γδ T Cells: First Line of Defense and Beyond

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Abstract

γδ T cells, αβ T cells, and B cells are present together in all but the most primitive vertebrates, suggesting that each population contributes to host immune competence uniquely and that all three are necessary for maintaining immune competence. Functional and molecular analyses indicate that in infections, γδ T cells respond earlier than αβ T cells do and that they emerge late after pathogen numbers start to decline. Thus, these cells may be involved in both establishing and regulating the inflammatory response. Moreover, γδ T cells and αβ T cells are clearly distinct in their antigen recognition and activation requirements as well as in the development of their antigen-specific repertoire and effector function. These aspects allow γδ T cells to occupy unique temporal and functional niches in host immune defense. We review these and other advances in γδ T cell biology in the context of their being the major initial IL-17 producers in acute infection.

INTRODUCTION

DETC: dendritic epidermal T cell

γδ T cells, αβ T cells, and B cells are the only cells that use somatic V, D, J gene rearrangement to generate diverse antigen receptors. Although the presence of αβ T cells and B cells and their roles in host immune defense were originally predicated on functional observations, the discovery of γδ T cells was unanticipated and resulted from the fortuitous identification of a T cell receptor (TCR) γ chain (1). The identification of the TCR δ gene was also serendipitous. In an attempt to understand the temporal expression of TCR α and β chains, Chien et al. (2) identified a region that is located within the TCR α locus but that rearranges early in thymic development. Further characterization of this locus showed that it contained a new TCR constant region gene, TCR δ.

γδ T cells are the first T cells to appear in the thymus during fetal thymic ontogeny. As the emergence and development of αβ T cells progresses, the relative proportion of γδ T cells decreases. In adult human peripheral blood, γδ T cells comprise between ~0.5% and 16% of total $CD3^+$ cells (with a mean of approximately 4%). A similar frequency of γδ T cells is also found in the organized lymphoid tissues (thymus, tonsil, lymph node, and spleen) and in the gut- and skin-associated lymphoid systems (3, 4), with no obvious tropism for epithelial microenvironments.

In adult mice, γδ T cells make up ~1–4% of total T cells in the thymus and the secondary lymphoid organs. Although similarly low frequencies of γδ T cells are present in the lung, these cells are more numerous in other mucosal sites: ~10–20% of total T cells in the reproductive tracks (5), ~20–40% of the intestinal intraepithelial T cells (6), and ~50–70% of skin dermal T cells (7, 8). Nearly all the T cells in the epidermal layer of the skin, the dendritic epidermal T cells (DETCs), express identical γδ TCRs (9). DETC-like γδ T cells have also been described in rats, but not in any other animal species.

Unlike mice and humans, cattle, sheep, and chickens have high numbers of γδ T cells among circulating lymphocytes (20–30% of the peripheral blood T cells) and in the skin (e.g., ~40% of bovine skin T cells) (10, 11). In these animals, γδ T cells express highly diverse TCRs regardless of their tissue localization.

γδ T cells and αβ T cells produce a similar set of cytokines and mount cytotoxic responses upon activation. However, αβ T cells are the main providers of "help" for the development of antibody as well as cytolytic αβ T cell responses. αβ T cells are also essential for eliminating infected cells. Accordingly, γδ T cell–deficient mice usually appear more immune competent than do αβ T cell–deficient mice. Nonetheless, correlations between different disease phenotypes and/or outcomes and the presence or absence of γδ T cells can be discerned, pointing to distinct functional roles for γδ T cells (12–15).

The frequency of γδ T cells expands dramatically (up to 60% of total T cells) in the blood of patients during infections (16–25) and among infiltrating T cells (up to 20–30% of total T cells) in early, but not in late, lesions of multiple sclerosis patients (26). The roles of γδ T cells as critical early responders and cytokine producers have been further demonstrated by Ferrick and colleagues (27), who showed with intracellular cytokine detection that γδ T cells were the major initial producers of interferon (IFN)-γ after *Listeria monocytogenes* infection and of IL-4 after *Nippostrongylus brasiliensis* infection. These cytokine-producing γδ T cells appeared days before the αβ T cell response developed (27).

More recently, γδ T cells have been identified as the major initial IL-17 producers in mouse models of infection and autoimmune diseases (7, 28–35). IL-17, which is largely made by T cells, is required to initiate the inflammatory response by inducing neutrophil maturation and recruitment from the bone marrow (36). Evolutionarily, this IL-17 requirement coincides with the appearance of the adaptive immune system. In acute infections, IL-17 production is often elicited without prior antigen exposure. A schematic representation of the γδ T cell IL-17 response relative to

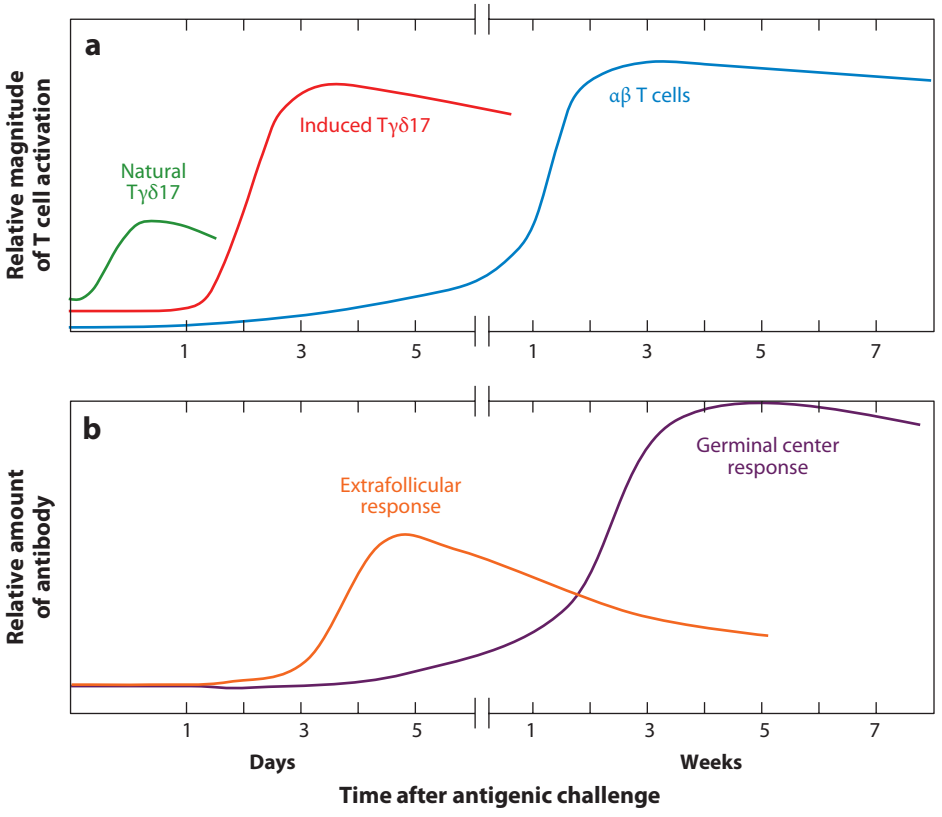

Figure 1

Kinetics of the development of (*a*) natural and induced γδ T cell IL-17 responses and of αβ T cell effector responses and (*b*) T-dependent B cell responses. (*a*) After stimulation, natural γδ T cells make IL-17 within 12 h; induced antigen-specific γδ T cells make IL-17 within 60 h. Naive αβ T cells require antigen-specific priming and take at least 5–7 days to develop effector function. (*b*) After encountering antigen, naive B cells proliferate, differentiate, and participate in either the extrafollicular response, where early antibodies are made within 3–4 days, or the germinal center response, which takes 1–3 weeks to develop (155).

that of αβ T cells and B cells is shown in **Figure 1**. Indeed, γδ T cells are distinct from αβ T cells in the generation of TCR diversity, in antigen recognition (reviewed in 37), in repertoire and effector fate development, and in their activation requirements (reviewed in 38, 39). These features allow γδ T cells to function as the first line of defense and to initiate the inflammatory response. We discuss these aspects and their implications in γδ T cell biology in this review.

THE GENERATION OF γδ T CELL RECEPTOR DIVERSITY

T and B cell antigen receptor diversity is conferred by V gene combinations and by the CDR3 regions formed by V(D)J junctions. In CDR3 regions, extensive length and sequence heterogeneity is the result of variations in the numbers of V, D, and J elements; of alternate open reading frames of D elements; and of random nucleotide (denoted by N) deletions and additions at gene fragment junctions.

CDR3: complementarity determining region 3

Concentration of Diversity at the CDR3 Regions of the TCR δ Chain

Although the sequence organization of the α/δ locus would allow a common pool of V gene segments to rearrange to either the α or the δ constant regions, most TCR α and TCR δ chains use different V genes and even different members of the same gene family. Whereas approximately 50 V genes are associated with TCR α, TCR δ–associated V genes number only about 10 in mice and 8 in humans. The TCR γ chain loci also have a small number of V gene segments. In mice, 6 V genes are associated with three constant region genes: Vγ1Jγ4Cγ4, Vγ2Jγ2Cγ2, and Vγ5, 6, 4, 7 that are rearranged to Jγ1Cγ1 [using the nomenclature of Heilig & Tonegawa (40)]. The human γ chain locus consists of four subgroups; VγI includes Vγ2, 3, 4, 5, and 8. Among the three other Vγ subgroups, only Vγ9 (from the VγII group) is functional [using the nomenclature of Lefranc & Rabbitts (41)]. The number of V gene combinations expressed by γδ T cells is further reduced because of Vγ and Vδ chain pairing requirements (42).

Nonetheless, among all immune receptor chains, TCR δs have the highest potential diversity in the CDR3 loop (approximately 10^{16} combinations for murine TCR δ) owing to the presence of multiple D gene segments (two in mice, three in human, and up to five in cattle) that can join together. Each D gene segment can be read in all three open reading frames, and N nucleotides can be inserted into the junctions of the joining segments. Thus, despite the limited diversity at the VJ junctions of TCR γ chains, the potential diversity generated at the combined CDR3 regions (approximately 10^{18} combinations) is still higher than that of αβ TCRs (~10^{16}) and immunoglobulins (Igs) (~10^{11}).

It is generally accepted that multiple V genes are evolutionarily advantageous. Yet structural analyses show that CDR3 loops of Igs, αβ TCRs, and γδ TCRs are critical for antigen binding (43–45). Furthermore, the CDR3 formed by V(D)J recombination appears to be crucial in determining Ig antigen specificity (46), despite the high level of diversity from V gene combinations in Igs (in mice, ~90,000 for Igs, compared with ~2,500 for αβ TCRs and ~70 for γδ TCRs). In this context, γδ TCRs should not be disadvantaged in their potential to recognize a diverse set of antigens.

