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Antigen recognition by human γδ T cells: pattern recognition by the adaptive immune system

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Introduction

T cell populations exist that express distinct T cell antigen receptors (TCRs). Following identification of the αβ TCR heterodimer protein complex [2, 77, 110], the genes encoding this TCR were cloned (reviewed in [124]). However, a third rearranging gene, at first mistakenly thought to encode TCRα, was isolated and later renamed TCRγ [144]. Separately, using chemical cross-linking techniques to identify CD3-associated subunits from T cells, we identified a population of T cells that expressed CD3 polypeptides but lacked TCRα or TCRβ transcripts. We showed that these cells expressed the protein product of the rearranging γ gene in association with yet another chain designated TCRδ [25]. The gene encoding TCRδ was then cloned providing the full primary structure of the distinct TCRγδ [6, 32, 78].

These distinct T cell antigen receptors evolved early in vertebrate phylogeny since both are found in primitive cartilaginous fish [132]. Although both TCRs are heterodimers that associate with the CD3 signaling complex, they vary greatly in the antigens that they recognize, the presenting molecules they use, and the way that they recognize these antigens.

Whereas antigen recognition by αβ T cells is now well understood, antigen recognition by γδ T cells has been hampered by the difficulty in identifying prominent antigens and antigen-presenting elements. Although γδ T cells clearly play an important role in human and murine immunity, it is not clear how γδ T cells monitor the host environment to decide when and where to respond to pathogens during infections or to host tissues during autoimmune responses or chemical injuries. Only in humans and mice have antigens been identified that are recognized by a significant proportion of γδ T cells. Few antigens have been identified for ruminant and avian γδ T cells, despite the high levels of γδ T cells that are present in these species. Alternately, in cases where broad antigen reactivity is noted, the antigen is unknown, as with the antigen in stressed keratinocytes that is recognized by murine dendritic epidermal γδ T cells [79]. Moreover, it is not clear if γδ T cells recognize antigens in the context of presenting molecules and, if so, the presenting pathways that are used. In

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some cases, they clearly do not use presenting molecules [150] or established intracellular processing pathways [114, 147]. Yet, from the available well-characterized examples, it is clear that the antigens for γδ T cells and how these antigens are recognized can be quite different from αβ T cells.

γδ T cells differ from αβ T cells in TCR diversity. Unlike αβ T cells, in mice and humans there are limited numbers of Vγ and Vδ gene segments (six Vγ and three major Vδ gene segments in humans, seven Vγ and seven major Vδ gene segments in mice) and J gene segments. Thus, there are limited numbers of CDR1 and CDR2 regions available for recognition. Human γδ T cells express one of six functional Vγ gene segments, five in the Vγ1 family (Vγ1.1, Vγ1.3, Vγ1.4, Vγ1.5, V γ1.8 also termed Vγ1, Vγ3, Vγ4, Vγ5, Vγ8 or VG1S1, VG1S3, VG1S4, VG1S5, VG1S8) and one in the Vγ2 family (Vγ2, also termed Vγ9 or VG2S1). More potential Vδ gene segments exist as most Vα gene segments can be expressed in γδ TCRs. However, the vast majority of human γδ T cells use one of three major Vδ gene segments, Vδ1, Vδ2, or Vδ3. Moreover, despite the presence of different γδ chain pairs at birth [116], after the age of 10 years most human γδ T cells express one V gene pair, Vγ2Vδ2 [120]. The remaining γδ T cells primarily express Vγ1Vδ1 TCRs. A small percentage of γδ T cells express Vγ2Vδ1 or Vγ1Vδ3 TCRs.

Adult human Vγ2 chains almost always use the Jγ1.2 junctional segment especially when paired with the Vδ2 gene segment. Moreover, the length of the Vγ2 CDR3 region is within 1 amino acid in 90% of Vγ2Vδ2$^+$ T cells [46]. Thus, even in "diverse" adult γδ T cells there can be relatively limited diversity in the junctional region.

Although there are a limited number of V gene segments, γδ T cells have more potential junctional diversity than αβ T cells due to the ability of their Vδ diversity segments to function in tandem [42]. In many situations, however, γδ T cells show limited junctional diversity. Because γδ T cells develop early in fetal life before terminal deoxynucleotidyl transferase is activated to insert N nucleotides in the junctional regions [171], γδ T cells may express invariant or canonical T cell antigen receptors.

The result of this unique γδ TCR expression is the existence of populations of γδ T cells that share a common TCR and thus are programmed to respond to a common antigen. For example, some human Vγ2Vδ2$^+$ T cells develop early in fetal life and express a canonical TCR [109]. T cells expressing this receptor are able to respond to nonpeptide prenyl pyrophosphate antigens [43]. Most murine dendritic γδ T cells that express invariant γδ TCRs respond to an unknown antigen on stressed keratinocytes [79]. The major example of a similar type of recognition in αβ T cells is the NKT cell population. These αβ T cells express an invariant Vα chain in humans and in mice, paired with a limited number of Vβ chains [11, 127]. αβ T cells expressing this receptor recognize self CD1d [11, 51]. The self reactivity to CD1d is the most prominent aspect of this type of recognition and has been shown to play important functional roles in tumor immunity and regulation of T cell differentiation and ongoing immune responses. It is likely that specialized functional roles also exist for γδ T cells expressing invariant T cell antigen receptors.

Three-dimensional structure of the Vδ domain of a human γδ TCR: implications for antigen recognition

Although the three-dimensional structure of an intact γδ TCR has not been obtained, we have determined the crystal structure to 1.9-Å resolution of the Vδ domain of a human γδ TCR designated ES204 (Vγ1.3-Jγ1.3/Vδ3-Dδ2-Jδ1) specific for the MHC class I molecule HLA-A2 [105]. Recognition appears to be independent of bound peptide [157], as in the case of other MHC-reactive γδ TCRs [31, 39, 147]. The structure provides direct evidence that γδ TCRs are structurally distinct from αβ TCRs, and is consistent with functional studies suggesting that recognition of certain antigens by γδ TCRs may resemble antigen recognition by antibodies, which also recognize antigens directly and without processing [31, 39, 114, 147, 150].

The TCR Vδ domain is structurally homologous to the V domains of both immunoglobulins and αβ TCRs with CDR loops disposed to form part of the antigen-combining site (Fig. 1). When Vδ is superposed onto representative V_H domains by overlapping 89 structurally equivalent framework residues, an average root-mean-square (r.m.s.) in α-carbon positions of 1.1 Å is obtained; a similar comparison with V_L domains (85 framework residues overlapped) gives an r.m.s difference of 1.7 Å. The r.m.s. difference is 1.4 Å with respect to Vα domains for 85 framework residues and 1.6 Å with respect to Vβ domains [64, 105] for 83 residues. The nearest match is to the V_H domain of a human IgM immunoglobulin [53] (r.m.s. difference of 0.9 Å). The framework structure of the ES204 Vδ domain is, therefore, closest to that of V_H, even though Vδ regions are most similar to Vα in terms of amino acid sequence [3] and are encoded in the same genetic locus as Vα [145].

