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MULTI-AUTHOR REVIEW

Peptide antigens for gamma/delta T cells

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Abstract $\gamma\delta$ T cells express adaptive antigen receptors encoded by rearranging genes. Their diversity is highest in the small region of TCR V–J junctions, especially in the δ chain, which should enable the $\gamma\delta$ TCRs to distinguish differences in small epitopes. Indeed, recognition of small molecules, and of an epitope on a larger protein has been reported. Responses to small non-peptides known as phospho-antigens are multi-clonal yet limited to a single $\gamma\delta$ T cell subset in humans and non-human primates. Responses to small peptides are multi-clonal or oligo-clonal, include more than one subset of $\gamma\delta$ T cells, and occur in rodents and primates. However, less effort has been devoted to investigate the peptide responses. To settle the questions of whether peptides can be ligands for the $\gamma\delta$ TCRs, and whether responses to small peptides might occur normally, peptide binding will have to be demonstrated, and natural peptide ligands identified.

Keywords $\gamma\delta$ T cells · T cell receptor · Peptide · Ligand · Antigen recognition

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Introduction

The adaptive immune system of jawed vertebrates includes lymphocytes expressing B cell receptors (BCR), $\alpha\beta$ T cell receptors ($\alpha\beta$ TCR) and $\gamma\delta$ T cell receptors ($\gamma\delta$ TCR). All of these receptors are encoded by rearranging genes, which combine variable and constant gene segments to generate diverse receptor molecules. The mechanisms that come into play in building this diversity, which is often random and can be nearly unlimited, have been extensively reviewed elsewhere [1]. It is thought that random receptor diversity is the evolutionary answer to a need to recognize a myriad of antigens expressed by the countless and rapidly changing pathogens that threaten vertebrate survival. The lymphocytes expressing BCRs (B cells) and $\alpha\beta$ TCRs ($\alpha\beta$ T cells) have been much studied, and they are considered key players in the adaptive immune responses. Thus, B cells produce antibodies, which opsonize and neutralize pathogens and their antigens, and $\alpha\beta$ T cells either help B cells to develop into antibody-producing plasma cells, or they target infected or otherwise injured cells directly, and eliminate them by virtue of their cytolytic activity. In contrast, it is less well known what $\gamma\delta$ T cells do, and whether they use their adaptive receptors to support adaptive immune responses or perhaps other functions of the immune system. Most investigators in the field believe, however, that knowing the ligands recognized by the $\gamma\delta$ TCRs will also provide answers to the current questions about the role of $\gamma\delta$ T cells in immune responses.

Lack of a paradigm in $\gamma\delta$ ligand recognition

Among the earliest studies concerned with $\gamma\delta$ TCR ligand recognition were those that compared $\alpha\beta$ and $\gamma\delta$ T cells for

their ability to recognize MHC differences. The results are confusing because, although γδ T cells did not specifically respond to allo-antigens and did not show MHC-restricted specificities involving conventional antigens, γδ T cells capable of recognizing classical MHC molecules and related cell-surface proteins, including the CD1 molecules, have been isolated. This collection could be mined by crystallographers interested in the problem of ligand recognition by γδ T cells, and the fact that the structure of the MHC-ligand might be already available should represent an advantage in resolving the structure of TCR–ligand complex. However, this collection has hardly been touched so far, and in fact, only a single crystal structure involving a murine γδ TCR in complex with the T10/22 molecules has been solved [2]. This structure showed an interesting molecular interaction very different from those of most MHC-restricted αβ TCRs and their MHC-ligands. Further structures of this type will be needed before generalizations about γδ ligand recognition can be made.

Based on structural comparisons between γδ TCRs and immunoglobulins, it has been suggested that γδ ligand recognition should be Ig-like [3]. Like the immunoglobulins, where the complementarity determining region 3 (CDR3) of the IgH chain makes a major contribution to receptor diversity and specificity, the TCR-δ CDR3 in the γδ TCRs contributes the lion's share to receptor diversity. Not surprisingly, then, TCR-δ CDR3 is solely implicated in the recognition of the T10/22 molecule, both structurally and functionally [2, 4, 5], and other studies provided further evidence for the importance of this particular portion of the γδ TCR in the recognition of additional ligands [6]. However, specific immunoglobulins can be raised by immunization with almost any antigen, whereas most antigens fail to elicit specific γδ T cells. Therefore, although physical γδ TCR-ligand interactions may be Ig-like, this attractive concept alone does not suffice in predicting what might be a γδ TCR ligand. Recent evidence indicates that receptor selection plays a major role in determining "allowed" specificities in peripheral γδ T cells [7]. Since γδ T cells develop in the thymus, such selection might occur here, and indeed, some studies provided strong evidence for thymic selection of these cells. The evidence is limited, however. So far, there are only two well-studied examples; the thymic selection of cells specific for T10/22 and of the TCR-invariant dendritic epidermal T cells (DETC) [8–10]. The T10/22-specific cells share a CDR3δ motif in their TCRs, which is for the most part contributed by a germline DNA segment (Dδ, reading frame 3) and thus shared by a relatively large number of clones. However, thymic selection was found to affect the functional development of these cells, such that TCR–ligand interactions were needed in the thymus for them to become producers of IFN-γ, rather than producers of IL-17A. The invariant TCR of the thymic precursors of DETC also appears to be selected. In this case, the putative ligand SKINT-1 [10], a molecule that belongs to the immunoglobulin superfamily of receptors [11], remains to be confirmed as a true ligand. Combining the notions of Ig-like receptor–ligand interactions and thymic selection one might speculate that γδ TCRs resemble natural Igs by having an inherent or selected specificity for auto-antigen, but such a concept requires more testing.