Comparison of the CDR3 Length Distributions of All Immune Receptor Chains

Computer modeling of antibodies suggests that CDR length has a profound effect on its shape, as a difference of even one amino acid can produce a significant change in the overall structure (47, 48). The CDR3 loops of the TCR α and β chains are nearly identical in length, and their length distributions are constrained. These length constraints may reflect a functional requirement for the α and β chains of TCRs to contact both MHC molecules and bound peptides. In contrast, the CDR3s of Ig heavy chains are long and variable, and those of Ig light chains are short and constrained. This difference in length distributions may reflect the fact that Igs are capable of recognizing small organic compounds as well as large protein molecules. Interestingly, the CDR3 lengths of TCR δ chains are long and variable, whereas those of the TCR γ chains are short and constrained (49) (**Figure 2**). Thus, on the basis of the lengths of key structural components for antigen binding, we can conclude that γδ TCRs are more similar to Igs than to αβ TCRs. Indeed, the mode of γδ TCR recognition of antigen resembles Ig antigen recognition (discussed in the section entitled Antigen Recognition).

γδ TCRs with Antibody-Like Antigen Recognition Properties: An Evolutionary Perspective

In all tetrapods examined, the TCR β and γ chains are each encoded at separate loci, whereas the genes encoding the α and δ chains are at a single (TCR α/δ) locus. Several commonly used Vδ

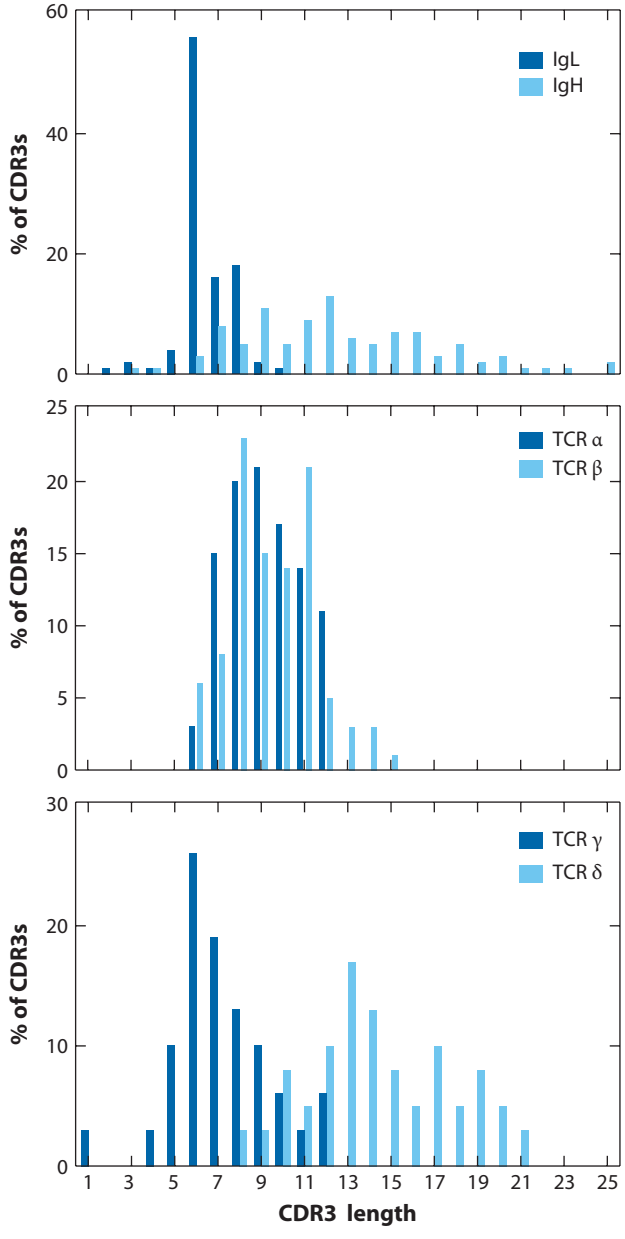

Figure 2

CDR3 length distributions of antigen-specific immune receptor chains. The CDR3 length is defined as four amino acids less than the number of amino acid residues between the J region–encoded GXG triplet, where G is glycine and X is any amino acid, and the nearest preceding V region–encoded cysteine as described in Rock et al. (49). Histograms show percentages of CDR3 sequences at given lengths in human chain families.

gene segments are in the Vα gene family. However, in both amphibians and birds, there are TCR δ isoforms that use V genes that appear indistinguishable from Ig heavy chain V (VH) (50, 51). Such V genes have been designated as VHδ.

MICA/MICB: MHC class I chain–related proteins A and B

PBL: peripheral blood lymphocyte

In the chicken, *Gallus gallus*, genes encoding VHδs are located at a second TCR δ locus that is unlinked to the conventional TCR α/δ locus (51). In the frog, *Xenopus tropicalis*, and a passerine bird, the zebra finch *Taeniopygia guttata*, the VHδs are located within the TCR α/δ loci, where they coexist with conventional Vα and Vδ genes (50, 51).

Marsupials also have a TCR locus unlinked to TCR α/δ (TCR μ) that uses antibody-related V genes. TCR μ is related to TCR δ, but it is highly divergent in sequence and structure (52, 53). TCR μ, which contains three extracellular domains, has evolved first in the ancestors of the cartilaginous fish in the form of NAR-TCR (54) and second in mammals as TCR δ (52). The NAR-TCR uses an N-terminal V domain related to the V domains found in IgNAR antibodies, which are unique to cartilaginous fish. The N-terminal V domains of TCR μ and NAR-TCR are unpaired. They may act as a single antigen-binding domain, analogous to the V domains of heavy chain–only antibodies found in sharks and camelids. Other than the bony fish and placental mammals, the presence of TCR chains that use antibody-like V domains (such as TCR δ using VHδ, NAR-TCR, or TCR μ) is widely distributed in vertebrates. These findings suggest independent solutions for generating TCR chains with antibody V domains in different vertebrate lineages. They may reflect the selective pressure for TCR chains that may recognize antigen in ways similar to that of antibodies in many different lineages of vertebrates.

Along this line, in jawless lampreys, adaptive immunity is mediated by variable lymphocyte receptors (VLRs), where the VLRA and VLRC types of receptors are expressed on T cell–like lymphocytes and the VLRB type of receptors are expressed on B cell–like lymphocytes. Comparing the gene expression patterns and tissue localization of VLRA- and VLRC-expressing cells, Cooper and colleagues (55) concluded that $VLRA^+$ and $VLRC^+$ cells resemble those of mammalian TCR $\alpha\beta^+$ and TCR $\gamma\delta^+$ T cells respectively. However, although the $VLRA^+$ cells may be the functional equivalent of αβ T cells, the VLRA type of receptor binds antigen directly (56).

ANTIGEN RECOGNITION

Consistent with the Ig-like recognition properties of γδ TCRs (discussed above) and with the lack of thymic maturation constraints imposed on the peripheral γδ T cell antigen–specific repertoire (discussed in the next section), γδ T cells can respond to a variety of stimuli irrespective of their molecular or genetic nature (**Table 1**). Immunization of mice with MHC-mismatched splenocytes generates MHC-reactive γδ T cell clones, including LBK5, specific for IE; LKD1, specific for IA (57, 58); and the G8 clone, specific for the nonclassical MHC class I molecules T10 and T22 (58, 59). Human γδ T cells that recognize MICA and MICB (MHC class I chain–related proteins A and B) (60) or CD1c (61) were derived from culturing γδ T cells with MICA- and MICB-expressing C1R cells or CD1c-expressing dendritic cells (DCs), respectively. G115, a human γδ T cell clone that was generated by culturing peripheral blood lymphocyte (PBL) γδ T cells with irradiated PBLs and B lymphoblastoid cells, recognizes ATP synthase and apolipoprotein A-1 (apoA-I) complex on tumor cells (62). In addition, stimulating lymph node cells from herpes simplex virus (HSV)–infected mice with HSV glycoprotein gI–expressing L cells generates herpes glycoprotein gI–reactive γδ T cell clones (63). Furthermore, the stimulation of human γδ T cells with tumor cells and *Mycobacterium tuberculosis* extract generates human γδ T cell clones that respond to low-molecular-weight phosphorylated molecules and alkylamines from host and microbial metabolic pathways (64–69).

Table 1 List of molecules shown to bind γδ TCRs or elicit a specific γδ T cell response[a]

Ligand/reactivity	γδ T cell	Source	Comments	Reference
Murine				
PE	0.02–0.4% γδ T cells (e.g., MA2)	B6 splenocytes	Interaction between MA2 TCR and PE is shown by direct binding	76
CD1d-cardiolipin		B6 splenic and hepatic T cells stimulated with cardiolipin		72
Ins2 B:9-23 (insulin peptide)	SP9D11 (Vδ10Vγ4)	Splenocytes from NOD TCR α transgenic mice immunized with ins2 B:9-23 peptide	Peptide response is independent of APCs	230
HSV-gI	Tg14.4	Lymph node of HSV-infected C3H mouse, restimulated with HSV-gI-transfected L cells	gI protein alone can stimulate Tgl4.4 clone	231, 63
HSP60 peptide	69BAS-122	Splenocytes from *Mycobacterium* PPD-immunized B10 mice	Transferring TCR transfers reactivity	232
Keratinocytes	DETC (e.g., 7–1),	Murine keratinocytes	Transferring TCR transfers reactivity	233
$IE^{b,k,s}$; not IE^{d}	LBK5	B10 nu/nu immunized with B10.BR splenocytes	Peptide/IE complexes stimulate LBK5	57, 82
IA^{d}	LKD1	B10.BR immunized with B10.D2 splenocytes		57
T10/T22	0.1–1% γδ T cells (e.g., G8), KN6	G8: BALB/c nu/nu immunized with B10.BR APCs KN6: C57BL/6 double-negative thymocytes	G8 TCR and T22 direct binding and cocrystal structure	45, 58, 89, 93
Qa-1/($Glu^{50}Tyr^{50}$)	DGT3	DBA2 lymphocytes primed with poly($Glu^{50}Tyr^{50}$)		234
Cardiolipin	69BAS-122, BNT-19.8.12	69BAS-122: C57BL/10 adult splenocyte BNT-19.8.12: B10 newborn thymocytes	Transferring TCR transfers reactivity	235, 236
β_2-glycoprotein 1	69BAS-122, BNT-19.8.12	69BAS-122: B10 adult splenocyte BNT-19.8.12: B10 newborn thymocyte	Transferring TCR transfers reactivity	235
Mycobacterium PPD	BNT-19.8.12	B10 newborn thymocyte	Transferring TCR transfers reactivity	236
Human				
CD1d-α-GalCer	9C2 clone; $V\delta1^{+}$ γδ T cells	PBMC γδ T cells	9C2 TCR and CD1d-α-GalCer direct binding and cocrystal structure	74
EPCR	LES	Clonally expanded γδ T cell from CMV-infected transplant recipient	LES TCR and EPCR direct binding	71
PE	0.025% PBMC γδ T cells (e.g., HX2)	PBMC γδ T cells	Transferring TCR transfers reactivity	76

