

Tissue-associated γδ T cells

Selective homing and retention. What processes lead to the formation of tissue-localized γδ T cell compartments that are well suited to the afferent phase of immune responses? In mice, the answer relates in part to the schedule of γδ T cell development. The first wave of γδ T cells homes to the epidermis, the second wave to the genital tract, and subsequent waves to other tissue sites, such as the lungs and the gut. Following interaction with SKINT1$^+$ mTECs in the fetal thymus, Vγ5Vδ1$^+$ DETC progenitors acquire expression of CCR10, which in part directs them to the epidermis[80], suggesting that successive waves of γδ T cells developmentally acquire specific chemokine receptors. In addition, γδ T cells seemingly engage in steady-state interactions that stabilize their retention within particular tissues[48,81], although the molecules responsible for this important process are largely unknown.

Microanatomical specialization and pre-programming. Within tissues, γδ T cell subsets display overt microanatomical localization. In mouse skin, IFNγ-producing DETCs are localized in a different but proximal niche from IL-17-producing dermal γδ T cells, and in the intestine IFNγ-producing IELs are segregated from IL-17-producing γδ T cells in the lamina propria. It is not known why γδ T cells that produce IFNγ and those that produce IL-17 are best suited to the outer and inner layers of the tissue, respectively. Nonetheless, the hard-wired commitment of γδ T cell compartments to particular functional programmes is important because it permits the rapid responsiveness that is essential for the afferent response. This is achieved by developmental pre-programming.

By monitoring the development of H2-T10- and/or H2-T22-specific γδ T cells in mice that do or do not express H2-T10 and H2-T22, it was possible to show that agonist encounter during thymic development committed cells to a differentiation pathway characterized by IFNγ production, rather than IL-17 production[82]. Similarly, Vγ5Vδ1$^+$ DETC progenitors that engage SKINT1$^+$ mTECs express IFNγ, whereas Vγ5Vδ1$^+$ thymocytes in FVB mice from Taconic farms, which lack the *Skint1* gene, continue to express IL-17. The selective impact of SKINT1 is achieved at least in part by the upregulation of the genes encoding nuclear factor of activated T cells (NFAT), nuclear factor-κB (NF-κB) and early growth response protein 3 (EGR3), which collectively promote the expression of the IFNγ-inducing transcription factor T-bet and suppress the expression of the IL-17-inducing transcription factor retinoic acid receptor-related orphan receptor-γt (RORγt) and the 'γδ marker gene' SRY-box 13 (*Sox13*)[83]. This induced gene-regulatory network is not limited to SKINT1-selected DETC progenitors, but is also detected in adult γδ thymocytes that are identified by their phenotypic similarity to SKINT1-selected Vγ5Vδ1$^+$ fetal thymocytes. However, the network in these cells is induced by molecules other than SKINT1 that remain to be identified.

This gene-regulatory network is relevant to the observations that the mouse peripheral γδ T cell population can be subdivided into CD27$^+$ γδ T cells that produce IFNγ and CD27$^-$ γδ T cells that produce IL-17, and that many such cells are also developmentally pre-programmed[84]. Interestingly, the SKINT1-induced gene-regulatory network can be induced in adult thymocytes by direct TCR stimulation[83], consistent with the idea that TCR agonists are primary agents of functional pre-programming. This is in striking contrast to the developmental impact of TCR agonists on αβ T cell progenitors, in which agonists usually promote apoptosis or the upregulation of FOXP3 (which suppresses effector function).

According to these studies, IL-17-producing γδ T cell progenitors might not have encountered TCR-binding agonists during development. However, they may still have received crucial differentiation signals, possibly via ligand-independent TCR signalling. Indeed, 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

Autoimmune regulator
(AIRE). A transcription factor that is expressed by medullary thymic epithelial cells (mTECs) and promotes the promiscuous expression of genes that are otherwise specific to individual peripheral tissues. Peptides derived from these tissue-specific antigens are presented by mTECs to developing αβ T cells, and any T cells with high-affinity TCRs may be clonally deleted as a means of central tolerance.