The structure of the γδ TCR genes predicts a high degree of adaptivity in the receptors [1] and indeed, numerous studies have documented peripheral TCR selection. These include human diseases such as polymyositis [12] and multiple sclerosis [13] as well as animal models of diseases including collagen-induced arthritis (CIA) [14], and what appears to be natural selection based on endogenous ligands in healthy individuals [15, 16]. However, how the selected TCRs might be functionally different is not clear, because their ligands remain unknown, and their binding properties and ability to activate the cells that express them cannot be assessed. In the mouse model of CIA, cells expressing selected TCRs (Vγ4Vδ4) also expressed the cytokine IL-17A at increased frequencies [14], suggesting a functional connection, but even prior to the immunization, the Vγ4+ subset contained IL-17A+ cells at slightly higher frequency than other subsets [14].

Because many of the putative ligands for the γδ TCRs are non-repetitive and too small to mediate TCR cross-linking, they must be anchored in some way to enable polyvalent interactions with the TCRs. Indeed, evidence for cellular presentation of the so-called phospho-antigens has been obtained [17, 18], although it is still unknown if distinct presenting molecules are involved. Furthermore, it has been reported that human γδ T cells recognize phospholipids when CD1d is present [19, 20], suggesting that CD1d might be the presenting molecule in this particular example. Cellular presentation of conventional peptide antigens, common in the specific responses of αβ T cells, has been suggested for γδ T cells [21]. Moreover, some data suggest that peptides having covalently attached non-peptidic ligands can, when bound to MHC, be recognized by γδ T cells [22]. If polyvalency is the only requirement for stimulatory ligands, small molecular moieties also might be bound to non-cellular structures and still be stimulatory.

Many of the observed γδ T cell responses are oligoclonal, involving cells with shared germline elements in their TCRs, several of the antigens recognized are very small and require presentation, and the γδ TCRs are selectable and highly adaptive. To rationalize these observations, we suggested that the γδ TCR might be antigen-restricted instead of being restricted by a presenting molecule, and that its variability might serve to adjust

to the variable cell surface context of an antigen that can be presented in many different ways [23]. However, non-MHC-restricted presentation of small molecules on the cell surface is not likely to be random, and specific molecular associations still might exist that are recognizable by the $\gamma\delta$ TCRs. Thus, a general paradigm that adequately describes TCR–ligand interactions with $\gamma\delta$ T cells remains to be established.

Proteins among the molecular moieties that stimulate $\gamma\delta$ T cells

Although currently there is a strong focus on human $\gamma\delta$ T cell responses to the non-peptidic phospho-antigens [24], proteins still might turn out to represent the bulk of the antigens recognized by $\gamma\delta$ T cells. Indeed, tetanus toxoid, a strong immunogen derived from a protein, the tetanospasmin of *Clostridium tetani*, might have been the first defined antigen reported capable of stimulating specific $\gamma\delta$ T cell responses [25, 26]. Others that followed include Ig λ light chain [27], certain heat shock proteins [28, 29], and the proteins staphylococcal enterotoxin A (SEA) [30], herpes simplex glycoprotein I [31] and—perhaps—insulin [32]. Furthermore, the less well-defined mycobacterial purified protein derivative (PPD) has been shown to elicit $\gamma\delta$ TCR-dependent responses [28, 33], but it was not conclusively determined if its stimulatory moiety is really a protein. More recently, the defined mycobacterial protein ESAT-6 was found to stimulate $\gamma\delta$ T cells [34], however, and this may not be the only mycobacterial protein recognized by $\gamma\delta$ T cells [35]. Finally, in addition to natural proteins, a 100-amino acids-long synthetic protein, poly GT (a random hetero-copolymer of roughly 50 glutamic acids and 50 tyrosines), was found to stimulate strong $\gamma\delta$ TCR-dependent responses in mice, with other related synthetic proteins being less stimulatory (poly GAT) or non-stimulatory (poly G, poly T) [36–39]. Because poly GT lacks a defined secondary structure, it resembles peptide antigens perhaps more than globular proteins.

In addition to soluble proteins, several cell surface-expressed proteinaceous ligands have been identified, including the classical MHC I and II molecules as well as the non-classical MHC I-related molecules T10/T22 in mice [40–42], and the more distant MHC I-like molecules MICA/B in humans [43, 44] as well as CD1c in humans [45] and CD1d in both mice and humans [20, 46]. Although the degree to which these various responses have been characterized varies greatly, the list in its entirety makes a good case that the $\gamma\delta$ TCRs in principle are capable of interacting with proteinaceous ligands. This raises the question of whether small peptides might also be recognized.