(*Continued*)

Table 1 (*Continued*)

Ligand/reactivity	γδ T cell	Source	Comments	Reference
CD1d-sulfatide	Vδ1$^+$ γδ T cells, DP10.7, AB18.1	PBMCs or IELs enriched with CD1d-sulfatide tetramer	Soluble TCR binds CD1d in sulfatide-specific manner and cocrystal structure	73, 75
MutS homolog 2	Vδ2$^+$ γδ T cells	PBMC γδ T cells expanded with anti-pan TCR γδ		237
tRNA synthetases, EcIF1, formimino-transferase cyclodeaminase	M88	Muscle-infiltrating γδ T cells in an autoimmune myositis patient	Transferring TCR transfers reactivity	70, 113
ULBP4	Vδ2$^-$ γδ T cells; TCRγ9/δ2	Tumor-infiltrating γδ T cells expanded with plate-bound ULBP4	Transferring TCR transfers reactivity	238
AS/apoA-I	G115 (Vγ9Vδ2)	PBMC γδ T cells cultured with irradiated PBLs and lymphoblastoid cells	G115 and AS/apoA-I interaction shown by direct binding	62
CMV-infected fibroblasts, HeLa, HT-29, and Caco-2	4–29, 5–3 (Vδ2$^-$)	PBMCs from CMV-infected transplant recipients, stimulated with irradiated PBMC, PHA		239
CD1c-expressing cells	JR.2, XV.1 (Vδ1 cells)	PBL stimulated with autologous CD1$^+$ DC and *M. tuberculosis* extract	Transferring TCR transfers reactivity; many Vδ1$^+$ γδ T cells are not CD1c-reactive	61
MICA/MICB	Clones 1, 2, 3, 4, 5 (Vδ1 cells)	Lymphocytes from human intestinal epithelial tumors cultured with irradiated C1R-MICA and C1R-MICB cells	Transferring TCR transfers reactivity	60, 240
Alkylamines	Vγ9Vδ2 clones	PBMC γδ T cells stimulated with alkylamine-enriched bacterial culture supernatants	Transferring TCR transfers reactivity	66
Aminobisphosphonates	Vγ9Vδ2 clones	PBMC or RA synovial γδ T cells stimulated with *M. tuberculosis* extract	Reactivity requires cell-cell contact	241–243
Phosphoantigens-BTN3A1	Vγ9Vδ2 clones	PBMC or RA synovial fluid γδ T cells stimulated with *M. tuberculosis* extract PBMC γδ T cells from patient with tuberculoid leprosy stimulated with *M. tuberculosis* extract Vγ9Vδ2-expressing clones: PBMC γδ T cells cultured with irradiated PBLs and lymphoblastoid cells	Reactivity requires cell-cell contact; direct binding between a Vγ9Vδ2 TCR and phosphoantigen-BTN3A1 complexes	65, 68, 69, 112, 244, 245
Listeriolysin O	TCR γδ$^+$ cells	PBMC γδ T cells stimulated with live listeriae	Proliferation in response to LLO peptide 470–508	246

(*Continued*)

Table 1 (*Continued*)

Ligand/reactivity	γδ T cell	Source	Comments	Reference
Tumor cells (e.g., Daudi, Molt-4), *M. tuberculosis* extracts, and mevalonate pathway metabolites	Panels of T cell clones expressing Vγ9Vδ2 (Vγ9Vδ2 cells)	γδ T cells isolated from RA synovial fluid, stimulated with *M. tuberculosis* sonicate Vγ9Vδ2-expressing clones: PBMC γδ T cells stimulated with irradiated PBMCs and PHA	20% of Vγ9Vδ2 clones do not react to MT; only slightly more than 50% of MT- or Molt-4-specific clones recognize the other specificities	64, 65, 100, 103, 106, 109, 110, 111, 112, 247, 248
SEA	Vγ9$^-$ clones	PBMC γδ T cells stimulated with irradiated PBLs and EBV-transformed B cells	Dose-dependent cytotoxic activity against SEA-coated target cells	249
Tetanus toxin	DF	Lymphocytes of a hyperimmunized donor, stimulated with tetanus toxin and autologous APC		250, 251

[a]Abbreviations: APC, antigen-presenting cell; AS/apoA-I, ATP synthase/apolipoprotein A-I; CMV, cytomegalovirus; DETC, dendritic epidermal T cell; EBV, Epstein-Barr virus; EcIF1, *E. coli* translation initiation factor 1; EPCR, endothelial protein C receptor; HSV, herpes simplex virus; MICA/MICB, MHC class I chain–related proteins A and B; PBL, peripheral blood lymphocyte; PBMC, peripheral blood mononuclear cell; PE, phycoerythrin; PHA, phytohemagglutinin; PPD, purified protein derivative; RA, rheumatoid arthritis; SEA, staphylococcal enterotoxin A; TCR, T cell receptor.

γδ T cell antigens have also been identified from pathological and disease settings, including those from expanded γδ T cells in polymyositis (70) and after cytomegalovirus (CMV) infection (71). In addition, some populations of γδ T cells in normal human PBMCs also recognize lipid molecules such as cardiolipin (a marker of damaged mitochondria), sulfatide (a myelin glycosphingolipid), or α-galactosylceramide (α-GalCer) in association with CD1d (72, 73, 74), noted ligands of natural killer T (NKT) cells.

More recently, phycoerythrin (PE), a photo-antenna protein of algae, was found to be recognized directly by specific human, murine, and bovine γδ T cells (76). PE is noted for inducing a strong (αβ) T cell–dependent antibody response (77–79). This observation, together with the identification of γδ T cells that recognize human histidyl-tRNA synthetase, a common autoantibody target (70), indicates an overlap between the γδ T cell and B cell antigen–specific repertoire. Along this line, a variety of peptides were found to induce a γδ TCR–dependent T cell response (80, 81), but direct interaction between the TCR and the peptide has yet to be demonstrated. Some γδ T cell antigens and their recognition requirements are discussed below, as each analysis illustrates different aspects of γδ T cell antigen recognition.

Antigen Recognition Requirements

Analysis of peptide/IE recognition. The availability of the LBK5 γδ T cell clone (specific for IE^{b,k,s} but not IE^d) (57) provided an opportunity to directly compare γδ and αβ T cell antigen recognition. LBK5 recognition of peptide/IE complexes occurs in a manner distinct from αβ TCR recognition of peptide/MHC. In particular, LBK5 recognizes the peptide/IE complex but is not peptide specific. Consistent with these observations, mutations of residues located on the α helical regions of the IE α and β chains that affect αβ T cell recognition of peptide/IE^k do not affect LBK5 activity. Instead, the epitope on IE^k for LBK5 is centered on the polymorphic residues 67 and 70 of the IE β chain (82, 83). This explains the fine specificity of LBK5, which is for IE^{b,k,s} but not IE^d. In addition, LBK5 does not respond to IE^k with an altered glycosylation

PE: phycoerythrin

at the α 82 site, even though a carbohydrate structure is not required for LBK5 recognition (83). This type of interaction is consistent with the general contention that electrostatic interactions confer specificity to biological recognition without necessarily being energetically favorable, a way to ensure the specificity of an interaction without requiring a high affinity (84).

Indeed, the affinity of the LBK5 γδ TCR for moth cytochrome peptide/IE^k is low, with an estimated dissociation constant (K_D) greater than 240 μM. This is lower than the affinities of most soluble αβ TCRs for peptide/MHC complexes (10–100 μM) (85, 86). However, in general, there are approximately 50,000–200,000 IE molecules on a cell surface that present a diverse repertoire of peptides, such that only 200–2,000 of them may bind the same peptide. Because the LBK5 γδ TCR recognizes IE molecules regardless of the amino acid sequences of the bound peptide, the cell-surface ligand density would be 25–1,000 times higher than the density of ligands for peptide/IE-specific αβ TCRs. Thus, the LBK5 interaction with IE on the cell surface would be of a high valency despite the individual low-affinity interaction.

Analysis of T10/T22, MICA recognition. T10 and T22 are β_2 microglobulin (β_2m)-associated molecules with a truncated MHC-like fold and lack a peptide-binding groove (87, 88). T10 and T22 do not bind peptide or any other moiety and instead are recognized directly by the G8 γδ TCR (45, 89, 90) (**Figure 3**). Surface plasmon resonance indicates that G8 interacts with its T10/T22 ligands with high affinity, with a K_D of 13 μM (90). In particular, the association rate (on rate) ($k_{on} = 6.53 \times 10^4\ M^{-1}s^{-1}$) was the fastest among TCR-ligand interactions reported to date.

MICA has a T10/T22-like structure (91) but does not associate with β_2m. Analysis of the crystal structure of a MICA-specific γδ TCR, δ1A/B-3, and that of G8 indicate that T10/T22 and MICA are likely not recognized similarly by their respective γδ TCRs. In fact, the interaction between MICA and soluble δ1A/B-3 showed the weakest interaction and the slowest association rate, K_D = 110–900 μM, k_{on} = 5–53 $M^{-1}s^{-1}$ (92), among all TCR-ligand interactions reported to date.