Box 1 | γδ T cells in disease and therapy

Inevitably, functionally pleiotropic γδ T cells can contribute to and ameliorate disease and are attractive targets for clinical manipulation. The capacity of some γδ T cell activities to limit αβ T cell functions is manifested in the increased incidence of αβ T cell-dependent glomerulonephritis and lupus in MRL–*lpr* mice that lack expression of the T cell receptor δ-chain[112]. By contrast, the rapid responsiveness of interleukin-17 (IL-17)- and IL-22-producing CD27$^-$ γδ T cells to infection is mirrored by the rapid response of dermal IL-17-producing γδ T cells to the epicutaneous application of imiquimod, which promotes a psoriasiform pathology[113,114]. IL-17-producing γδ T cells have also been identified in psoriatic lesions but not in unaffected human skin[115]. There are at least two interesting implications of these findings. First, disease may reflect a violation of the anatomical functional microsegregation that ordinarily excludes IL-17-producing γδ T cells from the epidermis (see main text). Second, immunopathology in adults may be provoked by cells that arose in the fetus. Hence, disease might be predisposed by a dysregulated persistence of fetal cells.

IL-17-producing γδ T cells are also implicated in experimental autoimmune encephalomyelitis (EAE)[116,117], the mouse model of multiple sclerosis, and emerging evidence demonstrates that γδ T cells are crucial for the development of type 1 diabetes in non-obese diabetic (NOD) mice (J. Markle, A.H. and J. Danska, unpublished observations). Moreover, by promoting inflammation, IL-17-producing γδ T cells may also exacerbate tumour progression in cases in which transformed cells are not eradicated by prior lymphoid stress-surveillance[118].

Such considerations suggest two distinct clinical approaches. On the one hand, the clear capacity of human γδ T cells to detect transformed cells via NKG2D and/or other pathways, to promote cytolysis, to present antigens and to mobilize other components of the immune systems provides an antitumour potential that can be readily invoked by clinically approved aminobisphosphonates that upregulate γδ T cell-activating phosphoantigens. This approach has been pursued at multiple centres, is largely safe and has implied efficacy. However, γδ T cells become irreversibly exhausted after chronic aminobisphosphonate treatment, which impinges on the application of adoptive γδ T cell immunotherapy. The second approach would be to limit the activities of γδ T cells in autoimmune and autoinflammatory diseases. Whereas it is commonplace, but challenging, to treat such diseases with agents that suppress immune effector functions, targeting γδ 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 fetus and are not absolutely required for adult immune function.

exposure to IL-1 and/or IL-23, which is also consistent with prior TCR signalling. However, the possibility that innate-like IL-17-producing γδ 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. Hence, the influence of TCR signalling on the development of IL-17-producing γδ T cells merits further study.

There is also a mouse γδ T cell subset that is pre-programmed towards IL-4 production. This pre-programming is mediated by interactions between signalling lymphocytic activation molecule (SLAM) on γδ T cell progenitors and SLAM-associated protein (SAP) on immature CD4$^+$CD8$^+$ αβ T cell progenitors[85]. Interestingly, these CD4$^+$CD8$^+$ αβ thymocytes also regulate the differentiation of IFNγ- and IL-17-producing γδ thymocytes in a process termed *trans*-conditioning that is in part mediated by lymphotoxin[86,87]. This and other mechanisms regulating γδ T cell development have been extensively discussed elsewhere[88].