TCR-dependent responses to small peptide antigens

Based on the concentrated diversity in the V–J junction of δ and the other TCR genes, together with a relative absence of diversity elsewhere in the TCRs, Davis and Bjorkman suggested that the $\gamma\delta$ TCRs might be capable of recognizing "diverse small molecules (such as peptides) embedded in much less diverse and larger MHC molecules" [1], but so far only a small number of studies have actually examined responses of $\gamma\delta$ T cells to defined peptide antigens (Table 1). Encouraged by the observation that purified recombinant HSP-60 could stimulate murine $\gamma\delta$ T cell hybridomas of a select set [28], as well as findings of others implicating HSP-60 as a possible antigen recognized by $\gamma\delta$ T cells in humans [29, 47], we decided to examine synthetic peptides representing portions of the HSP-60 protein for stimulatory activity. We quickly identified one particular sequence within mycobacterial HSP-60 represented by peptide 180–196, which stimulated strong TCR-dependent cytokine responses in these hybridomas [48]. All of the reactive hybridomas expressed Vγ1, most frequently associated with Vδ6 or Vδ4, but other Vδs were also seen [49]. Because the same hybridomas also produced small amounts of cytokine in the absence of the added peptide [28], and because some of the hybridomas responded as well to a matched peptide representing the homologous mammalian HSP-60 protein [48], it seemed possible that the spontaneous response is auto-reactive in nature, but this was not formally shown. Responsiveness to this peptide could be transferred with the rearranged TCR γ and δ genes of a peptide-reactive hybridoma [50], thus establishing TCR-dependence. To determine if the mycobacterial HSP-60 peptide also elicits a response of $\gamma\delta$ T cells in vivo, we immunized mice with it and subsequently examined the TCRs and peptide responses of Vγ1+ $\gamma\delta$ T cells from the immunized animals. An increase in the relative frequency of peptide-reactive cells and minor changes in their TCR-repertoire and structure were found, indicating that this small peptide indeed triggers select $\gamma\delta$ T cells in vivo [51].

It was not determined in what form this peptide is recognized. Hybridomas responded to it in the absence of added antigen-presenting cells (APCs) [49], suggesting that either APCs are not required at all, or that the hybridoma cell themselves can present the peptide. We observed the same with a TCR-deficient mouse T cell line (58α-β-) expressing a transgenic $\gamma\delta$ TCR of the peptide-reactive type [50], indicating that no other $\gamma\delta$ lineage-specific molecules are required for this response. However, others failed to find the response when human JURKAT cells were transfected with such a TCR [52]. This is reminiscent of recent studies with the response of primate $\gamma\delta$ T cells to phosphoantigens, which requires cellular presentation of

Table 1 Responses of $\gamma\delta$ T cells to specific peptides

$\gamma\delta$ T cell source	Protein source	Peptide origin, name (epitope)	MHC restriction	TCR-dependent	Reference[a]
Mouse	HSP-60	Mycobacterial (180–196)	No	Yes	[48–52]
		E. coli (181–197)			
		Mammalian (205–221)			
	Undefined	Several, corresponding to HSP-70 BiP motif	No	Yes	[55], unpubl.
	None	EY nonamer	No	Yes	[39], unpubl.
	Insulin B chain	B:9-23 [9–23]	No	Yes	[57]
Rat	Retinal S-antigen	PDSAg (341-354)	?	?	[65, 66]
	HLA-B	B27PD (125-138)	?	?	[65, 66]
Human	Tetanus toxin	C. tetani (1235-1246)	HLA-DRw53	Yes	[21]
	Ig λ light chain	Processed peptide	No	Yes	[67]
	Listeriolysin O	*L. monocytogenes (470-508)*	No	Yes	[68]

the phosphoantigens, but only primate-derived cells can act as presenters [53, 54]. Apparently, this reflects the specificity of the $\gamma\delta$ TCR itself, because the same requirement was observed in binding studies with soluble $\gamma\delta$ TCRs [18]. These findings suggest that the $\gamma\delta$ TCRs have some intrinsic ability to identify a rudimentary "self", akin perhaps to the intrinsic ability of $\alpha\beta$ TCRs to recognize MHC molecules.

In further experiments, we explored the limits of this particular peptide sequence and derivatives in terms of their ability to elicit $\gamma\delta$ T cell responses. The shortest HSP-60-derived peptides to retain stimulatory activity were seven amino acids in length, covering the sequence FGLQLEL [50]. A single amino acid substitution in this peptide (to FALQLEL) increased the stimulatory activity [50]. The biological significance of this peptide response is unclear. Because the FGLQLEL peptide roughly matched a motif shared by unfolded proteins, which bind to the molecular chaperone HSP-70 BiP [55], we tested unrelated peptides having this motif for their stimulatory activity as well, and found several that triggered hybridoma responses, albeit none as strong as those to the HSP-60 peptide (unpublished). It is thus conceivable that some $\gamma\delta$ T cells recognize epitopes in unfolded proteins.