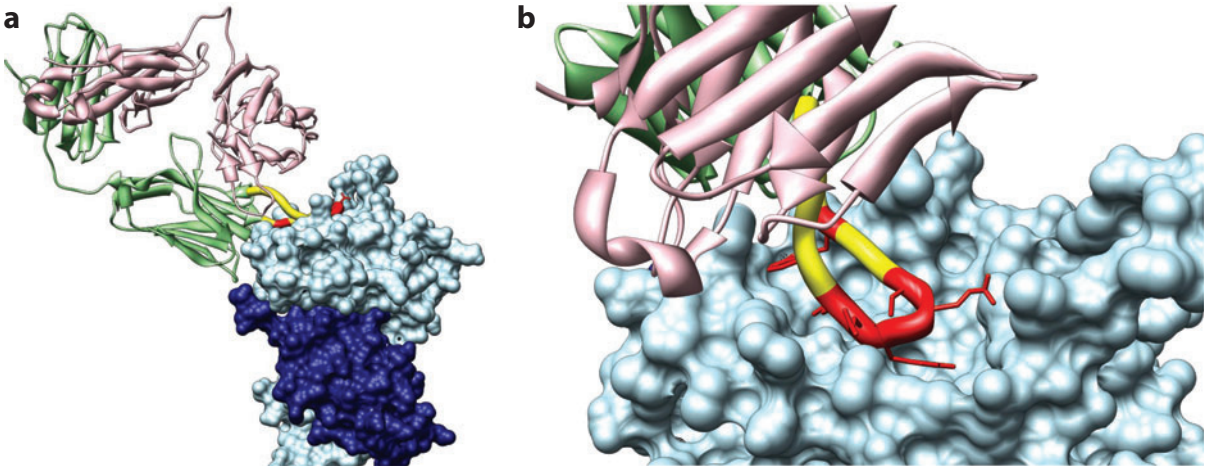

Figure 3

The interaction between T22 and the G8 γδ TCR. (*a*) The TCR γ (*pink*) and δ (*green*) chains, T22 (*light blue surface*), and β_2m (*dark blue surface*) are based on the published cocrystal structure of the G8 γδ TCR bound to T22 (PDB accession number 1YPZ) (45). The CDR3 loop of the TCR δ chain is shown, with the residues of the W-(S)EGYEL motif, which allow for T10/T22 binding. (*b*) The CDR3 δ loop in relation to the surface of T22 is shown. The CDR3 loop of the TCR δ chain is shown in yellow, with the residues of the W-(S)EGYEL motif, which allow for T10/T22 binding, shown in red. Reprinted with permission from *Immunological Reviews* (37).

Analysis of T10/T22-specific γδ TCRs. In addition to being ligands of G8, T10/T22 are ligands of KN6, which was derived from a normal C57BL/6 $CD4^{-}CD8^{-}$ thymocyte (93, 94). Moreover, ~0.1–1% of total spleen and intestinal intraepithelial lymphocyte (IEL) γδ T cells in naive mice can be stained specifically with a T22 tetrameric staining reagent (90). T10/T22-specific γδ TCRs are defined by a CDR3 δ motif that consists of a Vδ- or Dδ1-encoded Trp (W); a Dδ2-encoded sequence of Ser, Glu, Gly, Tyr, and Glu (SEGYE); and a P nucleotide–encoded Leu (L) (95). The W-(S)EGYEL motif is necessary and sufficient for T10/T22 binding, and sequence variations in the CDR3 regions around this motif modulate the affinity and the kinetics of T22 binding (95, 96). The cocrystal structure of the G8 γδ TCR bound to T22 (45) is shown in **Figure 3**.

In mice, most spleen and lymph node γδ T cells express Vγ1 and Vγ4, whereas Vγ7-expressing γδ T cells are more prevalent in the IELs. This bias in Vγ usage has led to the suggestion that Vγ-encoded residues enable these T cells to respond to antigens unique to their resident tissues (97). However, Shin et al. (95) found that most T10/T22-specific γδ T cells in the spleen express Vγ1 and Vγ4 and that those in the IELs express Vγ7, indicating that for this specificity the Vγ usage reflects the tissue origin but not the antigen specificity of the γδ T cells.

Recognition of CD1d/lipid complexes by human γδ TCRs. Population of human γδ T cells that are specific for CD1d-sulfatide and CD1d-α-GalCer were identified using a tetramer-based staining and isolation strategy. In both cases, γδ T cells with the capacity to bind to CD1d are modulated in a sulfatide- or α-GalCer-dependent manner (72–74). Interestingly, γδ T cells with such specificity mostly express Vδ1. The structure of a γδ TCR–CD1d–α-GalCer ternary complex indicates that the Vδ1 chain and, in particular, the germline-encoded CDR1δ loop dominated interactions with CD1d, and the TCR γ chain sat peripherally to the interface, with the CDR3 γ loop representing the principal determinant for α-GalCer specificity. Notably, the mode of γδ TCR–CD1d–α-GalCer recognition is markedly different from that of type I NKT TCR recognition of CD1d bound to α-linked or β-linked GalCer (74). A structure of a γδ TCR-CD1d-sulfatide complex also showed that Vδ1 dominated the interaction with CD1d. Nonetheless, all three CDR loops of Vδ1 were involved in CD1d recognition, and the CDR3 of the TCRδ chain contacted the sulfatide (75).

A microbial-encoded natural antigen recognized by murine and human γδ T cells. PE is a fluorescent molecule located on the tip of the photosynthetic antenna of cyanobacteria and red algae. PE stains ~0.02–0.4% of γδ T cells in normal unimmunized mice, stains ~0.025% of human and bovine peripheral γδ T cells, and is recognized directly by specific murine and human γδ TCRs (76).

Direct binding between a PE-specific γδ TCR (MA2) and PE showed a K_D of 2.7 μM as measured with surface plasmon resonance. Although PE is not a membrane-associated molecule, it is a hexameric protein that has tetrapyrrole chromophores covalently linked to cysteine residues. Scatchard analysis of the apparent affinity of PE binding to MA2 expressed on $58\alpha^{-}\beta^{-}$ cells showed a subnanomolar K_D (0.3 nM) with a half-life of approximately 20 min, underscoring the contribution of valency to γδ TCR–ligand interactions.

Most PE-specific γδ T cells in the mouse spleen express Vγ1 and Vγ4, and those in the IEL express Vγ7, again demonstrating that the Vγ usage reflects the tissue origin, not the antigen specificity, of γδ T cells. Mutagenesis studies indicated that the CDR3 regions of the TCR γ and δ chains encode PE binding determinants, with contributions from the CDR1 and/or CDR2 encoded on the Vδ gene. Although there are common CDR3 features shared among murine PE-specific γδ TCRs, these features are not shared by human PE-specific γδ TCRs, and there is no apparent sequence similarity between the human and the murine PE-specific γδ TCR sequences.

Thus, the antigen specificity, but not the particular antigen-specific TCR sequences, is conserved through evolution. This aspect is a defining characteristic of adaptive immune recognition.

Antigens Recognized by γδ T Cells in Pathophysiological Settings

Phosphoantigens and human Vγ9Vδ2 γδ T cells. γδ T cells make up approximately 4% of the PBL T cells in healthy human adults but can expand to 60% during a variety of infectious diseases (16–25). Most of the expanded γδ T cells express Vγ9 and Vδ2 TCRs [also referred to as Vγ2Vδ2 by the Seidman and colleagues nomenclature (98)], suggesting some of the specificities within this population may be important in responding to pathogenic challenges. Indeed, in vitro incubation of PBL with mycobacterial lysates induces an expansion of Vγ9Vδ2-expressing γδ T cells, and the stimulatory components are protease resistant and phosphatase sensitive (99). Subsequently, compounds containing 5′ triphosphorylated thymidine in the mycobacterial lysate were found to stimulate one such clone, G115 (67, 100). However, the structures of these small molecules were later disputed (101). Another Vγ9Vδ2 TCR clone, 12G12, derived from the lymphocytes of a tuberculoid leprosy patient, responds robustly to isopentenyl pyrophosphate (IPP), an intermediate in the human mevalonate pathway, and (E)-4-hydroxy-3-methyl-but-2-enyl-pyrophosphate (HMBPP), a microbial isoprenoid intermediate (69). These phospho-containing compounds are collectively called PAgs, and several microbial and plant PAgs produced through the deoxyxylulose pathway (102), such as hydroxy-methyl-butyl PP, show 10,000-fold higher activity than those from mammalian PAgs. In addition, although some Vγ9Vδ2 T cells respond poorly to PAgs, they can respond robustly to naturally occurring primary alkylamines, such as *iso*-butylamine that are secreted by bacteria and found in certain edible plants (66). Collectively, the reactivity of these Vγ9Vδ2 γδ T cells could allow them to perceive microbial products in diverse infectious contexts.

It is not surprising that the response pattern of Vγ9Vδ2 γδ T cells is heterogeneous. TCR repertoire studies (100, 103) as well as TCR transfer and mutagenesis analyses (65, 104) indicate that the amino acid residues located in the CDR3 regions of both the TCR γ and δ chains in addition to the Vγ and Vδ gene segments are essential for the response. In this context, the TCR δ 97 Val/Leu/Ile residue is necessary for the PAg reactivity. Whereas this amino acid is encoded by N nucleotides in adult γδ TCR, it is primarily germline or P nucleotide encoded in fetal γδ thymocyte TCRs (105). This suggests that reactivity toward PAg is favored by combinatorial/recombination constraints in a population of human fetal γδ T cells.

It was reported that γδ T cell clones responsive to mycobacterial lysates are also responsive to Burkitt's lymphoma Daudi cells, leading to the supposition that the antigenic component(s) in mycobacterial extract and Daudi are closely related (100). Indeed, IPP and dimethylallyl pyrophosphate are often overproduced in cells with elevated mevalonate pathway activity (e.g., in response to activation or transformation) (106, 107). Thus, the activation of PAg-responsive T cells by IPP and its close derivatives (such as ATP isopentenol) might underlie their reactivity toward tumor cells. However, as Scotet et al. (62) have noted, efficient activation of some PAg-reactive clones requires millimolar concentrations of IPP. Thus, tumor cell recognition likely also involves cell-surface components. Indeed, an entity related to the mitochondrial F1-ATPase, which is expressed on tumor cell surfaces, in complex with apoA-I was identified as an antigenic complex recognized directly by specific human γδ TCRs with a K_D of 0.81–1.5 μM (62). This observation suggests an unanticipated tumor recognition mechanism by γδ T cells that could be related to their ability to proliferate in response to PAgs.

Although PAg reactivity is TCR dependent, some attempts to show direct interaction between PAgs and specific TCRs have failed (108). Recent studies implicate the A1 isoform of butyrophilin-3 (BTN3, also named CD277), a member of the extended B7 receptor family, in PAg reactivity

(109). The response pattern of PAg-reactive clones was affected by blocking or knocking down BTN3 on target cells. Moreover, Vγ9Vδ2 PAg responses toward BTN3 knockdown target cells were restored after reexpression of full-length BTN3-A1 or of chimeric proteins composed of the extracellular part of BTN3-A3 and the intracellular part of BTN3-A1 (but not of the wild-type BTN3-A3 isoform). These observations suggested that Vγ9Vδ2 T cell activation by PAg is through the recognition of an allosteric change in BTN3-A1 extracellular domain, which is induced in response to intracellular accumulation of PAg (110, 111). Nonetheless, De Libero and colleagues' (112) biophysical and structural studies indicate that BTN3A1 binds IPP and HMBPP in a small cleft in the distal V-like domain and makes cognate interactions with soluble Vγ9Vδ2 TCRs. It is this molecular complex that stimulates specific γδ T cells. More studies are needed to reconcile these seemingly discrepant results.