Two origins of γδ T cells. As stated above, the biological significance of pre-programming is not fully understood. What, for example, is the benefit of extinguishing IL-17 potential in agonist-selected γδ T cells? Moreover, many peripheral naive γδ T cells do not display the molecular hallmarks of pre-programming and retain the potential for IL-17 production *in vivo* even when they have arisen from CD27$^+$ cells, which are more typically associated with IFNγ production. Collectively, such non-pre-programmed cells may constitute the adaptive γδ T cell compartment, which has specificity for diverse antigens, including phycoerythrin and herpes simplex virus glycoprotein (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 γδ thymocytes that fail to engage agonists with the 'non-committed' state of many other post-natal γδ thymocytes strongly suggests that the different γδ T cell subsets have distinct developmental origins. Possibly the IL-17-default position marks γδ T cell progenitors that will form the bulk of the innate-like cell population, whereas other mouse γδ T cell progenitors form the predominant reservoir of lymphoid-homing, adaptive γδ T cells (FIG. 1). Indeed, evidence was recently presented for the divergence of innate and adaptive T cell precursors before commitment to the αβ and γδ T cell lineages[89]. This study showed that γδ T cells that are rapidly responsive via innate co-stimulatory or cytokine receptors (see above) readily accommodate co-expression of TCRβ, consistent with the view that signalling through their own γδ TCR is the dominant force in their development. By contrast, TCRβ expression is selected against in those γδ T cells with more adaptive properties, because such cells (which have not undergone pre-commitment) might readily be diverted towards an αβ T cell fate by TCRβ expression and the consequent formation of a pre-TCR. The presence of discrete progenitors for innate and adaptive γδ T cells would readily explain why different gene-regulatory networks are found in distinct subsets of γδ thymocytes[90]. Moreover, most innate-like γδ T cells may be derived from fetal progenitors and might not be reconstituted from the bone marrow. This is certainly true for DETCs and for IL-17-producing CD27$^-$ γδ T cells, and it may also be the case for substantial numbers of human peripheral blood γδ T cells. This has profound clinical implications in terms of bone marrow transplantation, and it highlights the importance of ontogeny in γδ T cell biology.

γδ T cells and ontogeny

γδ 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 for adaptive γδ T cell responses are derived, the T cell compartment throughout the first year of life can be dominated by γδ T cells. As discussed above, mouse DETCs and many IL-17-producing γδ T cells are exclusively generated from fetal progenitors. Indeed, the development of mouse and human IL-17-producing γδ T cells is selectively promoted by IL-7, the expression of which is highest in neonates[91]. This may explain why human IL-17-producing γδ T cells are readily evoked from cord blood but are very difficult to evoke from the peripheral blood of healthy adults[91,92]. Moreover,

MRL–*lpr* mice
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 the apoptosis of activated lymphocytes. The MRL strain contributes disease-associated mutations that have yet to be identified.

Imiquimod
An imidazoquinoline-based compound that is sensed by TLR7. It is currently used for the treatment of basal cell carcinoma, but it has also been implicated in iatrogenic induction of psoriasis-like symptoms.

although DETCs and IL-17-producing γδ T cells are functionally distinct, they clearly share a capacity for life-long self-renewal. The same may be true of many human IFNγ-producing Vγ9Vδ2$^+$ cell populations in the peripheral blood that are also derived from fetal progenitors and that undergo substantial expansion in early life[93]. Understanding the self-renewal of these differentiated cells may inform the biology of Langerhans cells and microglial cells, which were also recently found to be derived exclusively from fetal progenitors[94].

This striking ontogeny of γδ T cells suggests that their primary contribution is to neonatal protection, when conventional αβ 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 the adult compartment, but is qualitatively distinct. In support of this hypothesis, human γδ T cells are functionally precocious compared with αβ T cells[95,96]. Moreover, in independent cases of CMV transmission *in utero*, a dramatic expansion of human fetal Vδ1$^+$ T cells with highly related TCRs has been reported, suggesting a common response to a single epitope[97].

In two instances of parasite infection in mice, γδ T cells were required for the protection of young mice but not adults[98,99]. However, even as adults, the combined deficiency of αβ and γδ T cells increased susceptibility to parasite infection compared with TCRβ deficiency alone, demonstrating that responsive γδ T cells persist in adults[100]. In addition, the frequent association of γδ T cells with IgE induction may reflect their role in early life, as B cells that have switched directly to IgE production rather than via IgG1 production are immature and most abundant in very young mice[101]. In sum, the greatest dependence of cell-mediated immunity on γδ T 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, γδ T cells of fetal origin may variably persist in adults, and these cells may function together with those derived from postnatal progenitors to make additional key contributions to immunoprotection and, conversely, to immunopathology.

γδ T cell responses to specific challenges

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

For example, mice infected intraperitoneally with vaccinia virus have increased numbers of IFNγ-secreting splenic γδ T cells by 2 days post infection[102]. Such cells show increased reactivity towards vaccinia virus-infected cells, but there is no evidence of virus specificity. Nonetheless, compared with control mice, γδ T cell-deficient mice have substantially higher virus titres immediately post infection and increased mortality. However, in vaccinia virus-infected *Tcrd*$^{-/-}$ mice that survive, immunity develops normally[102], consistent with γδ T cells making innate-like contributions to the primary response but having no involvement in adaptive memory.