The largest departure from the Vα framework occurs in the position of the c″ β strand (see [19] for strand nomenclature). In all known Vα structures [64], the c″strand is associated with the d strand of the adjacent β sheet through a number of backbone-backbone hydrogen bonds, resulting in a β strand topology different from that of antibody V domains. In Vδ, the c″ strand is hydrogen-bonded to the c' strand in the same β sheet, as in V_L and V_H domains and in Vβ. This structural difference between Vδ and Vα is due, at least in part, to an insertion of three residues at positions 57A-59 (numbering according to [92]) compared with 2C Vα and of four residues compared with 1934.4 Vα and A6 Vα. The insertion, which makes the sequence of human Vδ3 in this region equal in length to that of V_L or V_H rather than Vα, results in a displacement of the c″ β strand away from the d strand and toward the c' strand. A consequence of the c″ strand switch in Vδ relative to Vα is that the main chain of Vδ CDR2 is almost perpendicular to that of Vα CDR2 and nearly coincident with that of V_H CDR2 (Fig. 2). In contrast, the relative positions of CDR1 and CDR3 are nearly identical to those in Vα. Thus, the overall topology of that portion of the ES204-combining site formed by the Vδ CDRs displays key features of both antibody V_H and TCR Vα domains.

Although the framework structure and CDR2 loop conformation of ES204 Vδ are most like that of V_H, its CDR1 conformation clearly resembles that of Vα (Fig. 2). In contrast to V_L and V_H domains, whose CDR1s are mainly stabilized by the hydrophobic side chain of residue 29 which intercalates between two β-pleated sheets, Vδ3 CDR1 is mainly stabilized by hydrophobic interactions between highly conserved residues Tyr24 and Leu33, as in the case of Vα. The conservation of hydrophobic residues at positions 24 and 33 in both Vα and Vδ sequences suggests that the CDR1 conformation described here is common to most α and δ chains and represents a canonical structure [33] for this CDR loop.

An analysis of the CDR3 length distribution of antibodies and TCRs has revealed that the length profiles of γδ TCRs are more similar to those of antibodies than of αβ TCRs [137]. In particular, TCR δ and immunoglobulin H chains have the longest and most variable CDR3 lengths (6–21 residues for δ chains and 1–25 residues for H

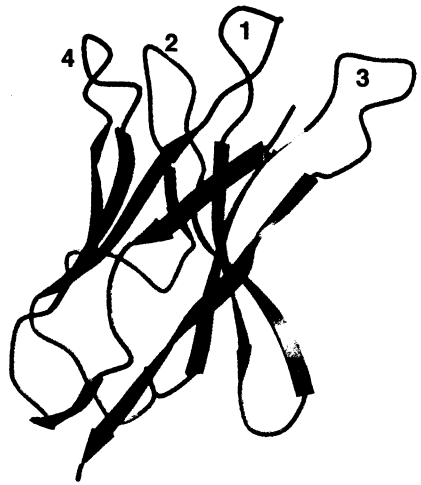

Fig. 1. Ribbons diagram of the TCR Vδ domain [105]. The CDR loops are numbered *1*, *2* and *3*; HV4 is labeled *4*

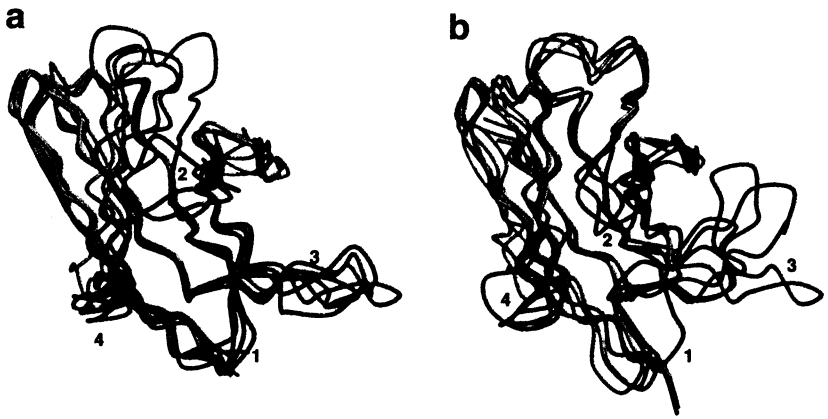

Fig. 2. The antigen-binding loops of the TCR Vδ domain. (**a**) overlap of Vδ with three Vαs [64] to show relative dispositions of CDRs. The Vδ and Vα CDRs are labeled *1*, *2* and *3*; HV4 is numbered *4*. Vδ CDR1 is colored *green*, CDR2 *red*, CDR3 *pink* and HV4 *purple*; the corresponding regions of Vα are *blue*. (**b**) Overlap of Vδ with three antibody V_H domains. The CDR and HV4 loops are labeled and colored as in (**a**)

chains), whereas the CDR3s of α, β, γ and light chains are notably shorter and less variable. However, in terms of its relative position in the TCR-combining site, Vδ CDR3 resembles Vα CDR3 more than V_H CDR3 (Fig. 2). Unlike V_H CDR3s, which are directed away from the core of the domain, Vδ CDR3 folds back toward CDR1 and CDR2, as in Vα. The ES204 CDR3 loop is ten residues long, which is about the median length for δ chains [137], and forms part of a relatively flat binding surface (Fig. 1), as observed in αβ TCRs [64]. Longer Vδ CDR3s are likely to project from

the surface of the domain, thereby creating protuberances or crevices which may facilitate the binding of γδ TCRs to a wider variety of antigenic surfaces than αβ TCRs.

Most of the residues in the putative Vγ-contacting surface of the ES204 Vδ domain are equivalent by homology to those involved in VαVβ and V_LV_H dimerization, which indicates that the geometry of VγVδ association is likely to be similar to that of VαVβ and V_LV_H. However, this surface is considerably more hydrophobic than the corresponding surface of Vα domains [64] and includes the following substitutions relative to 1934.4 Vα: Gln37 → Ile38, Glu41 → Tyr42, Pro43 → Phe44, Arg48 → Tyr49 and Glu93 → Leu92. The change of Gln37 in Vα to Ile38 in Vδ3 would result in the loss of a pair of side chain-side chain hydrogen bonds with Vγ Gln39 in the γδ heterodimer that are invariant in VαVβ (and V_LV_H) structures. In other Vδ sequences, position 38 is Gln or Lys. There is a similar lack of conservation of Vγ Gln39, which may be also Arg or Lys, but which is nearly always Gln in Vβ. This variability at positions Vγ 39 and Vδ 38 may result in the preferential pairing of certain γ and δ chains, as observed in early fetal ontogeny [103].

Antigens for γδ T cells

Nonpeptide antigens

Foreign prenyl pyrophosphate antigens

The best characterized class of antigens for γδ T cells are the prenyl pyrophosphate antigens or phosphoantigens. These antigens are small, nonpeptidic compounds with short alkyl and alkenyl chains linked to phosphate or pyrophosphate moieties. The prototypic antigen that we identified is isopentenyl pyrophosphate [163], but there exist a number of other compounds that are probable precursors of isopentenyl pyrophosphate or have longer chain lengths. Since prenyl pyrophosphates are essential metabolites required for life, they are produced by bacteria and protozoal parasites as well as by humans. There are likely to be a number of different foreign prenyl pyrophosphate compounds as well as self compounds that are present in non-Hodgkin's B cell lymphomas and, perhaps, in activated T cells (see below).

We demonstrated that recognition of prenyl pyrophosphate antigens is mediated by the antigen recognition site of the Vγ2Vδ2 TCR (reviewed in [115]). Thus, when peripheral blood mononuclear cells are exposed to prenyl pyrophosphate antigens, there are large expansions of Vγ2Vδ2$^+$ T cells. Only T cell clones expressing Vγ2Vδ2 TCR respond to prenyl pyrophosphates with secretion of cytokines, proliferation, calcium flux, and cytotoxicity [104, 114, 164]. Vγ2Vδ2$^+$ T cell clones that recognize prenyl pyrophosphates include T cells expressing invariant Vγ2Vδ2 TCRs as well as T cells expressing junctionally diverse Vγ2Vδ2 TCRs [43, 116, 164]. Monoclonal antibodies (mAbs) to Vγ2Vδ2 TCR block functional responses to prenyl pyrophosphates [164]. Importantly, when we transfected the Vγ2Vδ2 TCR into TCR$^-$ Jurkat cells, we found that transfer of the Vγ2Vδ2 TCR conferred reactivity to prenyl pyrophosphate antigens on recipient cells, confirming that recognition is mediated by the Vγ2Vδ2 TCR [28]. Reactivity requires both the Vγ2 and the Vδ2 chains. Mutation of the N-encoded CDR3 region of the Vγ2 chain can abrogate reactivity, whereas Vγ2 chains using different Jγ regions can still recognize prenyl pyrophosphate antigens, although the fine specificity of the receptor is altered [26].