We also re-examined poly GT (in the current nomenclature for amino acids, this random hetero-copolymer would instead be designated poly $E^{50}Y^{50}$), and found that the same hybridomas that had shown reactivity with the HSP-60 peptides responded to it, as well as to several derivatives (e.g., poly GAT), and even a nonamer peptide with a non-random sequence of alternating glutamic acids and tyrosines [39]. Taken together, these studies established that short peptide molecules can elicit responses via $\gamma\delta$ TCRs. Because the hybridomas examined were derived from non-immunized animals, their responses might reflect some natural reactivity lacking a high degree of specificity. Since these responses are polyclonal, peptide antigens of a wide variety might meet the criteria required for stimulation. A biological role for these peptide responses was not determined, however.

Recently, a different type of peptide response involving murine $\gamma\delta$ TCRs was discovered. When examining the TCR-β chain repertoire of insulin peptide B:9-23-specific $\alpha\beta$ T cells in NOD mouse model of type I autoimmune diabetes [56], one in a substantial collection of peptide reactive hybridomas was found to express $\gamma\delta$ TCR. In contrast to the above-described peptide reactive $\gamma\delta$ hybridomas, this clone (named SP9D11) expressed a TCR consisting of Vγ4 paired with Vδ10, and no auto-reactivity was seen with this cell [57]. In addition to the B:9-23 peptide, the SP9D11 hybridoma also responded to purified islets, but did not recognize intact or denatured insulin, or cells from another tissue used as control. Transfer of the TCR of this clone to another cell confirmed TCR-dependence of the peptide response. The hybridoma response was specific for the B:9-23 peptide. However, truncations and amino acid substitutions revealed that the carboxy-terminal amino acids 21 and 22 were not necessary for the response whereas the amino-terminal ones could not be changed. As has been seen with the $\alpha\beta$ T cell responses to this peptide [58], substitution of the tyrosine in position 16 with alanine abrogated the $\gamma\delta$ response. Interestingly, the same happened when the cysteine in position 19 was substituted with alanine [57], a change that does not affect the $\alpha\beta$ T cells [58]. This could indicate that the cysteine itself is recognized by the $\gamma\delta$ TCR, or that its ability to dimerize the peptide is essential for stimulation. A dimeric peptide might be able to cross-link $\gamma\delta$ TCRs directly. Another interesting finding of this study was that individual isolated hybridoma cells could respond to the B:9-23 peptide [57]. This suggested that the peptide might be recognized without any cellular presentation, either in soluble form or perhaps bound to the surface of the culture plate, or that the responding cells are able to auto-present. To resolve this, direct binding studies will be required.

The SP9D11 hybridoma was derived from a mouse immunized with the B:9-23 peptide. In subsequent experiments, we used non-immunized NOD mice to generate further $\gamma\delta$ T cell hybridomas. Several of these responded to the B:9-23 peptide as well [57]. This indicated that $\gamma\delta$ T cells with this particular specificity also develop spontaneously, at least in NOD mice. However, these cells mainly expressed Vγ1+ TCRs in contrast to the Vγ4Vδ10 TCR of hybridoma SP9D11, which was derived from a peptide-immunized mouse [57]. Furthermore, their peptide specificity is shared with $\alpha\beta$ T cells and B cells [56, 59, 60]. However, unlike $\alpha\beta$ T cells and B cells, the $\gamma\delta$ T cell response to insulin might be focused on this epitope alone. Importantly, there is now convincing evidence that insulin B:9-23 and closely related peptides occur naturally, contained within intra-islet dendritic cells, which engulf insulin granules and produce and present the peptides [61]. Thus, the B:9-23 peptide is a promising candidate as a natural peptide ligand for $\gamma\delta$ T cells.

Perhaps the B:9-23-specific $\gamma\delta$ T cells have some regulatory role or can become, like the $\alpha\beta$ T cells and B cells with this specificity, involved in the autoimmune response that leads to type I diabetes in this mouse strain. Some time ago, it was reported that inhaled insulin induces $\gamma\delta$ T cells capable of suppressing diabetes development in NOD mice [32], and we recently found that $\gamma\delta$ T cells, including those expressing Vγ1, infiltrate the islets of prediabetic NOD mice (unpublished data). However, in neither case was the specificity of the $\gamma\delta$ T cells determined.

Protein- or peptide-specific reactivity of $\gamma\delta$ T cells also has been reported in animal models of mucosal tolerance. Thus, mice and rats that inhaled or ingested the protein ovalbumin (OVA) developed tolerance to this antigen, which could be transferred with $\gamma\delta$ T cells [62–64]. The suppressive effect of the transferred $\gamma\delta$ T cells was limited to responses against OVA [62]. Orally induced $\gamma\delta$ T cells in rats suppressed experimental autoimmune uveitis (EAU) [65, 66]. These cells were induced by feeding the rats with short peptide antigens, including one derived from the retinal soluble antigen (S–Ag) and one derived from the HLA-B27 molecule (B27PD), which was also tolerogenic in this model. The in vivo induced $\gamma\delta$ T cells also specifically proliferated in response to the peptides in vitro, albeit only in the presence of irradiated $\alpha\beta$ T cells with the same specificity. These responses could be inhibited with antibodies against CD8 and MHC II molecules. It was not determined if the $\gamma\delta$ T cells actually recognize the peptide antigens via their TCR, however.