EPCR: endothelial protein C receptor

γδ T cells from a myositis patient. In a rare case of autoimmune myositis, muscle fiber was destroyed by monoclonally expanded γδ T cells (113). A clone, M88 (Vγ1.3Vδ2), which was established from these expanded cells, responds to protein extracts prepared from muscles as well as other mammalian cells and bacteria in a TCR-dependent manner. It was demonstrated that bacterial as well as mammalian translation apparatus proteins, including several tRNA synthetases and the *Escherichia coli* translation initiation factor (EcIF1), are M88 targets. The ability of these molecules to stimulate M88/58$\alpha^-\beta^-$ cells is conformation dependent, and the CDR3s of both the TCR γ and δ chains are essential for antigen recognition (70). This demonstration of M88 reactivity to a variety of molecules is a good example of poly-specificity of adaptive immune recognition.

Although it is unclear whether these tRNA synthetases and translational apparatus molecules are exposed in pathological conditions, autoantibodies against nuclear or cytoplasmic antigens, including antibodies to alanyl tRNA synthetase, are found in approximately 20% of patients with myositis. In this context, human histidyl-tRNA synthetase, a stimulatory antigen for M88, is also targeted by autoantibodies known as anti-Jo-1 (114). This observation reveals a link between γδ T cell and antibody responses in autoimmune myositis and suggests a possible mechanism for γδ T cell involvement in this and other autoimmune diseases.

γδ T cells in a cytomegalovirus-infected patient. Déchanet-Merville and colleagues (115) identified and isolated a γδ T cell clone, LES (Vγ4/Vδ5), that represented 25% of blood T cells of a lung transplant patient who suffered from CMV infection. The LES clone responded to CMV-infected fibroblasts and endothelial cells as well as to certain human tumor cell lines in a TCR-dependent manner. LES was shown to recognize endothelial protein C receptor (EPCR), a trans-membrane protein with 20% identity to MHC class I and CD1 molecules. Soluble LES TCR interacts directly with EPCR with a K_D around 90 μM. However, cellular EPCR expression does not confer LES recognition. Nonetheless, CMV infection of these EPCR-expressing cells augments their ability to stimulate LES (71).

The Size of the γδ T Cell Antigen–Specific Repertoire

In unimmunized mice, 0.1–1% and 0.02–0.4% of total γδ T cells are T10/T22- and PE-specific, respectively (76, 90). In humans, ~0.025% of peripheral γδ T cells recognize PE (76). If the frequencies of other antigen-specific γδ T cells are in a similar range as those of T22- and PE-specific γδ T cells, then the numbers of distinct γδ T cell antigens would be 10^3 to 10^4, which is considerably smaller than the estimated αβ T cell antigen–specific repertoire (~10^6). Along this line, the size of the B cell antigen–specific repertoire was originally estimated based on antigen-specific B cell frequencies: 0.004–0.007% of total B cells recognize nitrophenyl,

dinitrophenyl, and trinitrophenyl. These numbers were obtained using antigen-specific spleen foci formation as a readout (116, 117) and are likely to be underestimates, as this assay requires extensive proliferation of individual clones. In fact, FACS analysis showed that in naive mice, 0.1% of the murine B cells are PE-specific and 0.02% are allophycocyanin-specific (79). Based on these values, the size of the B cell antigen–specific repertoire would consist of approximately 1,000 to 5,000 antigens, which is in the same range as that of γδ T cells. In this context, it has been proposed that the immune repertoire can offer efficient protection against about 10^3 to 10^4 distinct infections relevant for the survival of a given species (118–120). Based on this argument, Zinkernagel & Hengartner (121) proposed that "in mice. . .immunity is generated from a starting number of about 100 to 1000 antigen-specific precursor [αβ] T and B cells" (p. 252). Although this range turns out to be much higher than the frequencies of naive antigen-specific αβ T cells, it is surprisingly close to that of antigen-specific γδ T cells in naive animals.

THE DEVELOPMENT OF ANTIGEN-SPECIFIC REPERTOIRE AND FUNCTION

γδ T cells, like αβ T cells, require development in the thymus before entering the periphery. Analysis of γδ T cell development in the thymus provides important clues about likely γδ T cell targets and functions as discussed below.

The Contribution of Thymic Maturation to the Peripheral γδ T Cell Repertoire and Effector Function

Gene rearrangement, but not thymic selection, is responsible for the canonical TCR sequences expressed on fetal γδ thyomocytes. γδ T cells are the first T cells that appear during fetal thymic ontogeny. The same V gene rearrangements and the junctional sequences for the first and second waves of γδ T cells (Vγ5Jγ1, Vδ1Dδ2Jδ2 and Vγ6Jγ1, Vδ1Dδ2Jδ2) are present in the fetal thymus of TCR $\delta^{-/-}$ mice and wild-type mice (122), suggesting that these restricted γδ TCR rearrangements are not due to selection. However, the appearance of the Vγ5Vδ1 cells as DETCs in the skin of adult mice and their ability to react to keratinocytes appear to require interaction with thymic antigen(s) (123–125), although it is unclear what ligand(s) DETC TCRs recognize. Regardless, the relatively homogeneous junctions in fetal γδ TCRs may be formed in V(D)J joining using a short homology at the breakpoint, as proposed for neonatal B cells (126–128).

γδ thymocyte development is not contingent on encountering cognate antigen in the thymus. Early studies of the role of thymic selection in the establishment of a functional γδ T cell repertoire analyzed transgenic KN6 and G8 γδ T cell development on B6 ($T10^b/T22^b$), BALB/c ($T10^d$), or $\beta_2m^{-/-}$ (no cell-surface T10/T22 expression) backgrounds. There were fewer or no transgenic T cells in the thymus and/or periphery in B6 mice compared with those in the BALB/c mice, and the transgenic T cells showed a reduced ability to secrete IL-2 and proliferate when stimulated in vitro. It was concluded that γδ T cells are subject to ligand-driven thymic positive and negative selection much like αβ T cells (129–132). However, using the same lines of G8 transgenic mice, Schweighoffer & Fowlkes (133) showed that the G8 transgenic T cells could mature in $\beta_2m^{-/-}$ mice, indicating that positive selection is not required for γδ development.

Analyzing the same specificity in normal mice with a T22 tetrameric staining reagent showed that similar frequencies (0.1–1% of total γδ T cells) of T10/T22-specific γδ T cells are found in the thymus, spleen, and IEL of B6, BALB/c, and $B_2m^{-/-}$ mice (32). These results indicate

that the expression of cognate ligand in the thymus does little to affect the peripheral T10/T22-specific γδ T cell repertoire. Consistent with this finding, approximately 0.85% of the TCR δ sequences from out-of-frame rearrangements or CD3ε$^{-/-}$ thymocytes have a CDR3 motif that is necessary and sufficient for T10/T22 binding (95), as described in the section above entitled Antigen Recognition. This frequency is well within the range of the normal frequency of T10/T22-specific γδ T cells in the periphery, suggesting that once the antigen specificity is generated by V(D)J recombination, it is only marginally modified by thymic selection.

Although γδ T cells can develop without ligand-mediated signals, signaling through the TCR is required for γδ T cells to mature and exit the thymus (134). It was demonstrated that all VγVδ pairs, except for the Vγ5Vδ1 TCR of DETCs, could dimerize and mediate signals without ligand engagement (32). TCR dimerization might be sufficient to drive the development of γδ thymocytes, which have a low signaling threshold. TCR dimerization may also enhance the signaling efficiency of peripheral γδ T cells upon antigen recognition. It was reported that γδ T cell signal transduction in response to anti-CD3ε cross-linking is superior to that of αβ T cells (135). Regardless, cell-surface marker expression and turnover rate analysis suggest that most γδ T cells in wild-type mice have not encountered ligand during development or in the periphery and that an antigen-naive γδ T cell repertoire is actively maintained in peripheral lymphoid organs by turning over rapidly, and by not prolonging the life span of γδ T cells that have encountered antigen (32).

The antigen-specific γδ IEL repertoire is not biased toward thymic antigens. Both αβ and γδ T cells are found among small intestinal IELs. It was commonly assumed that γδ IELs, like αβ IELs, develop from thymocyte precursors that have interacted strongly with self-ligand in the thymus (136). Nonetheless, T10/T22-specific γδ T cells are present with comparable frequencies in the IEL compartment of B6, BALB/c, and β_2m$^{-/-}$ mice. Furthermore, the small intestinal homing receptor CCR9 is preferentially expressed on γδ thymocytes that have yet to encounter ligand, whereas γδ thymocytes with high affinity for self-ligand are CCR9^{lo} (137). These results indicate that although self-reactivity is present within the IEL compartment, it is biased against a high-affinity self-reactive repertoire. Furthermore, the number of αβ IELs fluctuates wildly in response to microbial colonization, but under the same experimental conditions, γδ T cells in the intestinal epithelium seem barely to vary (138–141). This suggests that thymic output, rather than peripheral cues, play a greater role in establishing the γδ IEL repertoire.

Thymic ligand encounter determines γδ T cell effector fate. To test whether encountering ligand during thymic development affects γδ T cell function in the periphery, Jensen et al. (32) used expression of CD122 (the common β chain of the IL-2 and IL-15 receptors) to approximate γδ T cells that have and have not encountered ligand. They analyzed CD122^{hi} and CD122^{lo} γδ T cells isolated from the spleen and lymph nodes and found that upon TCR cross-linking, CD122^{lo} (antigen-naive) cells produced IL-17 but not IFN-γ. Conversely, CD122^{hi} (antigen-experienced) cells produced IFN-γ and not IL-17. Surprisingly, the differential ability to make IL-17 versus IFN-γ is already apparent in γδ thymocytes (32). These results indicate that γδ thymocytes that have not engaged ligand have the potential to develop into IL-17-producing cells, and the encounter of cognate ligand drives γδ thymocytes toward adopting the IFN-γ effector fate. Along these lines, Kisielow and colleagues (142) identified SCART1 and SCART2, scavenger receptor proteins that are highly expressed on γδ lymphocytes. Upon strong TCR stimulation, SCART2 levels on SCART^{+} cells decrease considerably. SCART2^{hi}, but not SCART2^{lo}, γδ T cells make IL-17 (142). Ribot et al. (143) demonstrated that γδ T cells expressing CD27 (a TNF receptor family member) make IFN-γ, whereas CD27^{-} cells produce IL-17. Stimulation through the TCR is known to drive the expression of CD27 on T cells (143).