Prenyl pyrophosphate antigens are directly presented to Vγ2Vδ2+ T cells [114]. Unlike proteins or lipid antigens, we found no evidence for antigen uptake or processing of these unique antigens. Instead, the prenyl pyrophosphate antigens can directly associate with both professional and non-professional antigen-presenting cells (APCs) where the antigens are then recognized by the Vγ2Vδ2 TCR on γδ T cells [114]. Recognition does involve presentation by APC since soluble antigens are unable to activate Vγ2Vδ2+ T cells [104, 114]. These findings suggest that there is an antigen-presenting molecule for the prenyl pyrophosphate antigens. However, such a presenting element does not belong to the classical MHC class I, MHC class II, or CD1 families [114]. Because so many human cell lines can present these antigens regardless of their source, the putative antigen-presenting element would be predicted to be nonpolymorphic and widely distributed.

Vγ2Vδ2+ T cells and microbial infections in humans

Recognition of these nonpeptide prenyl pyrophosphate antigens is likely to occur in a number of human infections. Infections with mycobacteria and other bacteria, and with certain protozoal parasites that produce prenyl pyrophosphate antigens are associated with large increases in Vγ2Vδ2+ T cells in the blood of infected individuals (Table 1). These expansions generally occur rapidly with elevated levels of γδ T cells

Table 1. Expansion of human γδ T cells in response to infection

Infection		γδ T cells		References
		Normals	Patients	
		Mean (max) % of T cells		
Bacterial	Tuberculosis	6	14 (35)	[5]
		2	6 (17)	[84]
	TB contacts	5	10 (18)	[165]
	TB meningitis	3	4 but Vγ2Vδ2	[48]
	Leprosy Reversal reaction	5	29[a]	[111]
	Tularemia	3	33	[161]
		7	31(48)	[123]
	Salmonellosis	5	18 (48)	[74]
	Brucellosis	4	29 (48)	[14]
	Q-fever (*Coxiella burnetii*)	4	16 (30)	[148]
	Ehrlichiosis	5	57 (97)	[30]
	Meningitis (*H. influenzae*)	7	27 (37)	[133]
	Meningitis (*N. meningitidis*)	7	25 (42)	[133]
	Meningitis (*S. pneumoniae*)	7	35 (46)	[133]
	Listeriosis	2	12 (33)	[90]
Protozoal parasites	Acute malaria	4	16 (26)	[81]
	(non-endemic)	5	16 (27)	[140]
		3	18 (46)	[149]
	Malarial paroxysm	4	11 (27)	[121]
	Toxoplasmosis	4	9 (15)	[146]
	Visceral leishmaniasis	8	44	[134]
	Leishmaniasis	3	13 (18)	[141]
	Localized leishmaniasis	4	20[a]	[111]

[a] % γδ T cells among CD3+ T cells in skin lesions

Table 2. Bacterial, parasitic, and plant lysates that stimulate Vγ2Vδ2+ T cells in vitro[a]

Bacteria	Species	Reference
Mycobacteria	***M. tuberculosis***	[35, 44, 82, 94, 111, 119]
	M. phlei	Unpublished data
	M. leprae	Unpublished data
	M. smegmatis	[36, 72, 164]
	M. fortuitum	[36, 122, 164]
	M. avium	[36, 57, 72], unpublished data
	M. bovis BCG	[36, 72], unpublished data
	M. vaccae	[72]
	M. marinum	[36]
	M. chelonae	[36]
	M. scrofulaceum	[72]
Gram-negative rods	***Escherichia coli***	[44, 97, 167], unpublished data
	Pseudomonas aeruginosa	[97, 167]
	Xanthomonas maltophilia	[167], unpublished data
	Salmonella typhimurium	Unpublished data
	Yersinia pseudotuberculosis	Unpublished data
	Yersinia enterocolitica	[80, 169], unpublished data
	Francisella tularensis	[123]
	Campylobacter jejuni	[13]
Gram-positive cocci	***Staphylococcus aureus***	[118], unpublished data
	***Streptococcus pyogenes* Group A**	[12, 118], unpublished data
	***Streptococcus agalactiae* Group B**	[12]
	***Streptococcus* Group C**	[12]
	Enterococcus faecalis Group D	[12], unpublished data
	Streptococcus Group F	[12]
Gram-positive rods	***Listeria monocytogenes***	[44, 71, 90, 117]
	Corynebacterium diphtheriae	Unpublished data
Protozoa		
Malaria	***Plasmodium falciparum***	[9, 61, 65, 66, 155]
Toxoplasma	***Toxoplasma gondii***	[160]
Leishmania	***Leishmania braziliensis***	[141]
	Leishmania amazonensis	
Plant		
Mistletoe	**Mistletoe from apple or pine trees**	[59]

[a] Bold type = biologically active in vitro, non-bold type = no activity upon testing in vitro

(26–39% of blood T cells) noted in tularemia infections by 8–10 days [123]. In two patients with neonatal listeriosis, 70-fold and 110-fold increases in the absolute numbers of γδ T cells were noted by day 5 and day 15 to 15% and 27% of blood T cells, respectively [90]. In ehrlichiosis, up to 97% of blood T cells were Vγ2Vδ2+ T cells by days 5–12 after infection [30]. These expansions can last up to 4 months after infection [14]. Thus, profound increases can occur in γδ T cells that express one V gene pair, Vγ2Vδ2, and these increases can persist for some time after infection.

Bacteria and parasites that stimulate in vivo expansions contain soluble antigens since their lysates can stimulate Vγ2Vδ2 expansion in vitro (Table 2). All Gram-

Table 3. Production of nonpeptide antigens for γδ T cells by different pathogens

	Secrete biological activity	Pyrophospho monoesters	Nucleotide conjugates	References
Mycobacterium tuberculosis	–	IPP?, TUBAg 1–2	TUBAg 3–4	[35, 36, 163]
Mycobacterium bovis	–	IPP?, TUBAg 1–2	TUBAg 3–4	[35, 36]
Mycobacterium bovis BCG	–	IPP?, TUBAg 1–2	TUBAg 3–4	[35, 36]
Mycobacterium avium	+/-			Unpublished data
Mycobacterium smegmatis	++	IPP, TUBAg 1–2	+	[163]
Mycobacterium fortuitum	++	IPP, TUBAg 1–2	TUBAg 3	[122, 163]
Mycobacterium phlei	++			Unpublished data
Escherichia coli	+	++	–	Unpublished data
Yersinia enterocolitica	+	TUBAg1	-?	Unpublished data
Xanthomonas maltophilia	+			Unpublished data
Francisella tularensis	?			[123]
Staphylococcus aureus	–			Unpublished data
Plasmodium falciparum	??	MALAg 1–2	–	[9]

negative bacteria and mycobacteria tested were able to stimulate Vγ2Vδ2+ T cells, including both pathogenic and non-pathogenic species. Many but not all Gram-positive cocci were also stimulatory ([12], and Morita, personal observation). Similarly, lysates of protozoal parasites also stimulated γδ T cells. Plant extracts also stimulate γδ T cells [60], whereas fungi and yeast do not stimulate Vγ2Vδ2+ T cells. We have also shown that an early expansion of Vγ2Vδ2+ T cells occurs in most children due to an environmental factor [120]. Given our present knowledge, this is likely due to early bacterial exposures.