Specific recognition of short peptides by human $\gamma\delta$ T cells was also reported. One study involved cloned $\gamma\delta$ T cells from the synovial fluid of a patient with rheumatoid arthritis. These clones specifically responded to tetanus toxin-derived peptides, and they expressed Vγ9Vδ2 while lacking CD4 and CD8 expression, the predominant phenotype of $\gamma\delta$ T cells in human peripheral blood [21]. Unlike the polyclonal MHC non-restricted reactivity of Vγ9δ2+ $\gamma\delta$ T cells to mycobacterial antigens, peptide responsiveness was limited to individual clones. Epitope mapping defined the specificity of one of these clones to a portion of the toxin represented by an 12mer peptide covering amino acids 1235–1246. The response to these peptides required APCs, and a screening of partially HLA-matched donors revealed that one $\gamma\delta$ T cell clone (1.4) required a match in HLA-DRw53. HLA-DRw53 is a non-polymorphic class II MHC molecule, which might serve as a presenting element in the peptide response. The clones that responded to the toxin peptide in this study shared the broader reactivity of Vγ9 Vδ2+ cells with mycobacterial antigens [21]. In contrast, the earlier described $\gamma\delta$ T cells with specificity for tetanus toxoid were CD8+ and expressed a different TCR (Vγ1+) [25]. The response of these cells also appeared to be MHC II-restricted, but it was not determined if they could recognize short peptide antigens.

$\gamma\delta$ CTL clones from a Burkitt's lymphoma patient, which recognized a B cell lymphoma Ig λ light chain idiotype [27], also responded to cells transfected with a signal sequence-deficient mutant of the SUP-B17 Ig λ chain. Target cells transfected with this construct expressed the truncated protein only intracellularly. Nevertheless, the Ig λ-specific $\gamma\delta$ CTL clones lysed the transfected cells as well as others expressing the full-length construct on their surface [67]. Since purified tumor Ig and peptides corresponding to the hypervariable regions of the SUP-B17l chain failed to sensitize target cells to lysis by the $\gamma\delta$ clones, the data suggested that antigen processing is required to generate a peptide that can be effectively recognized by the $\gamma\delta$ T cells.

Finally, in cultures of human peripheral blood mononuclear cells (PBMC) stimulated in vitro with live *Listeria monocytogenes* bacteria, $\gamma\delta$ T cells developed that were capable of recognizing listeriolysin O (LLO) and a peptide covering LLO amino acids 470–508 [68]. The specific response to this peptide antigen by the $\gamma\delta$ T cells required peptide pulsed autologous PBMC, whereas HLA-DR4 matched PBMC, which supported $\alpha\beta$ T cells responding to the same peptide, failed to support the $\gamma\delta$ response. A specific restricting element was not identified. As with the murine responses to insulin [57], it is interesting to note that LLO-reactive human $\gamma\delta$ and $\alpha\beta$ T cells seem to share an epitope specificity with regard to this bacterial toxin [68]. However, while the $\gamma\delta$ response appears to be focused on this particular epitope, $\alpha\beta$ T cells also recognize other LLO epitopes. As noted by the authors, LLO 470–508 is highly homologous to other toxins of Gram-positive bacteria, perhaps indicating that the focus of the $\gamma\delta$ T cell response includes an entire spectrum of related toxins.