By contrast, γδ T cell deficiency in mice infected with West Nile virus (an emerging mosquito-borne pathogen) impairs responses to both primary and secondary infection. This is not because γδ T cells form memory cells in these animals, but because they make a crucial contribution to the quality of the memory CD8$^+$ T cells that are generated during the primary infection[103]. An analogous influence of γδ T cells exists over CD4$^+$ T cell memory generated during intravaginal infection by herpes simplex virus type 2 (REF. 104). In humans, γδ T cell population expansion is strongly associated with CMV infection[105,106], and γδ T cell clones derived from CMV-infected individuals secrete cytokines in response to both infected cells and tumour cells[19].

Likewise, γδ T cells are a crucial source of rapid IL-17 production in response to diverse bacterial infections, and *Tcrd*$^{-/-}$ mice show substantially increased susceptibility to infections by, for example, *Nocardia* spp., *Klebsiella* spp., *Listeria* spp., *Escherichia coli*, *Salmonella* spp., *Mycobacterium* spp. and *Pseudomonas* spp.[107]. In addition, *Tcrd*$^{-/-}$ mice have impaired responses to infection by certain parasites, including *Plasmodium* spp., during which IFNγ production by γδ T cells may be more important than IFNγ production by NK cells or αβ T cells[108,109]. Beyond infection, γδ T cells confer resistance to particular regimens of chemical carcinogenesis and to certain spontaneously arising tumours in transgenic mice, through mechanisms that have not been clarified in depth[110].

As mentioned above, the primary manifestation of γδ T cell deficiency in mice is often an inflammatory pathology that reflects exaggerated αβ T cell responses. Understandably, this has been taken as evidence that γδ T cells exert a regulatory effect on conventional T cells, possibly consistent with their production of TGFβ and LAG3 in the gut. However, it can also be explained by the rapid response to and limitation of infection by γδ T cells that thereby limits αβ T cell activation. In general terms, this may not be a unique means of regulation, as some human αβ T_{Reg} cells exert their effects by killing antigen-presenting cells in a phenomenon of linked suppression[111]. Importantly, this mechanism would be consistent with the sustained contribution of γδ T cells to protective immunosurveillance in adults. Naturally, the aggregate functional pleiotropy of γδ T cells has clinical implications, both in the cells' potential to cause and to regulate particular immunopathologies and in their utility as targets for immunotherapy to treat cancer and chronic infection (BOX 1).

Conclusions: six good reasons for γδ T cells

This Review has considered data, much of it recently published, in the context of six explanations for the unique contributions of γδ T cells in the immune system. Although much remains to be learnt, there is a sufficient basis to draw some conclusions. First, γδ TCRs engage a distinct constellation of antigens from αβ TCRs, thereby widening the scope of immune responsiveness. This may

Autoinflammatory diseases
Diseases that are characterized by seemingly unprovoked pathological activation of the innate immune system in the absence of overt autoantibodies or autoreactive T cells.

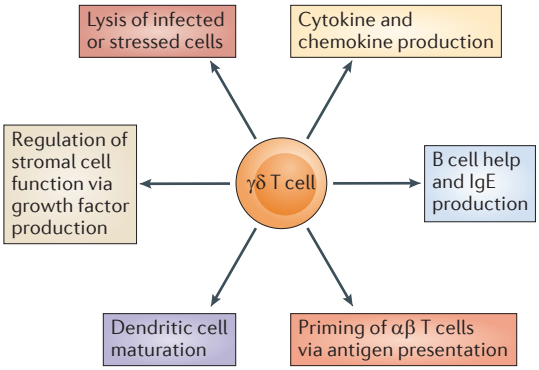

Figure 3 | **Six of the best γδ T cell functions.** An increasing body of literature now demonstrates that γδ T cells can have an important central role in defending the host against a broad range of infectious and sterile stresses. This is achieved through six main mechanisms. One, γδ T cells can directly lyse and thereby eliminate infected or stressed cells through the production of granzymes. Two, they can produce a diversified set of cytokines and chemokines to regulate other immune and non-immune cells. Three, they can provide help for B cells and promote the production of IgE. Four, they can present antigens for αβ T cell priming. Five, they can trigger dendritic cell maturation. And, six, they can regulate stromal cell function through the production of growth factors.