Mycobacteria produce at least five different phosphoantigens as determined by ourselves and others that all appear related to the isoprenoid synthetic pathway (Tables 3 and 4). These antigens can be divided into two chemical classes, pyrophosphomonoesters and nucleotide-conjugated monoesters. Bacterial species differ in the array of antigens present and in the secretion of the antigens (Table 3). For instance, most Gram-negative bacteria do not appear to have nucleotide-conjugated monoesters. Four of the antigens have been structurally identified and include isopentenyl pyrophosphate [163] and a related structure, 3-formyl-1-butyl pyrophosphate [10]. This antigen, originally termed TUBAg 1, is also present as nucleotide conjugates of uridine triphosphate (TUBAg 3) [122] and deoxythymidine triphosphate (TUBAg 4) [35]. Gram-negative bacteria appear to have only the TUBAg 1 pyrophosphomonoesters (unpublished observation). Two other antigens produced by malarial parasites exist that appear distinct from the bacterial antigens based on their retention times [9].

Non-mevalonate, deoxyxylulose pathway for isopentenyl pyrophosphate synthesis as a probable source of antigens

Previously described prenyl pyrophosphates do not account for all of the different antigens observed. One likely source for undiscovered prenyl pyrophosphates or prenyl precursors that we [115] and others [10, 88] have proposed, is the alternative, de-

Table 4. Characterized nonpeptide antigens for Vγ2Vδ2+ T cells

	Specific biological activity (μM)[a]	Alkaline phosphatase sensitive	Nucleotide pyrophosphorylase sensitive
Organic phosphoester			
Monoethyl pyrophosphate (ME-P-P)	1–3	+	–
Isopentenyl pyrophosphate (I-P-P)	1–3	+	–
DMAPP, FPP, GPP, GGPP[b]	30–300	+	–
TUBAg 1 (3-formyl-1-butyl-PP)	0.001–0.005?	+	–
TUBAg 2 (X-P-P)	0.001?	+	–
MALAg 1–2	?	+	–
2,3-Diphosphoglycerate	300–600	+	–
Xylose-1-Phosphate	1000	+	–
Ribose-1-Phosphate	2000–3000	+	–
Glycerol-3-Phosphate	1000	+	–
Francisella tularensis lysates	?	+	
Mistletoe	?	+	
Nucleotide conjugated			
Ethyl-ATP (E-P-P-P-A)	1–3	–	+
Isopentenyl-ATP (I-P-P-P-A)	1–3	–	+
TUBAg 3 (3-formyl-1-butyl-P-P-P-U)	0.001?	–	+
TUBAg 4 (3-formyl-1-butyl-P-P-P-dT)	0.001–0.005?	–	+ (liberates TUBAg1)

[a] Concentration of compound required for half maximal proliferation of Vγ2Vδ2+ T cell clones
[b] DMAPP dimethylallyl pyrophosphate, FPP farnesyl pyrophosphate, GPP geranyl pyrophosphate, GGPP geranylgeranyl pyrophosphate

oxyxylulose pathway for isopentenyl pyrophosphate synthesis. Although not fully elucidated, broad aspects of this biosynthetic pathway have been characterized (Fig. 3) [138, 139]. As we previously noted [115], many eubacteria lack the critical HMG-CoA reductase enzyme used in the mevalonate pathway of most eukaryotic organisms (Table 5). Instead, some, though not all, eubacteria use a non-mevalonate pathway for isopentenyl pyrophosphate synthesis that involves deoxyxylulose-5-phosphate (Fig. 3). This can be demonstrated by the labeling pattern of isoprenoid compounds, the presence of enzymes of the deoxyxylulose pathway, or the absence of enzymes of the mevalonate pathway in bacteria whose whole genome has been sequenced (Table 5).

The 3-formyl-1-butyl pyrophosphate antigen [10] may be a downstream intermediate directly in this pathway or in a branch off this pathway. The basic structure of the carbon chain is similar to isopentenyl except that an aldehyde group is attached to a terminal carbon residue, resulting in a single bond instead of a double bond between C3–C4 (Fig. 3). This probable precursor of isopentenyl pyrophosphate is reported to have about a 1,000-fold higher specific bioactivity than self isopentenyl pyrophosphate [10]. The nucleotide-conjugated antigens could either be derived from 3-formyl-1-butyl pyrophosphate as a side product or function like the upstream precursor, 4-diphosphocytidyl-2-C-methylerythritol (Fig. 3), as an intermediate in the synthesis pathway. Nucleotide-conjugated antigens may be side products since Gram-negative rods do not appear to produce such antigens (unpublished observation).

The recognition of intermediates in the deoxyxylulose synthetic pathway provides insights into the recognition of prenyl pyrophosphate antigens in vivo. First, expan-

Table 5. Alternative deoxyxylulose pathway for isopentenyl pyrophosphate synthesis is present in some eubacteria, apicomplexan parasitic protozoa, and plant chloroplasts[a]

	Deoxyxylulose pathway[b]					Mevalonate Pathway
	dxs	dxr	ygbP	ychB	ygbB	HMG-CoA reductase
Prokaryotes						
Eubacteria						
Mycobacterium tuberculosis	+	+	+	+	+	–
Mycobacterium leprae	+	+	+	+	+	
Yersinia pestis	+	+	+	+	+	
Escherichia coli	+	+	+	+	+	–
Haemophilus influenzae	+	+	+	+	+	–
Bacillus subtilis	+	+	+	+	+	–
Helicobacter pylori	+	+	+	+	+	–
Treponema pallidum	+	+	+	+	+	–
Chlamydia pneumoniae	+	+	+	+	+	–
Chlamydia trachomatis	+	+	+	+	+	–
Synechocystis sp PCC6803	+	+	+	+	+	–
Aquifex aeolicus	+	+	+	+	+	–
Thermotoga maritima	+	+	+	+	+	–
Pseudomonas aeruginosa	+	+	+	+	+	+
Enterococcus faecalis	+		+	+	+	+
Streptococcus pyogenes	+		+	+		+
Streptococcus pneumoniae	+		+	+	+	+
Staphylococcus aureus	+		+	+		+
Borrelia burgdorferi	–	–	–	–	–	+
Mycoplasma pneumoniae	+	–	+	–	–	–
Mycoplasma genitalium	+	–	–	–	–	–
Rickettsia powazekii	+	–	–	–	–	–
Archebacteria						
Pyrococcus horikowskii	–	–	+	+	+	+
Archeoglobus fulgidus	–	–	–	–	–	+
Methanobacterium	–	–	–	–	–	+
Methanococcus jannaschii	–	–	–	+	+	+
Aeropyrum pernix	–	–	–	–	–	+
Eukaryotes						
Apicomplexan Parasites						
Plasmodium falciparum	+	+	+	+		-
Plants						
Arabidopisis thaliana	+	+	+	+	+	+
Mentha x piperita	+	+		+		+
Fungi/molds						
Saccharomyces cerevisiae	–	–	–	–	–	+
Nematoda						
Caenorhabditis elegans	–	–	–	–	–	+
Mammals	–	–	–	–	–	+

[a] Genomes of different bacteria were searched using the BLAST program for the presence of HMG-CoA reductase from *Pseudomonas mevaloni* or deoxyxylulose pathway enzymes from *E. coli* or other bacterial species. BLAST scores greater than 80 and matching the complete protein (where the complete genome is known) or large fragments of the protein (for genomes that are only partially sequenced) were considered positive. For Gram-positive cocci, enzymes from *Enterococcus faecalis* was used for searches