Features of the peptide responses by γδ T cells

Interactions of peptides with proteins are often found in cell biology. A pentapeptide unit has emerged as a common minimal amino acid sequence in peptide protein interactions and in immune recognition [69]. The number of reported peptide responses of γδ T cells is small, but some features are already evident. First, peptides recognized by γδ T cells can be derived from both autologous and heterologous sources [48]. Hence, they might play roles in health and disease, in sterile and in infectious inflammation, and in the regulation of auto-reactivity and the detection of pathogens. Such a diverse range of peptide antigens fits well with the nearly unlimited potential of structural diversity of the γδ TCRs. Second, there might be few constraints with regard to the size of the recognized peptides. Peptides representing natural amino acid sequences as large as 38 amino acids [68], and as short as seven amino acids in length were found to stimulate γδ TCR-dependent responses [50]. Of note, in the studies with larger peptides, secondary peptide processing was not excluded. Unlimited peptide size would be incompatible with presentation of the larger peptides by MHC class I molecules, but presentation by MHC class II molecules and other molecules that do not restrict peptide size remain possibilities. Third, amino acid composition and sequence of the peptides determine responses. In all reported examples, only certain peptides representing an individual epitope within a larger protein sequence stimulated the γδ response. As mentioned earlier, with the HSP-60-derived peptides, we found that only certain truncations and amino acid substitutions were tolerated, whereas others reduced or completely abrogated the γδ response [50]. Similarly, the γδ TCR-dependent response to the insulin peptides B:9-23 was sensitive to amino-terminal truncations but permissive of carboxy-terminal truncations (up to amino acid 21), and substituting amino acids in positions 16 or 19 with alanine rendered the peptide non-stimulatory [57]. Some of our data suggested that amphipathic properties of a peptide matter, and we found that sequence un-related peptides still might stimulate certain γδ hybridomas within the larger set of HSP-60 peptide-reactive cells, as long as they contained hydrophobic or aromatic amino acids in every second position. The same hybridomas also responded to poly GT, and a nonamer peptide with alternating glutamic acids and tyrosines, EY^9 (unpublished data). In sum, these observations indicate that the responses are peptide-specific, albeit with a range of specificities from polyclonal, and including entire groups of similar peptides, to clonal, and limited to one or a few related peptides. Finally, reports vary greatly with regard to antigen presentation requirements. Similar to the originally reported response of human γδ T cells to tetanus toxoid [25], the response of human γδ T cells to a peptide antigen derived from tetanus toxin required APCs [21]. It was also found to be HLA-DR4-restricted, suggesting a mechanism of antigen presentation involving MHC II [21]. In contrast, the response of human γδ T cells to a peptide derived from listeriolysin O did not appear to be MHC-restricted [68]. Similarly, there was no APC-requirement or MHC-restriction in the responses to HSP-60-derived [70] and insulin-derived peptides by murine γδ T cell hybridomas [57]. Here, it remains possible that the hybridoma cells themselves present peptides to one another. However, in the reactivity of one particular hybridoma (SP9D11) with the insulin peptide B:9-23, it was shown that isolated individual cells responded to the peptide, eliminating a requirement for intercellular presentation [57]. Notably, this peptide might represent a special case as it can dimerize by forming a disulfide bridge between cysteines in position 19. Suggestively, a peptide in which the cysteine was substituted by an alanine failed to stimulate the hybridoma [57]. Thus, available data might be best explained by a model in which peptide antigen presentation by professional APCs and MHC molecules is optional and limited to certain peptides only. Others might be presented by cells in an MHC non-restricted fashion, either involving other specialized cell-surface molecules or perhaps merely non-specific binding interactions between reactive peptides and cells [23], and multivalent peptides might even be able to stimulate directly, by cross-linking the TCR in the absence of any kind of presentation [39, 57].

Significance of the peptide responses

Presently, it is difficult to assess the significance of peptide responses by γδ T cells because it has not yet been demonstrated that small peptides bind to the γδ TCR itself or form a complex with a presenting molecule, and it remains to be seen if naturally occurring small peptides can be recognized. The first requirement pertains to the mechanism of peptide recognition as well as the utility of peptides in eliciting specific responses of γδ T cells. Thus, it is important to determine the affinity of binding interactions between γδ TCRs and small peptides as well as possible additional requirements such as the molecular context in which peptides might be recognized. It must also be determined if peptides indeed bind to the variable portion of the γδ TCRs, and if such interaction depends on the diverse V–J junctions. If peptides bind to the γδ TCR and elicit clonal or subset-specific responses, they might be used for immune modulation and other therapeutic purposes, via the functions of the targeted γδ T cells. Determining if γδ TCRs have evolved to recognize small peptide antigens is a more difficult task and will require the

isolation of naturally occurring peptide ligands. As with $\alpha\beta$ T cells, these might include break down products of larger proteins, but it is also conceivable that small proteins such as peptide hormones or defensins can be recognized.

Summary and conclusions

There is no compelling reason why $\gamma\delta$ T cells should not recognize small peptide antigens. Small peptide antigens representing epitopes of a variety of proteins produced antigen-specific $\gamma\delta$ TCR-dependent responses. The peptides are mostly recognized in the context of the eukaryotic cell surface, which might provide species-specificity. However, the precise mechanism(s) and the biological significance of peptide recognition via the $\gamma\delta$ TCR remain to be determined.

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Note added in proof Recently, a systematic approach to the identification of peptide antigens recognized by human gammadelta TCRs was reported as well as a stimulatory epitope on the oxidative stress response regulatory protein (OXYS), expressed by BCG.

Xi, X., Zhang, X., Wang, B., Wang, J., Huang, H., Cui, L., Han, X., Li, L., He, W., Zhao, Z. (2011) A novel strategy to screen Bacillus Calmette-Guerin protein antigen recognized by gammadelta TCR. PLoS ONE 6(4)e18809