Similar rules seemingly apply to fetal γδ T cell development. The first wave of fetal Vγ5Vδ1 thymocytes requires TCR engagement for development and seeding the adult epidermis (the DETCs) (123–125). This ligand engagement is dependent on the expression of Skint-1, a B7-related molecule, on fetal thymic epithelial cells. In wild-type mice, DETCs make IFN-γ; in contrast, DETCs on a Skint-1-deficient background are primarily committed toward an IL-17 effector phenotype (123, 144).

Although most γδ T cells make IL-17 or IFN-γ, a population of Thy1^{dull} γδ thymocytes that express TCRs encoded by Vδ6.3/6.4 and Vγ1Jγ4 make IL-4 in addition to IFN-γ, IL-10, and IL-3 upon activation (145, 146). Transcriptome analysis suggests a common molecular program among these Thy1^{dull} Vγ1^{+}Vδ6.3^{+} γδ T cells and the αβ TCR–expressing CD1d-restricted NKT cells (147), including the expression of the BTB–zinc finger transcription factor PLZF (promyelocytic leukemia zinc finger protein), suggesting that these cells have experienced strong TCR-mediated signaling during development (148, 149). Indeed, the Vγ1^{+}Vδ6.3^{+} γδ T cells are overrepresented in the periphery of mice with defects in TCR signaling (147, 148, 150).

Although the presence or absence of intrathymic ligand-TCR-mediated signaling seems to correlate with the IFN-γ or IL-17 effector function of γδ T cells from the thymus, lymph nodes, and spleen, this is not the case for γδ IELs. γδ thymocytes that have not encountered a thymic ligand may have a greater potential to home to the gut, and γδ IELs are uniformly CD122^{lo} (137). However, these cells do not make IL-17. Instead, they constitutively express high levels of Eomes and granzyme A and B (151) and are cytolytic in ex vivo redirected lysis assays (152).

Natural and Induced γδ Effector Cells

Several studies indicate that substantial numbers of γδ T cells in the peripheral mucosal tissues, such as the dermal layer of the skin, the peritoneal cavity, and possibly the lung in normal mice, produce IL-17 rapidly after infection or immune challenge (reviewed in 39). Reconstitution studies suggest that these cells are derived from fetal or postnatal γδ thymocytes (8, 153). Indeed, fetal γδ thymocytes have IL-17-producing capability, and the number of thymic IL-17-producing γδ T cells seems to peak at the perinatal period and decrease thereafter (154). This situation is reminiscent of B1 cells and their production of natural antibody. B1 cells develop primarily during fetal and postnatal life, and they produce natural antibodies without being exposed to specific antigens (155). We therefore designated these γδ T cells as "natural" IL-17-producing γδ T cells (Tγδ17) (also referred to as "innate" Tγδ17 in some publications).

In contrast, most γδ T cells in secondary lymphoid organs show no demonstrable effector function. More than 90% of the lymph node γδ T cells have the CD44^{lo}CD62L^{hi} phenotype (32, 76), typical of naive αβ T cells. These cells respond to immune challenge and develop effector function. Within two days after influenza virus infection, there is a significant increase in T22 expression on myeloid cells and an increased number of T22-specific γδ T cells with an activated phenotype (CD69^{hi} and CD62L^{lo}) in the regional lymph node (156). In addition, antigen-specific γδ T cells respond to immunization and differentiate into IL-17-producing cells within 60 h after encountering antigen (76). We designate IL-17-producing γδ T cells that develop in response to immune challenge as "induced" Tγδ17. The development of natural and induced Tγδ17 cells is discussed in more detail below and is presented schematically in **Figure 4**.

Natural Tγδ17 cells gain effector function in the thymus. The observation that some γδ thymocytes have effector-like properties is somewhat surprising, as most αβ T cells gain effector capability in the periphery. In fact, the ability to make IL-17 appears to be a default property of uncommitted (to either αβ or γδ T cell fate) early thymocytes. In Rag1-deficient

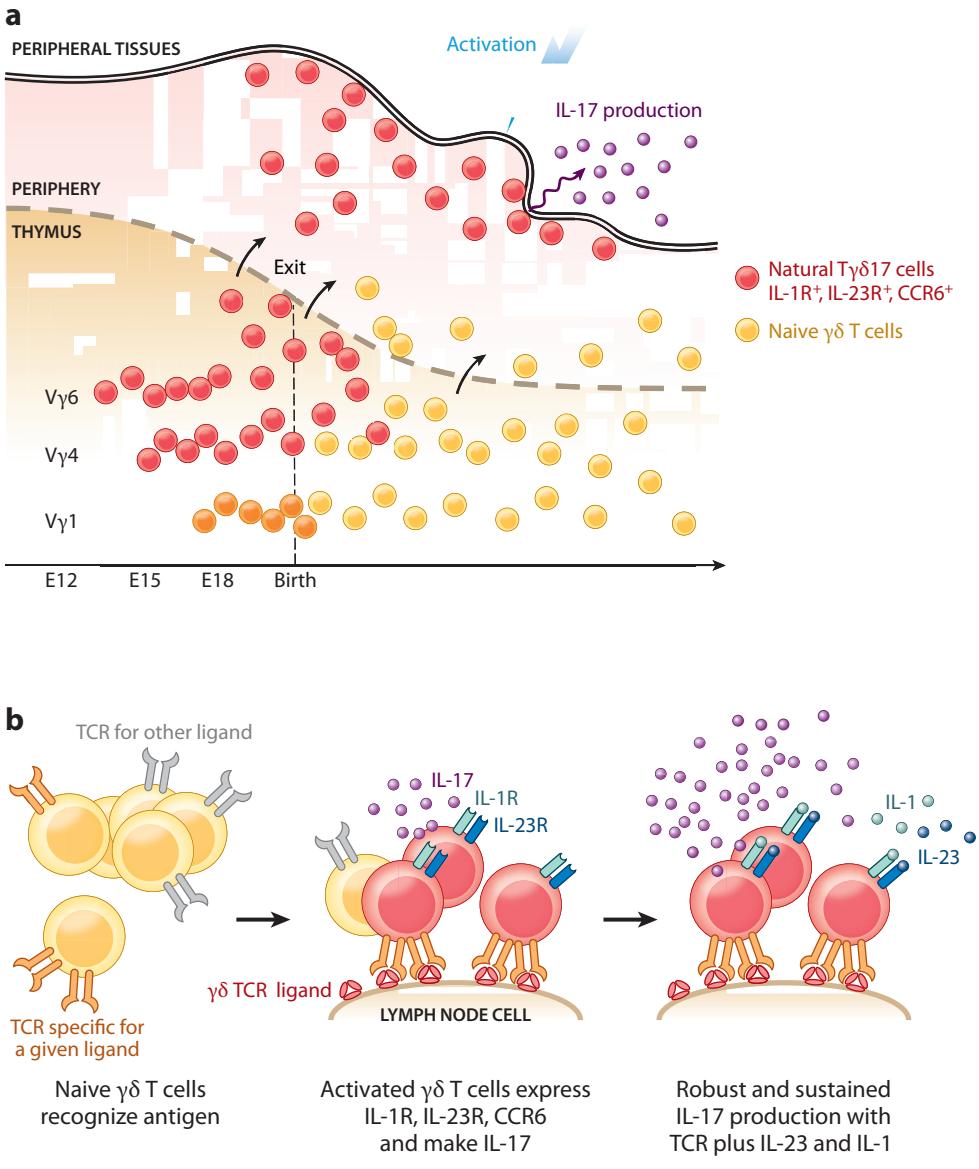

Figure 4

Models for the development of (*a*) natural and (*b*) induced IL-17-producing γδ T cells (Tγδ17s). (*a*) Commitment and differentiation of natural Tγδ17s occur in the thymus. Natural Tγδ17s exit the thymus as IL-1R$^+$IL-23R$^+$CCR6$^+$ effector cells and populate the peripheral barrier tissues such as the peritoneum and dermal layer of skin, where TCR triggering and/or IL-1 and IL-23 induce their production of IL-17 (153). (*b*) Induction of naive γδ T cells to produce IL-17 in the secondary lymphoid organs. Upon antigen recognition, naive γδ T cells are activated and gain effector functions, including IL-17 production and the expression of IL-1R, IL-23R, and CCR6. This responsiveness to IL-1 and IL-23 of activated antigen-specific γδ T cells further enhances and perpetuates IL-17 production by induced Tγδ17 cells (76).

thymi, IL-17A-producing cells can be found in the double-negative DN1 cells that express intracellular CD3 (157). Moreover, strong TCR signaling appears to divert γδ thymocytes from an IL-17-producing fate to an IFN-γ-producing (32, 144) or IL-4-producing (148, 149, 158) fate.

Kang and colleagues together with the Immunological Genome Project Consortium reported that natural Tγδ17 cells in adult thymus preferentially express Vγ4. Their IL-17-producing capacity are programmed by a gene regulatory network consisting of transcription factors SOX4, SOX13, TCF1, and LEF1 and not by conventional TCR signaling. SOX4 and SOX13 directly regulate the two requisite Tγδ17 cell–specific genes *Rorc* and *Blk*, whereas TCF1 and LEF1 countered the SOX proteins and induced genes of alternate effector. In addition, the development of natural Tγδ17 cells is also regulated by the transcription factor ETV5 (159–161). [Note that Kang and colleagues use the Raulet/Garman Vγ gene usage nomenclature (162); in their report, $V\gamma2^+$ cells are IL-17 producers. These are referred to here as $V\gamma4^+$ cells, using the Heilig & Tonegawa nomenclature (40).] The involvement of SOX13 in the generation of $V\gamma4^+$ natural Tγδ17 cells can also be inferred from two observations: (*a*) A $CD45.1^+$ B6 substrain (which has a spontaneous SOX13 mutation) shows a deficiency in $V\gamma4^+$ natural Tγδ17 cells (163) and (*b*) the engagement of TCR and Skint-1^+ thymic epithelial cells suppresses the expression of SOX13 and RORγt and regulates the gene network of Egr3, NFAT (nuclear factor of activated T cells), and NF-κB that commits cells to IFN-γ production (144).

Other studies suggest that signaling through lymphotoxin-β receptor/RelB (164), B lymphoid kinase (Blk) (165), the Notch/Hes1 pathway (166), transforming growth factor β (TGF-β) receptor (167), and prostaglandin I_2 receptor (168) is important. Conversely, signaling through Stat3, IL-6, or IRF-4 (33, 164, 166, 169, 170) is not required for the development of natural Tγδ17 cells. IL-17-producing γδ thymocytes have also been analyzed extensively in terms of their cell-surface markers and gene expression patterns. These cells are mostly $CD44^{hi}$ and express IL-23R, SCART2, and CCR6, but they express only low levels of CD122 or CD27.