[b] *dxs*, deoxyxylulose-5-phosphate synthase, *dxr*, deoxyxylulose-5-phosphate reducto-isomerase, *ygbP*, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase, *ychB*, 4-diphosphocytidyl-2C-methyl-D-erythritiol kinase, *ygbB*, 4-diphosphocytidyl-2C-methyl-D-erythritol 2-phospho cyclase

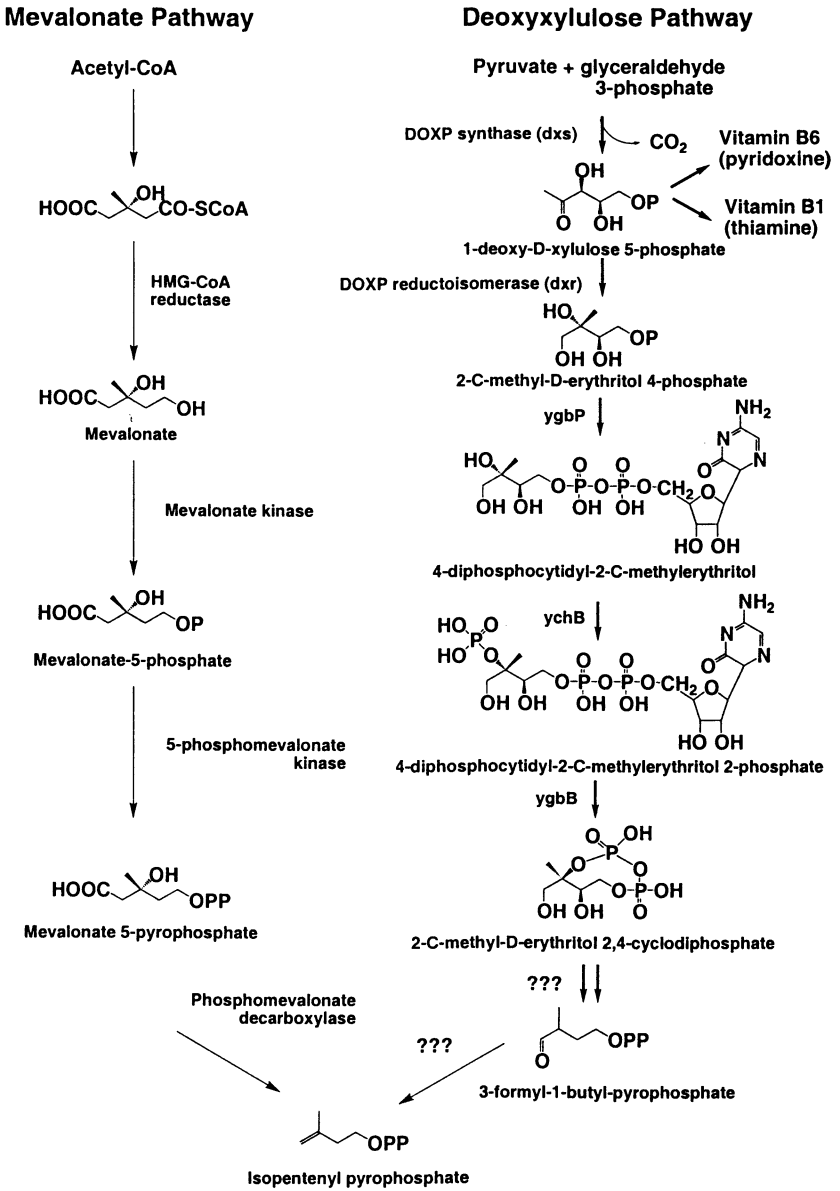

Fig. 3. Schematic diagram of the synthetic pathways for isopentenyl pyrophosphate. Most eukaryotic animals and archebacteria use the classical mevalonate pathway. Eubacteria, apicomplexan protozoa, and plant chloroplasts use an alternative non–mevalonate pathway in addition to or instead of the classical mevalonate pathway

sion of Vγ2Vδ2+ T cells appears to be more common with bacteria that exclusively use the deoxyxylulose pathway (Table 1). Thus, expansions are commonly seen with infections with mycobacteria, Gram-negative rods, and other bacteria using the deoxyxylulose pathway, but not with mycoplasma or *Borrelia burgdorferi* infections that lack this pathway. Moreover, bacterial lysates from eubacteria thought to use the deoxyxylulose pathway were stimulatory for Vγ2Vδ2+ T cells [88]. The major exception to this hypothesis, is the biological activity found in some streptococcal and staphylococcal bacteria ([12], and unpublished observation) and the expansion of Vγ2Vδ2+ T cells in *Streptococcus pneumoniae* meningitis (Table 1). Streptococcal and staphylococcal species express both the HMG-CoA enzyme of the mevalonate pathway (Table 5) and enzymes of the deoxyxylulose pathway (Table 5). In some cases, such as Enterococci, the bacteria lack biological activity, whereas other Gram-positive cocci clearly have biological activity. We would predict that differences in which pathway is predominately used may determine the degree of stimulating activity each bacterial species has for γδ T cells.

Second, the recognition of compounds from the deoxyxylulose pathway also explains why some eukaryotic parasitic protozoa can stimulate Vγ2Vδ2+ T cells. Two of the parasitic diseases associated with Vγ2Vδ2+ T cell expansions in vivo, malaria and toxoplasmosis, are caused by organisms that use the deoxyxylulose pathway. We would predict that human *Eimeria* infections would also be associated with expansions of Vγ2Vδ2+ T cells. Lysates from these parasites share with plant mistletoe extracts the ability to induce Vγ2Vδ2+ T cell expansions in vitro. Linking these two different types of organisms is the presence of plastid bodies in their cytoplasm [100]. Members of the phylum Apicomplexa, *Plasmodium*, *Toxoplasma*, and *Eimeria*, all have unique apicoplast organelles containing circular DNA related to green algal plastid (chloroplast) DNA [100]. The deoxyxylulose synthase enzyme required for the deoxyxylulose pathway is targeted to these plastid bodies [89]. The malaria organism, *Plasmodium falciparum*, contains all four of the known enzymes in this pathway (Table 5) and can be killed by antibiotics that block the deoxyxylulose pathway [89]. Thus, the presence of plastids and chloroplasts that were originally derived from green algae, correlates with bioactivity. However, there may be an exception since *Leishmania* parasites that may use the mevalonate pathway [131], also stimulate γδ T cell expansions in vivo (Table 1) and in vitro (Table 2). Although skin lesions in *Leishmania* infections have high levels of Vδ2+ T cells [1], it is not clear that the expanded cells in the blood express Vγ2Vδ2 TCRs or that the antigens are phosphoantigens [141].

Third, the preferential recognition of foreign prenyl pyrophosphate precursors would provide specificity to nonpeptide antigen recognition. If γδ T cells could not distinguish between self antigens and foreign antigens, there would be the chance of developing autoimmune responses. With the much higher specific activity of bacterial compared with self prenyl antigens, recognition would be specific for foreign bacteria as long as there are only low amounts of self antigens and especially if these self antigens were sequestered in the cytoplasm of cells.