References

1. Davis MM, Bjorkman PJ (1988) T cell antigen receptor genes and T cell recognition. Nature 334:395–402
2. Adams EJ, Chien Y-H, Garcia KC (2005) Structure of a gd T cell receptor in complex with the nonclassical MHC T22. Science 308:227–231
3. Rock EP, Sibbald PR, Davis MM, Chien Y-H (1994) CDR3 length in antigen-specific immune receptors. J Exp Med 179: 323–328
4. Shin S, El-Diwany R, Schaffert S, Adams EJ, Garcia KC, Pereira P, Chien Y-H (2005) Antigen recognition determinants of gd T cell receptors. Science 308:252–255
5. Adams EJ, Strop P, Shin S, Chien YH, Garcia KC (2008) An autonomous CDR3delta is sufficient for recognition of the nonclassical MHC class I molecules T10 and T22 by gammadelta T cells. Nat Immunol 9:777–784
6. Chen H, He X, Wang Z, Wu D, Zhang H, Xu C, He H, Cui L, Ba D, He W (2008) Identification of human T cell receptor gammadelta-recognized epitopes/proteins via CDR3delta peptide-based immunobiochemical strategy. J Biol Chem 283:12528–12537
7. Bonneville M, O'Brien RL, Born WK (2010) Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. Nature Rev Immunol 10:467–478
8. Jensen KDC, Su X, Shin S, Li L, Youssef S, Yamasaki S, Steinman L, Saito T, Locksley RM, Davis MM, Baumgarth N, Chien Y-H (2008) Thymic selection determines gammadelta T cell effector fate: antigen-naive cells make interleukin-17 and antigen-experienced cells make interferon gamma. Immunity 29:90–100
9. Lewis J, Girardi M, Roberts SJ, Barbee SD, Hayday AC, Tigelaar RE (2006) Selection of the cutaneous intraepithelial gd + T cell repertoire by a thymic stromal element. Nat Immunol 7:843–850
10. Boyden LM, Lewis JM, Barbee SD, Bas A, Girardi M, Hayday AC, Tigelaar RE, Lifton RP (2008) Skint1, the prototype of a newly identified immunoglobulin superfamily gene cluster, positively selects epidermal gammadelta T cells. Nat Genetics 40:656–662
11. Hayday AC (2009) Gammadelta T cells and the lymphoid stress-surveillance response. Immunity 31:184–196
12. Pluschke G, Rüegg D, Hohlfeld R, Engel AG (1992) Autoaggressive myocytotoxic T lymphocytes expressing an unusual γ/δ T cell receptor. J Exp Med 176:1785–1789
13. Shimonkevitz R, Colburn C, Burnham JA, Murray RS, Kotzin BL (1993) Clonal expansions of activated γ/δ T cells in recent-onset multiple sclerosis. Proc Natl Acad Sci USA 90:923–927
14. Roark CL, French JD, Taylor MA, Bendele AM, Born WK, O'Brien RL (2007) Exacerbation of collagen-induced arthritis by oligoclonal, iL-17-producing gd T cells. J Immunol 179:5576–5583
15. Sim G-K, Augustin A (1991) Extrathymic positive selection of $\gamma\delta$ T cells. Vγ_4Jγ1 rearrangements with "GxYS" junctions. J Immunol 146:2439–2445
16. Sim G-K, Augustin A (1991) Dominant expression of the T cell receptor BALB invariant δ (BID) chain in resident pulmonary lymphocytes is due to selection. Eur J Immunol 21:859–861
17. Morita CT, Beckman EM, Bukowski JF, Tanaka Y, Band H, Bloom BR, Golan DE, Brenner MB (1995) Direct presentation of nonpeptide prenyl pyrophosphate antigens to human $\gamma\delta$ T cells. Immunity 3:495–507
18. Wei H, Huang D, Lai X, Chen M, Zhong W, Wang R, Chen ZW (2008) Definition of APC presentation of phosphoantigen (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate to Vgamma2V-delta2 TCR. J Immunol 181:4798–4806
19. Russano AM, Agea E, Corazzi L, Postle AD, De Libero G, Porcelli SA, De Benedictis F, Spinozzi F (2006) Recognition of pollen-derived phosphatidyl-ethanolamine by human CD1d-restricted gamma delta T cells. J Allergy Clin Immunol 117: 1178–1184
20. Agea E, Russano A, Bistoni O, Mannucci R, Nicoletti I, Corazzi L, Postle AD, De Libero G, Porcelli SA, Spinozzi F (2005) Human CD1-restricted T cell recognition of lipids from pollens. J Exp Med 202:295–308
21. Holoshitz J, Vila LM, Keroack BJ, McKinley DR, Bayne NK (1992) Dual antigenic recognition by cloned human $\gamma\delta$ T cells. J Clin Invest 89:308–314
22. Speir JA, Abdel-Motal UM, Jondal M, Wilson IA (1999) Crystal structure of an MHC class I presented glycopeptide that generates carbohydrate-specific CTL. Immunity 10:51–61
23. Born WK, O'Brien RL (2009) Antigen-restricted gammadelta T-cell receptors? Arch Immunol Ther Exp (Warsz) 57:129–135
24. Morita CT, Lee HK, Wang H, Li H, Mariuzza RA, Tanaka Y (2001) Structural features of nonpeptide prenyl pyrophosphates that determine their antigenicity for human gamma delta T cells. J Immunol 167:36–41
25. Kozbor D, Trinchieri G, Monos DS, Isobe M, Russo G, Haney JA, Zmijewski C, Croce CM (1989) Human TCR-γ^+/δ^+, CD8$^+$ T lymphocytes recognize tetanus toxoid in an MHC-restricted fashion. J Exp Med 169:1847–1851
26. Kozbor D, Cassatella MA, Lessin S, Kagan J, Finver S, Faust J, Trinchieri G, Croce CM (1990) Expression and function of gammadelta- and alphabeta-T cell receptor heterodimers on human somatic T cell hybrids. J Immunol 144:3677–3683
27. Wright A, Lee JE, Link MP, Smith SD, Carroll W, Levy R, Clayberger C, Krensky AM (1989) Cytotoxic T lymphocytes specific for self tumor immunoglobulin express T cell receptor δ chain. J Exp Med 169:1557–1564