From the studies of Kang and colleagues (159, 160, 161), one can infer that in adult animals IL-17 effector-like γδ thymocytes may contribute directly to the pool of natural Tγδ17 cells in the periphery. Gray et al. (8) showed that neonatal thymocytes can reconstitute dermal γδ T cells after irradiation. However, other reconstitution studies indicate that after lethal irradiation, only cells from fetal liver reconstitute the natural Tγδ17 cells in the periphery (153). In addition, using *Indu-Rag1* mice (171), where deficient *Rag1* expression could be induced in adult mice, Prinz and colleagues (153) showed that after irradiation, natural Tγδ17 cells in the periphery were not regenerated when TCR rearrangement was initiated only in adult mice. Instead, natural Tγδ17 cells were generated from E15.5 fetal thymi transplanted under the renal capsule (153). A similar set of fetal γδ thymocytes was reported to be the cells that give rise to the early IL-17 responders in the peritoneal cavity after *E. coli* infection (154). Taken together, these results suggest that more than one population of γδ thymocytes, at least with respect to their developmental appearance, may have the capability to become natural Tγδ17 cells.

These observations raise the possibility that γδ thymic emigrants expand into natural Tγδ17 effector cells in the neonatal lymphopenic environment and then take residence in peripheral tissues, where they persist throughout adult life. Naive αβ T cells undergo homeostasis-driven proliferation that is dependent on signaling through both cytokine receptors and the TCR (172, 173). In this context, guanine nucleotide exchange factor VAV1 signaling, a requirement for γδ TCR–mediated activation (174), but not Toll-like receptor (TLR) signaling, is essential for both the expansion and effector function of natural Tγδ17 cells in the peritoneum (175). Moreover, although a rapid IL-17 response can be elicited from natural Tγδ17 cells with IL-1 and IL-23, or by IL-23 together with TLR agonists without explicitly triggering the TCR (176, 177), this response is inhibited by cyclosporine A (CsA) and FK506 (76). These compounds disrupt the calcineurin-NFAT signaling

circuit that is activated by signaling through the antigen receptor (178). Taken together, these observations suggest that TCR-mediated signaling is required for natural Tγδ17 function.

Antigen encounter drives the development of Tγδ17s. The identification of PE as a γδ T cell antigen allows for the tracking and analysis of PE-specific γδ T cells in an immune response to determine the requirements for γδ T cells to mount an effector response. It was demonstrated that antigen recognition activated naive γδ T cells to differentiate toward an IL-17-producing phenotype: Within 24 h after PE immunization, PE-specific γδ T cells in the draining lymph nodes increased in number (approximately threefold) and showed activated phenotypes. Activated PE-specific γδ T cells express RORγt 48 h after immunization and, after another 12 h, IL-17. Encountering antigen in the context of an immune response induces the expression of inflammatory cytokine receptors such as IL-1R and IL-23R on specific γδ T cells (76), and the receptors for IL-1 and IL-23 are expressed only on γδ T cells that have been activated through the TCR (76, 175), and only the activated γδ T cells can respond to IL-1 and IL-23 without explicitly triggering the TCR. Importantly, neither the inflammatory cytokines alone nor the engagement of TCR alone induces a sustained IL-17 response that can match the magnitude of that induced by TCR stimulation in the presence of inflammatory cytokines (76, 177). This suggests that cytokine receptor signaling provides a second signal to TCR engagement to perpetuate the IL-17 response in inflammation and can also serve to prevent a prolonged and robust IL-17 response without inflammation.

A notable difference in how γδ T cells and αβ T cells acquire effector function in a primary response is the ability of PE-specific γδ T cells to produce IL-17 in the absence of clonal expansion (76). Despite the paucity of γδ T cells in the secondary lymphoid organs, the initial antigen-specific γδ T cell frequency (0.02–1% of total γδ T cells in naive animals) is much higher than that of antigen-specific αβ T cells before clonal expansion (0.0001% to 0.001% of total αβ T cells) (76, 90, 179–181). In fact, even without clonal expansion, the number of PE-specific γδ T cells in the draining lymph nodes is comparable to that of some antigen-specific αβ T cells at the height of clonal expansion in an immune response (181). The high initial frequency and the lack of a clonal expansion requirement would allow γδ T cells to mount a swift and substantial response when they encounter antigen for the first time.

TUNING OF THE γδ T CELL RESPONSE

Regulation of Self-Ligand Recognition

As discussed above, the peripheral γδ T cell antigen–specific repertoire is not purged of self-reactive cells. Indeed, some γδ T cell antigens are constitutively expressed on cells in healthy tissues, and their ability to trigger specific γδ T cells' effector function is initiated and/or enhanced in infections and/or other altered physiological states. These mechanisms include increases in ligand expression as with T10/T22 (90, 156); acquisition of new microbial or host components, such as the association of cardiolipin with CD1d molecules (72); exposure of intracellular molecules to γδ T cells due to cell death or transformation, such as tRNA synthetases (70) and F1-ATPase/apoA-1 (62); improved recognition when expressed on infected cells, such as EPCR (71); and recruitment by molecules such as Skint-1 (182) or BTN3-A1 (109) (summarized in **Figure 5**).

The γδ T cell response can be enhanced and prolonged by the presence of inflammatory cytokines (reviewed in 39, 183). TLRs, Dectins, and NLRs may act as TCR costimulators in human or primate γδ T cells or just as stimulators in murine or bovine γδ T cells (for a recent review, see 184). The engagement of inhibitory and activating receptors, many of which are shared by natural killer (NK) cells and memory αβ T cells, may fine-tune the γδ T cell

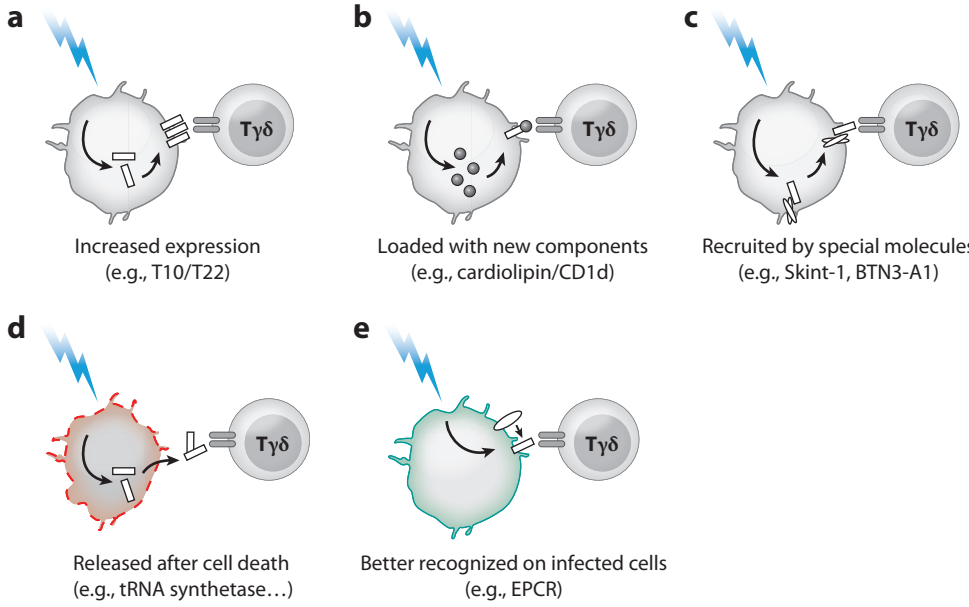

Figure 5

Modalities of self-antigen recognition by γδ T cells. Recognition of self molecules by γδ T cells can be initiated/enhanced by (*a*) increasing surface expression such as T10/T22 on monocytes after influenza virus infection; (*b*) acquiring new components, of either microbial or host origin, such as the association of cardiolipin with CD1d; (*c*) being recruited by B7 receptor–related molecules such as Skint-1 or BTN3-A1; (*d*) being released due to cell death, like tRNA synthetase; and (*e*) being better recognized on infected cells, like EPCR (endothelial protein C receptor).

activation threshold as well. Modulation of γδ T cell activation by the killer Ig-like receptors (KIR2D, KIR3D), C-type lectins (CD94/NKG2A-C, NKG2D), and several costimulators shared with αβ T cells (e.g., LFA1, CD2, CD27, CD28, 2B4) has been reviewed elsewhere (e.g., Reference 185) and is not discussed here. Human peripheral blood γδ T cells cultured in vitro with phytohemagglutinin (PHA) and IL-2 can upregulate several natural cytotoxicity receptors (NCRs) that are normally restricted to NK cells (such as NKp30, NKp44, and NKp46) (186). The expression of these NCRs greatly enhances recognition of lymphoid tumors by activated γδ T cells. In addition, although the low-affinity FcγRIII receptor CD16 is primarily found on NK cells, it is also expressed by a minor fraction of $CD3^+$ effector T cells, most of which bear the γδ TCR (187, 187a). It was reported that the expression of CD16 by human CMV–induced γδ T cells allows TCR-independent triggering of IFN-γ responses by opsonized virions and efficient inhibition of human CMV replication (188). There have been numerous reviews on cell-surface molecules, which can modulate γδ T cell activity, and we do not cite them individually.

The γδ T cell response may also be regulated by receptor-ligand pairs not commonly used by other lymphocytes. The requirement of BTN3 in TCR-mediated activation of human PAg-responsive γδ T cells is such an example (109, 111). Murine DETCs utilize a costimulation pathway involving the junctional adhesion molecule-like (JAML) receptor. Engagement of JAML by coxsackie adenovirus receptors, which are normally masked within the epithelial tight junctions but exposed when the epithelial barrier is disrupted, allows DETCs to respond rapidly to wounded epithelia (189). This type of regulation would allow γδ T cells to perceive activation contexts uniquely and to function accordingly.

NCR: natural cytotoxicity receptor

JAML: junctional adhesion molecule-like

Memory Response?

αβ TCR–deficient mice, but not γδ TCR–deficient mice, develop poor recall responses after secondary challenges with intracellular bacteria or protozoa (190, 191). This argues against the ability of murine γδ T cells to mediate efficient anamnestic responses, at least in these infection models. Nonetheless, it was reported that mice orally infected with *Listeria monocytogenes* induce a natural Tγδ 17 response in the mesenteric lymph nodes. Oral *L. monocytogenes* reinfection induces a higher magnitude (but not faster kinetics) of γδ T cell response, including cells expressing both IL-17 and IFN-γ (192).