Self prenyl pyrophosphate antigens presented by non-Hodgkin's B cell lymphomas and activated T cells

Besides recognizing foreign prenyl pyrophosphate or prenyl pyrophosphate precursor antigens, Vγ2Vδ2+ T cells also recognize certain non-Hodgkin's B cell lymphomas. The human B cell lines, Daudi [57] and RPMI 8226 [152], can stimulate the proliferation of Vγ2Vδ2+ T cells from blood and can stimulate IL-2 release from a Vγ2Vδ2 TCR transfectant. Although IL-2 production by the Vγ2Vδ2 TCR transfectant establishes that this recognition is mediated by the γδ TCR [28], it is possible that the antigen recognized is not a phosphoantigen but is, instead, a protein, peptide or another class of nonpeptide antigen. Yet, the strict concordance between reactivity to exogenous *M. tuberculosis* antigens and reactivity to Daudi by a large panel of Vγ2Vδ2+ T cell clones suggests that the antigens are likely to be prenyl pyrophosphate antigens [43]. Initial studies that suggested that the antigen on Daudi was human hsp65 could not be verified by others ([152], and Bukowski and Morita, unpublished observation).

The source of possible prenyl pyrophosphate antigen in the lymphoma cells is unclear. One ready source of antigens would be overproduction of prenyl pyrophosphates by the mevalonate pathway or by an alteration in intracellular trafficking of a putative phosphoantigen-presenting molecule, which results in the presentation of endogenous prenyl pyrophosphates such as IPP or longer chain prenyl pyrophosphate at the cell surface. Unlike eubacteria and apicomplexan protozoa, there is no evidence for the use of the non-mevalonate pathway of isopentenyl pyrophosphate synthesis in humans as a possible source of antigens.

Reactivity to self antigens may extend beyond the two B cell lymphomas. Reactivity to other lymphomas [58] and to activated T cells [73] may be masked by inhibitory NK receptors expressed by γδ T cells. However, it is possible that this reactivity reflects activation through an activating NK receptor rather than through the Vγ2Vδ2 TCR as noted with Vδ1+ T cell recognition of MICA/MICB [7] (see below).

Alkylamine antigens

We have recently discovered a second class of nonpeptide antigens for Vγ2Vδ2+ T cells, the alkylamine antigens [27]. These antigens are composed of short alkyl chains linked to an amine moiety. Straight or branched alkyl chains of from one to five carbons coupled to a single amine moiety stimulate the expansion of Vγ2Vδ2+ T cells from peripheral blood, the proliferation of γδ T cell clones expressing the Vγ2Vδ2 TCR and the release of IL-2 from transfectants expressing the Vγ2Vδ2 TCR [27]. Although similar in structure to synthetic alkyl phosphate and pyrophosphate analogs of prenyl pyrophosphate antigens, differences in the alkyl chain requirement were found. Thus, methylamine was not stimulatory, whereas methylpyrophosphate and methylphosphate were stimulatory. Also, *iso*-amylamine was stimulatory, whereas *iso*-amylpyrophosphate was not. Thus, the structural constraints on the alkyl chains of alkylamines are similar but not identical to the constraints on the alkyl chains of phosphate and pyrophosphate compounds.

Similar to prenyl pyrophosphate antigens, recognition of alkylamines appears to require cell-cell contact, although the presenting cell need not be a professional APC. There is no evidence for alkylamine internalization or processing. Recognition is clearly through the Vγ2Vδ2 TCR as evidenced by the ability of the Vγ2Vδ2 transfectant to respond to these compounds. Known antigen-presenting molecules such as MHC class I or class II or CD1 are not required for alkylamine recognition, suggesting that if a presentation element is required, it is a novel molecule.

Natural sources of alkylamines include bacteria and certain foods including tea. Some bacteria secrete alkylamine compounds. For example, *Proteus morganii* pro-

duces large amounts of *iso*-butylamine and *iso*-amylamine. Supernatants from this bacteria contain alkylamines that stimulate the expansion of Vγ2Vδ2+ T cells [27]. Other bacteria such as *Bacteroides fragilis, Clostridium perfringens, Salmonella typhimurium*, and *Listeria monocytogenes* are reported to produce alkylamines that have the potential to stimulate γδ T cells [27].

A second source of alkylamines are plants. One potential source of antigens in humans is tea. Tea contains the amino acid, L-theanine, that can be broken down to give ethylamine and glutamate. Indeed, acid hydrolyzed tea extracts were stimulatory for Vγ2Vδ2+ T cells due to the presence of ethylamine [27]. Alkylamines are also found in apples and wines as well as human breast milk and amniotic fluid. Clearly, alkylamines represent a new class of nonpeptide antigens, that may be of foreign and self origin, that activate the major pool of circulating γδ T cells expressing Vγ2Vδ2 TCR.

CD1c-restricted self lipid antigens

Whereas the nonpeptide prenyl pyrophosphate and alkylamine antigens are major antigens stimulating human Vγ2Vδ2+ T cells, no major antigen had been described for the major tissue subset of γδ T cells that express Vγ1 or Vγ2 gene segments paired with the Vδ1 gene segment. Vδ1+ T cells are abundant in tissues such as the gut (~40% of γδ T cells), spleen (~71% of γδ T cells), and thymus (~70% of γδ T cells) and are also present on 10–20% of peripheral blood γδ T cells [52, 86, 154]. We have recently reported evidence for CD1c recognition by this subset of γδ T cells [156]. Coupled with previous reports [54, 125], there are now 6 independently derived CD1c-reactive Vδ1+ T cell clones, suggesting that CD1c may be an important antigen-presenting molecule for Vδ1+ T cells.

CD1 proteins are non-MHC-encoded proteins with similar structure to MHC class I proteins and are composed of a heavy chain associated with β_2-microglobulin (reviewed in [130]). CD1 proteins represent a third lineage of antigen-presenting molecules specialized for the presentation of lipids and glycolipids. Unlike MHC class I or class II proteins, CD1 proteins have little sequence polymorphism [128]. The structure of CD1 has been shown to be similar to MHC class I with the major difference being the presence of two large hydrophobic pockets in the CD1 antigen binding groove in place of the smaller pockets that bind peptide side chains in MHC class I molecules [170]. The acyl chains of lipids and glycolipids bind into the hydrophobic pockets [50] and the protruding carbohydrate or carboxylic acid moieties [24, 112, 129] and the CD1 α helices are recognized by CD1-restricted TCR [67]. By binding foreign lipids, CD1c and other CD1 molecules can present foreign lipids and glycolipids to T cells [8, 126].

Recognition of CD1c by γδ T cells occurs in the absence of exogenous foreign antigens, suggesting that recognition may be dependent upon self lipids loaded into the CD1c molecule. The recent finding that mycobacterial lipids presented by CD1c to αβ T cells were mycobacterial hexosyl-1-phosphoisoprenoids suggests that structurally related mannosyl-1-phosphodolichols found in mammalian cells are candidate self antigens [113].

γδ T cell recognition of CD1c resembles αβ NKT cell recognition of CD1d [12, 91]. However, differences were noted between the two types of CD1-reactive cells. The CD1c-reactive γδ T cell clones were $CD4^-8^-$ and did not express the NKR-P1A

C-type lectin NK receptor [156] that are expressed on CD1d-reactive Vα14+ αβ T cells express [51]. Importantly, CD1c-reactive γδ T cells produced IFN-γ, but not IL-4, unlike NKT cells that produce both cytokines. Such CD1d-reactive αβ T cells are required to mediate IL-12-induced tumor immunity in mice [40], can polarize other T cells to a Th2 phenotype [29, 153], and play regulatory roles in listerial infection [162] and Th2 responses [41]. We speculate that CD1c-reactive γδ T cells also have unique immunological functions related to the Th1 phenotype and may interact with B cells or dendritic cells, as these are the main cells that express CD1c.