underlie a particularly significant contribution of adaptive γδ T cells. At the same time, the overt inclusion of autoantigens among their specificities illustrates a 'beneficial autoimmunogenicity' that may be based in part on the novel perspective that constitutive TCR engagement can facilitate rapid responses to stress or infection. Such afferent actions functionally and kinetically distinguish γδ T cells from most conventional T cells and rely in large part on the capacity of the γδ TCR to recognize self surface moieties expressed by cells within tissues. Moreover, the use of adaptive (somatically recombined) TCRs in the afferent phase potentially offers greater breadth and selectivity in the types of stimulus that γδ T cells can respond to, in comparison with the use of germline-encoded pattern-recognition receptors (PRRs) expressed by DCs or NK cells. Thus, γδ T cells may be better than PRR-dependent sensing at distinguishing between pathogenic and benign challenges and therefore at determining whether an immunogenic or tolerogenic response should ensue. Consistent with this, several adaptive responses are severely impaired in the absence of γδ T cells. Perhaps γδ T cells are well placed to eventually substitute for DCs in the role of afferent sensing.

Strictly speaking, γδ T cells do not have unique functions, but they can offer distinct combinations of functional potentials, such as cytolysis, IgE induction, antigen presentation and the production of growth factors (FIG. 3). The disposition of distinct functions among particular subsets, particularly within tissues, permits different complexions of the γδ T cell response. Thus, γδ IELs may promote the exclusion of infectious agents or toxins by eradicating targeted cells, by using IgE-mediated expulsion mechanisms, by recruiting other cells and by promoting tissue regrowth. Conversely, subepithelial responses may promote an integrative microbicidal innate and adaptive immune response to counter agents that have penetrated the basement membrane. Such a capacity to form functional compartments in particular anatomical niches may underlie a further crucial contribution of γδ T cells to immunity. However, it is possible that such roles may be increasingly subsumed by unconventional αβ T cells at human body surfaces. By contrast, αβ T cells may not be able to substitute for the functional potency of γδ T cells in newborns or even in the fetus, in which intrinsic (as opposed to maternal) mechanisms of immunoprotection are increasingly considered important.

Even in neonates, however, the growing set of data suggests that γδ T cells are disproportionately responsive to particular infectious challenges, notably CMV, *M. tuberculosis* and *Plasmodium falciparum*. The TCR Vγ genes show intraspecies and interspecies divergence that may reflect co-evolution with specific pathogens[1]. Moreover, although we have emphasized that the expansion of rare, antigen-specific γδ T cell clones is not the hallmark of γδ T cell biology, both *M. tuberculosis* and CMV infections seemingly induce the selective expansion of clones that share reactivity for relevant PAMPs or for molecular markers of infected cells. Thus, events early in life drive the expansion of specific γδ T cell populations and, although polyclonal, these population expansions result in 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 γδ 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 γδ 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 that rapidly convert them into innate-like cells. Given this, a particular emphasis should be placed on clarifying γδ T cell biology in CMV infection, tuberculosis and malaria, as these diseases are of great clinical significance and seem so effective at driving the early expansion of γδ T cell populations and the conversion of these cells into innate-like cells. These studies may test the utility of the mouse as a model for detailed aspects of γδ T cell biology. Indeed, like NK cells, γδ T cells show species-specific variation that is in part a reflection of their highly variable gene structures[1].

It is evident that some immunology college courses still pay little attention to γδ T cells. This is completely unjustified given the six signatory roles of γδ T cells reviewed here. We look forward to greater consideration, more extensive investigation and improved clinical manipulation of γδ 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 themselves.

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Acknowledgements
We thank many colleagues, within our laboratory and beyond it, including B. Silva-Santos, J. Kisielow, P. Fisch, L. Lefrancois, R. Tigelaar and W. Born, for thoughtful input and clarification of data, and we thank the Wellcome Trust and Cancer Research UK for funding. We apologize to those whose findings we may have inadvertently overlooked or that were a victim of space constraints.

Competing interests statement
The authors declare no competing financial interests.