In primates, Vγ9Vδ2 T cells show rapid recall-like expansion after *M. tuberculosis* infection of Bacillus Calmette-Guérin (BCG)-vaccinated animals, which correlates with protection against fatal tuberculosis (193). More recently, memory-like expansion of Vγ9Vδ2 T cells has been reported in primates after subclinical systemic infection and reinfection with attenuated *L. monocytogenes* strains (194). In humans, BCG-vaccinated adults show enhanced Vγ9Vδ2 T cell responsiveness to mycobacteria (195). Human CMV infection or reactivation in immunosuppressed patients is associated with clonal expansion of γδ T cells (196, 197). The observation that γδ T cell expansion correlates with resolution of the infection and lower incidence of solid tumors in immunosuppressed patients suggests that these γδ T cells acquire memory in order to play protective antiviral and antitumor roles (198). Nevertheless, in vitro Vγ9Vδ2 proliferative responses depend on T helper cells (199), and the recall-like expansion of Vγ9Vδ2 T cells after secondary mycobacterial or listerial challenges could result from enhanced activation and proliferation of conventional IL-2-producing αβ T cells rather than from actual priming of γδ T cells. Moreover, the direct causal links between γδ recall expansion or memory switch and enhanced protection against subsequent infectious challenges in macaques and humans have not been formally established. Therefore, even in primates, the ability of anamnestic γδ T cell responses to confer enhanced immune protection remains to be determined.

FIRST LINE OF DEFENSE AND BEYOND

The response of γδ T cells in infection models and pathological situations, including IL-17 production, has been expertly reviewed (200–202) and is not repeated here. In most instances, human γδ T cells elicited in infectious or tumor contexts perform pro- or anti-inflammatory functions through mechanisms that are shared with conventional αβ T cells. Therefore, it appears that few, if any, effector functions are entirely unique to human γδ T cells. Thus, as in mice, in humans the main distinguishing features of γδ T cell responses most likely lie in how these cells are triggered to act and the temporal expression of their effector responses. Nevertheless, we highlight a few unusual/unexpected functions of human γδ T cells and some unique roles played by murine γδ T cells at the skin and in the intestine. Both tissues require a correct balance between immunity and tolerance for proper function.

Human γδ T Cell Function

Human γδ T cells can produce various functional responses. Vγ9Vδ2 T cells expanded with live BCG or mycobacterial lysates can produce Th1 cytokines, granulysin, LL-37, and other antimicrobial peptides and can also exhibit cytolytic activity after coculture with mycobacteria-infected human monocytes. BCG-specific Vγ9Vδ2 T cell lines or clones also inhibit intracellular BCG growth in monocytes by secreting granzyme A (203).

Vγ9Vδ2 γδ T cells stimulated with PAg and primary B cells or EBV-transformed B cell lines acquire the ability to process and present antigens. These activated γδ T cells express lymph node

homing receptors (CCR7) and become professional antigen-presenting cells (APCs) (204–206). Although these results suggest a specific role for Vγ9Vδ2 T cells in the activation of the αβ T cell response, the capacity of γδ T cells to act as efficient APCs has yet to be confirmed in vivo. In addition, γδ T cells were reported to perform immunosuppressive functions in breast cancer patients via a blockade of DC maturation and induction of cell cycle arrest of T cells and DCs (207, 208).

Murine γδ T Cells as the First Line of Defense

Dermal γδ T cells have been implicated in the commensal-mediated balance between immune response and tolerance. In particular, dermal IL-17-producing γδ T cells (but not IL-17-producing αβ T cells) in germ-free mice are significantly reduced when compared with those from specific pathogen–free (SPF) mice, suggesting that the commensal microbiota in the skin affects the development of IL-17-producing γδ T cells (209). Similarly, microbial colonization in the intestine leads to the accumulation of natural IL-17-producing γδ T cells in the peritoneum (175). Indeed, dermal γδ T cells are the major source of IL-17 in mouse models of psoriasis (7) and in the lesions of psoriasis patients (7, 210). This is despite the scarcity of γδ T cells in human skin (211). In addition, intestinal IL-17-expressing γδ T cells were implicated in driving chronic intestinal inflammation in mice with reduced T regulatory cell numbers and functional capacity (212). These observations are consistent with the supposition that γδ T cells sense the microbiome to contribute to the immunity-tolerance balance in the barrier tissues.

Directly ex vivo, γδ IELs from normal mice can be triggered with anti-TCR antibody in a redirected lysis assay (152), suggesting that these cells can eliminate pathogen and infected cells readily. Furthermore, the lack of γδ T cells, but not αβ T cells, allows the rapid transit of orally administered microbes, including *Yersinia pseudotuberculosis*, *Toxoplasma gondii*, and *Salmonella typhimurium* (151, 213, 214). The absence of γδ T cells is also correlated with a significantly higher amount of penetrant resident microbiota in the small intestine immediately after environmental acquisition of new bacterial species (214). TCR $\delta^{-/-}$ mice have altered epithelial tight junctions and permeability, which could lead to the rapid migration of pathogenic microbes (213). Along this line, signaling through herpes virus entry mediator expressed on epithelial cells in the colon by the engagement of CD160 expressed on IELs has been reported to control epithelial permeability and bacterial dissemination after infection (215). Although both αβ and γδ IELs express CD160, $CD160^+$ γδ IELs become more prevalent after *Citrobacter rodentium* infection, a mouse model for acute attaching/effacing enteropathogenic *E. coli* infection in humans (215). Penetrant bacteria may also stimulate the γδ IEL antimicrobial response to limit bacterial invasion of mucosal tissues (214). Although histology analysis shows that γδ IELs are located beneath or between adjacent intestinal epithelial cells, in vivo microscopy shows that γδ IELs migrate actively within the intraepithelial compartment and into the lamina propria, suggesting an efficient way for γδ IELs to monitor the epithelial layer (216).

CONCLUDING REMARKS

γδ T cells were identified in the mid-1980s, and their participation in the early stages of immune defense was demonstrated nearly two decades ago. It was postulated then that these cells are involved in establishing the immune response to infections. The essential role of IL-17 in the initiation of the inflammatory response and the identification of γδ T cells as the major IL-17 producers in acute infections support this notion. Two classes of IL-17-producing γδ T cells have been identified: the natural Tγδ17 cells—which acquire effector function and inflammatory cytokines and chemokine-responding capability in fetal and/or neonatal thymus to mount an

immediate response—and the subsequent induced Tγδ17 cells—which develop rapidly in direct response to antigenic challenges without the requirement of clonal expansion. The regulation of early neutrophil infiltration is important to reset the cytokine milieu, and engulfment of dying neutrophils contributes to reprogramming macrophage cytokine secretion patterns, which affects the development of the adaptive immune response (217). Because γδ T cells play a major role in regulating neutrophil influx at the beginning of this process, they likely affect the subsequent development of αβ T cell and B cell responses.

In addition, the characterization of TCR ligands and the role of γδ TCRs in triggering γδ T cell activation suggest unique mechanisms for these cells to function in several settings. In particular, the Ig-like mode of antigen recognition and the lack of thymic constraint on the γδ T cell antigen–specific repertoire allow γδ T cells to recognize antigens of different molecular and genetic natures, including small native epitopes on structurally diverse molecules that may be intracellular but are exposed on the cell surface or extracellularly in broad pathophysiological contexts. Moreover, exploitation by γδ T cells of unusual costimulatory pathways involving innate receptors specific for a microbial or altered physiological state may strengthen their anti-infectious and antitumor functions and provide them with a unique ability to sense the loss of epithelial barrier integrity. Finally, the recent identification of γδ T cells specific for classical B cell antigens, such as PE, provides new insights into the possible regulation of αβ T cell–dependent antibody responses by γδ T cells.

Nevertheless, these observations leave us with several unanswered questions and raise exciting possibilities that should stimulate future studies. Although the underlying principle of γδ TCR antigen recognition is now better understood, the antigen specificity of the natural Tγδ17s is still unknown. Resolution of this issue should provide important insights into their unique modalities of pathophysiological detection. Whether natural γδ effector cells exist in other species, particularly in humans, remains a matter of debate. Although perinatal Vγ9Vδ2 T cells express activated/memory markers and have IFN-γ and TNF-α production potential that is consistent with Th1-like programing, an intrathymic commitment toward a Th1 effector type remains to be established. Similarly, although human cord blood–derived γδ T cells can produce IL-17 in response to innate signals (218), the existence of IL-17-precommitted γδ T cells in adults has yet to be supported by strong experimental evidence. In addition, although human γδ T cells show no obvious tropism for epithelial microenvironments, dermal γδ T cells are the major IL-17-producing cells in the lesions of psoriasis patients. It is important to know whether human γδ T cells also have a role, like their murine counterparts, in regulating immunity-tolerance balance in the barrier tissues.

The presence of γδ T cells in mucosae and epithelia, which are in direct contact with the external milieu, along with their ability to respond in acute infections and Ig-like antigen recognition mode make them well suited to recognize microbial components. Nonetheless, examples of direct recognition of microbial antigens remain scarce. Instead, most known γδ T cell reactivities are directed toward host antigens expressed in tumors and, in some cases, pathogen-infected cells. The prevalence of host-derived γδ T cell antigens may result from biases in the experimental approach used to identify them, in which γδ T cells were stimulated with tumor cells or host cells. Nonetheless, human CMV infection in immunosuppressed patients elicits dramatic clonal expansion of γδ T cells. Interestingly, most, if not all, of the dominant γδ T cell clones studied so far display dual reactivity toward CMV-infected normal cells and uninfected transformed cells and recognize a heterogeneous array of endogenous molecules that are upregulated in response to infection and cellular dysregulation (J. Déchanet, personal communication). The ensuing dual reactivity of γδ T cells toward infected and tumor cells could underlie the cross-protection against solid tumors mediated by CMV-elicited γδ T cells, as suggested by epidemiological studies (198). However, much work remains to be done to confirm and generalize these observations in other infection systems, especially during the initial response immediately after the infections.

Although current γδ T cell research has been focused largely on their role in the initiation of the inflammatory response, numerous studies show that γδ T cells emerge late in infections after pathogen numbers decline. In these cases, an exaggerated pathology and prolonged inflammatory responses have been correlated with the absence of γδ T cells (190, 219–228). Overly exuberant immune responses can cause damage through immune effectors and because of the energy allocated away from other physiological functions (229). A better understanding of the function of γδ T cells late in infection and of their integration into the resolution program should inform our understanding of the overall maintenance of host immune competence.

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