Recognition of CD1c by γδ T cells was mediated by the γδ TCR since transfection of the reactive γδ TCR into the TCR^- Jurkat tumor conferred reactivity to CD1c [156]. However, unlike αβ NKT cells and Vγ2Vδ2+ T cells, CD1c-reactive clones expressed several Vγ gene segments [54, 125, 156] and there was no evidence for expression of an invariant or canonical receptor when the TCRs of two CD1c-reactive γδ T cell clones were studied [156]. More CD1c-reactive γδ T cells will have to be examined to determine if there is a subpopulation of CD1c T cells expressing an invariant receptor.

Besides producing Th1 cytokines, we found that CD1c-reactive γδ T cells were cytolytic. Cytolysis was primarily through the perforin pathway with some clones also killing through the Fas/Fas ligand pathway [156]. CD1c-reactive γδ T cells expressed the antimicrobial granulysin protein. In conjunction with perforin, this protein can kill mycobacteria released from infected cells [158]. Thus, CD1c-reactive γδ T cells have the potential to kill infected CD1c-bearing dendritic cells and to secrete Th1 cytokines.

Given the presence of CD1c-reactive T cells in normal individuals, up-regulation of CD1c could represent an innate danger signal through the activation of these CD1c-reactive γδ T cells. In this model (Fig. 4), infection with bacteria causes the up-regulation of CD1c expression through direct signalling or by the release of compounds that either activate endothelial or myeloid cells or stimulate bacteria-specific T cells to produce granulocyte-macrophage colony-stimulating factor. The increased expression of self CD1c could then activate Vδ1+ T cells to secrete Th1 cytokines and to lyse infected cells and kill the released intracellular bacteria. Thus, activation of γδ T cells could play a role in directing conventional αβ T cells to a Th1 phenotype by the early secretion of Th1 cytokines or in controlling intracellular bacterial infections by lysing infected cells and killing the released bacteria. CD1c-reactive γδ T cells could also play regulatory roles by eliminating CD1+ dendritic APCs.

Non-classical MICA/B and TL MHC Class Ib proteins

Other potential antigen-presenting molecules for human Vδ1+ T cells are the MICA and MICB molecules. Like CD1 molecules, MICA/B have little sequence polymorphism [4]. MICA/B are encoded in the MHC locus but are expressed under different control than most MHC-encoded presenting molecules. Importantly, although MICA has structural similarities to MHC class I, MICA probably does not present peptides because it has a limited peptide binding groove and its α1 and α2 domains are tilted relative to the α3 domain exposing the underside of the α1/α2 platform [106]. MICA/B are expressed in the gut epithelium [68] and on tumor cells [69]. Upon heat shock, expression of *MICA/B* is up-regulated as predicted by the presence of promoter heat shock elements [68].

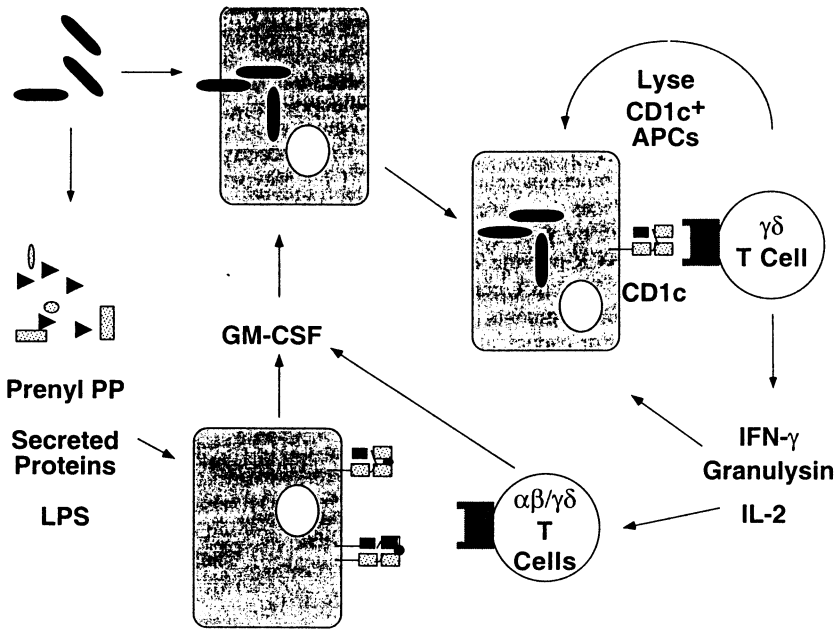

Fig. 4. Proposed model for CD1c recognition by Vδ1+ T cells. Infection with bacteria leads to the production of secreted bacterial proteins, lipopolysaccharides, and prenyl pyrophosphates. These compounds can activate myeloid cells and endothelial cells to produce GM-CSF. Additional GM-CSF is produced by αβ and Vγ2Vδ2+ γδ T cells responding to secreted proteins and prenyl pyrophosphates. The production of GM-CSF can induce monocytes to differentiate into CD1+ dendritic cells. CD1c-reactive Vδ1+ T cells would then recognize CD1c expressed on dendritic cells. Activated Vδ1+ cells release Th1 cytokines influencing the development of αβ T cells. Activated Vδ1+ cells also can kill CD1c+ APCs. If these cells harbor bacteria, the released bacteria can be killed by granulysin, (*PP* pyrophosphate, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *APCs* antigen-presenting cells, *LPS* lipopolysaccharide)

Since MICA is expressed in the intestinal epithelium, where gut γδ T cells are present in elevated numbers, gut Vδ1+ T cell clones expressing diverse TCR were studied and found to recognize MICA [70]. Recognition could be inhibited with the addition of anti-γδ TCR mAbs [70]. However, this recognition was found to be mediated by the NKG2D receptor [7]. The NKG2D receptor is an activating C-type lectin NK receptor that is expressed on γδ T cells, CD8 αβ T cells, and CD56+ NK cells [7]. Importantly, engagement of the NKG2D receptor allowed the specific lysis of MICA+ targets and mAbs to NKG2D blocked killing by γδ T cells. Thus, it remains to be definitively determined if Vδ1+ T cells recognize MICA through the γδ TCR. Instead of functioning as an antigen-presenting molecule, MICA may function as a stress-induced danger signal that is recognized by NKG2D-bearing NK and T cells.

Recognition of murine nonclassical class Ib TL molecules has also been reported [16, 18]. In these cases, the TL MHC class Ib molecules, T10 and T22, are clearly recognized by the γδ TCR as demonstrated in transgenic TCR models [18, 47]. γδ T cell recognition of TL does not require peptide processing machinery [147] probably because TL molecules cannot bind peptides [39, 95, 166]. T10 TL proteins are

induced on T cells, B cells, and other splenic cells following exposure to LPS or ConA [38]. Moreover, a population of γδ T cells that recognize TL exist in mice, constituting about 0.3–0.4% of splenic γδ T cells as evidenced by their staining with a T22 tetrameric reagent [38]. Thus, recognition of TL proteins may allow γδ T cells to respond to infections that up-regulate TL on APCs or, alternatively, to regulate activated αβ T cells and B cells that have up-regulated their expression of TL.

MHC peptide complexes/protein antigens

There is little evidence that significant numbers of γδ T cells recognize foreign peptides presented by MHC class I or class II molecules. Only a few isolated examples of MHC-restricted peptide recognition have been reported in humans [83, 102]. Also, there are few alterations in γδ T cell development in mice that have disrupted expression of MHC class I (through disruption of β_2M) [37] or MHC class II [15].

A few γδ T cells expressing Vδ1 TCRs appear to recognize allo-MHC molecules in the absence of foreign antigen. Although γδ T cells proliferated to allopresentor cells in limiting dilution analysis, few clones showed specificity for allo-MHC where, under similar conditions, CD8 αβ T cells were easily shown to be allospecific [93]. Isolated examples of γδ T cell reactivity has been described for several MHC class I alleles including HLA-A2 [157], HLA-A24 [34], HLA-B27 [45], and unspecified class I molecules [101, 135]. In the case of HLA-B27 recognition, reactivity was found to be altered by changes in an amino acid located at the base of the F pocket in the antigen-binding groove. This type of change may alter the type of peptides loaded [45]. Recognition of HLA-A2 [157] also was affected by residues altering peptide binding. α2 helix mutations that face into the binding groove (residues 152 and 156) and therefore not contacted by αβ TCR CDR [49, 63] were found to alter γδ T cell recognition. In none of these examples has TCR involvement been demonstrated by gene transfer. Therefore, it is possible that activating NK receptors could account for some of these examples of allo-MHC class I reactivity.

γδ T cell reactivity to allo-MHC class II has also been reported for murine I-E^k [108, 147], human HLA-DQ [22], and human HLA-DR2/DR8 [62]. When murine γδ T cell recognition of murine I-E^k was carefully studied [147], major differences were noted compared with αβ T cell recognition. Mutations on the α helices of I-E^k that lie on the diagonal of αβ TCR binding, did not affect γδ TCR recognition [64]. Moreover, there was no evidence that peptide binding was required. Indeed, γδ T cells could recognize empty MHC class II molecules attached to plastic [147]. Taken together, recognition of murine I-E^k by γδ T cells most resembled immunoglobulin recognition of protein since there was no evidence for a requirement for antigen processing or peptide binding.

Other examples of direct protein recognition have been reported. Murine γδ T cell reactivity to the gI glycoprotein of the herpes simplex virus involves direct recognition of the gI protein without processing or presentation [87, 150], and is important in the protection from herpes simplex infection of the eyes [151]. Thus, there are several examples of γδ T cell recognition of intact proteins in mice.

There are few characterized examples of protein recognition by human γδ T cells. Although γδ T cell recognition of a human λ immunoglobulin chain has been reported [168], the λ chain could be recognized even if expressed as an intracellular form, suggesting that a peptide was recognized. There was no evidence for MHC class I or

class II presentation of this peptide [99]. Staphylococcal enterotoxin A (SEA) reactivity by Vγ2+ T cells has also been reported [107, 143]. This appears to be classical superantigen recognition but has been difficult to establish definitively [142], and the SEA binding site for Vγ2 chains has not been determined. The 65-kDa heat shock protein (hsp65) recognition has also been reported for a non-Vγ2Vδ2 T cell clone [76]. This clone also recognized the human homolog of hsp65 [75]. Vγ2Vδ2+ T cells have also been reported to recognize hsp65 [57, 96], although this reactivity was probably due to phosphoantigens.

In mice, hsp65 proteins and peptides enhance the spontaneous production of cytokines by the Vγ1 subset of murine γδ T cells [21]. However, direct TCR transfection of a Vγ1 TCR into the TCR- Jurkat tumor line did not confer hsp65 recognition [98]. Moreover, although enhanced cytokine production requires the expression of the T cell antigen receptor complex, the variable recognition domains of the γδ TCR were not required, suggesting that the TCR dependence of this response reflects the requirement for surface expression of the CD3 signaling complex rather than cognate recognition of the hsp65 antigen [159]. Further supporting this hypothesis, spontaneous cytokine production clearly requires vitronectin binding to the $\alpha_V\beta 3$ integrin that is expressed on this T cell subset [136]. Thus, the available evidence would suggest that T cell cytokine release is triggered by $\alpha_V\beta 3$ integrin binding to vitronectin [23]. hsp65 peptides may enhance this cytokine production, but this enhancement is not due to recognition of hsp65 by the γδ TCR.

Conclusions

Pattern recognition by the adaptive immune system: γδ T cells as the intrinsic arm of the adaptive immune system

The best characterized examples of antigen recognition by γδ T cells now involve recognition of nonpeptide antigens or recognition of CD1c or the class Ib molecule, TL. From these examples, a new paradigm for γδ T cell antigen recognition is beginning to emerge. This paradigm is the use of the adaptive γδ TCR in an innate fashion to recognize nonpeptide antigens or changes in the expression of self molecules.

The recognition of nonpeptide prenyl pyrophosphates and precursor compounds or alkylamines may be an example supporting this new paradigm. There is now strong evidence that the major prenyl pyrophosphate antigen is an isopentenyl pyrophosphate precursor from the deoxyxylulose pathway (see above). This pathway is found only in eubacteria, apicomplexan parasites, and the chloroplasts of plants. Thus, the presence of this antigen in the blood or tissue is a strong indication of an infection. The recognition of this metabolic intermediate of pathogens by the γδ TCR allows all Vγ2Vδ2+ T cells to respond to infections. As Vγ2Vδ2+ T cells constitute 2–5% of blood T cells, a remarkably high precursor frequency of T cells, 1 T cell in 20 to 1 in 50, can respond to the prenyl pyrophosphates or alkylamines produced by bacteria or parasites. This type of recognition shares great similarities with innate immune recognition [55, 85]. First, the expression of antigen-specific γδ TCR is commonly fixed in the genome. In the case of prenyl pyrophosphate recognition, recognition is largely independent of junctional diversity and is primarily dependent on germ-line-encoded V and J gene segments. Thus, diverse Vγ2Vδ2 TCR from adults

can recognize prenyl pyrophosphate and alkylamine antigens. Moreover, during fetal development, Vγ2Vδ2 T cells often express an invariant Vγ2 chain that also recognizes prenyl pyrophosphate antigens, allowing newborn infants to respond [46, 109, 119]. Other γδ T cells, especially prevalent in mice, recognize common antigens through canonical or invariant γδ TCRs, whose development is germ line determined by the enzymatic action of the recombination machinery and by the lack of TdT during early T cell development. Second, the recognition is to a conserved molecular pattern found in pathogens. Third, there is no requirement for antigen processing or for professional APCs so activation can be immediate.

Other examples of such innate or intrinsic T cell recognition are human γδ T cell recognition of self CD1c molecules, murine dendritic γδ T cell recognition of stressed keratinocytes, invariant Vα14+ αβ T cell recognition of self CD1d molecules, and, potentially, murine γδ T cell recognition of TL. For CD1c recognition, we have proposed that a subset of Vδ1-expressing γδ T cells recognize CD1c expressed on dendritic cells and B cells in the absence of foreign antigens. Such recognition of a nonpolymorphic antigen-presenting molecule, without the requirement for foreign antigen, might allow activation of T cells even before the clonal expansion of foreign antigen-specific T cells.

In all these examples, the γδ TCR is formed by rearranging gene segments sometimes with and sometimes without VDJ junctional diversity. Since such antigen receptors are considered part of the acquired or adaptive immune system, their function as proposed here involves the recognition of antigens displaying certain structural patterns or of antigen-presenting molecules without foreign antigens. As such, this intrinsic reactivity spans the gap between innate recognition that does not utilize diverse antigen receptors and adaptive recognition that does.

The advantage of such intrinsic T cell recognition is its ability to focus T cell responses earlier in immune responses and more broadly than would be normally possible. γδ T cell-derived cytokines can be released. γδ T cells can kill infected cells and deliver microbicidal granulysin [156]. Unlike innate responses, there is the potential for amplification of this response by γδ T cell proliferation and the development of γδ T cell memory. γδ T cells can also aid innate immune cells in directing αβ T cells specific for conventional peptide antigens to develop Th1 or Th2 phenotypes [56]. If they recognize self stress antigens or other "danger" signals, γδ T cells could detect an array of insults, both infectious and chemical, and respond by secreting growth factors to aid tissue regeneration [17] or inhibitory cytokines to regulate ongoing innate or adaptive immune responses [20]. In performing these functions, γδ T cells may constitute the intrinsic arm of the adaptive immune system.

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