28. O'Brien RL, Happ MP, Dallas A, Palmer E, Kubo R, Born WK (1989) Stimulation of a major subset of lymphocytes expressing T cell receptor $\gamma\delta$ by an antigen derived from *Mycobacterium tuberculosis*. Cell 57:667–674
29. Holoshitz J, Koning F, Coligan JE, De Bruyn J, Strober S (1989) Isolation of $CD4^-$ $CD8^-$ mycobacteria-reactive T lymphocyte clones from rheumatoid arthritis synovial fluid. Nature 339: 226–229
30. Rust CJJ, Verreck F, Vietor H, Koning F (1990) Specific recognition of staphylococcal enterotoxin A by human T cells bearing receptors with the Vγ9 region. Nature 346:572–574
31. Johnson RM, Lancki DW, Sperling AI, Dick RF, Spear PG, Fitch FW, Bluestone JA (1992) A murine $CD4^-$, $CD8^-$ T cell receptor-$\gamma\delta$ T lymphocyte clone specific for Herpes simplex virus glycoprotein I. J Immunol 148:983–988
32. Harrison LC, Dempsey-Collier M, Kramer DR, Takahashi K (1996) Aerosol insulin induces regulatory CD8 gammadelta T cells that prevent murine insulin-dependent diabetes. J Exp Med 184:2167–2174
33. Happ MP, Kubo RT, Palmer E, Born WK, O'Brien RL (1989) Limited receptor repertoire in a mycobacteria-reactive subset of $\gamma\delta$ T lymphocytes. Nature 342:696–698
34. Li L, Wu C-Y (2008) CD4+ CD25+ Treg cells inhibit human memory gammadelta T cells to produce IFN-g in response to M. tuberculosis antigen ESAT-6. Blood 111:5629–5636
35. Brodin P, Rosenkrands I, Andersen P, Cole ST, Brosch R (2004) ESAT-6 proteins: protective antigens and virulence factors? Trends Microbiol 12:500–508
36. Vidovic D, Roglic M, McKune K, Guerder S, MacKay C, Dembic Z (1989) Qa-1 restricted recognition of foreign antigen by a $\gamma\delta$ T-cell hybridoma. Nature 340:646–650
37. Dembic Z, Vidovic D (1990) Specificity and function of Qa-1 restricted $\gamma\delta$ T cells. Res Immunol 141:600–602
38. Vidovic D, Dembic Z (1991) Qa-1 restricted gamma delta T cells can help B cells. Curr Top Microbiol Immunol 173:239–244
39. Cady CT, Lahn M, Vollmer M, Tsuji M, Seo SJ, Reardon CL, O'Brien RL, Born WK (2000) Response of murine $\gamma\delta$ T cells to the synthetic polypeptide poly-$Glu^{50}Tyr^{50}$. J Immunol 165:1790–1798
40. Matis LA, Fry AM, Cron RQ, Cotterman MM, Dick RF, Bluestone JA (1989) Structure and specificity of a class II alloreactive $\gamma\delta$ T cell receptor heterodimer. Science 245:746–749
41. Matis LA, Cron R, Bluestone JA (1987) Major histocompatibility complex-linked specificity of $\gamma\delta$ receptor-bearing T lymphocytes. Nature 330:262–264
42. Bonneville M, Ito K, Krecko EG, Itohara S, Kappes D, Ishida I, Kanagawa O, Janeway JCA, Murphy DB, Tonegawa S (1989) Recognition of a self major histocompatibility complex TL region product by $\gamma\delta$ T cell receptors. Proc Natl Acad Sci USA 86:5928–5932
43. Groh V, Steinle A, Bauer S, Spies T (1998) Recognition of stress-induced MHC molecules by intestinal epithelial $\gamma\delta$ T cells. Science 279:1737–1740
44. Wu J, Groh V, Spies T (2002) T cell antigen receptor engagement and specificity in the recognition of stress-inducible MHC class I-related chains by human epithelial $\gamma\delta$ T cells. J Immunol 169:1236–1240
45. Porcelli S, Brenner MS, Greenstein JK, Balk SP, Terhorst C, Bleicher P (1989) Recognition of cluster of differentiational antigens by human $CD4^-$ $CD8^-$ cytolytic T lymphocytes. Nature 341:447–450
46. Huber S, Sartini D, Exley M (2003) Role of CD1d in Coxsackievirus B3-induced myocarditis. J Immunol 170:3147–3153
47. Fisch P, Malkovsky M, Klein BS, Morrissey LW, Carper SW, Welch WJ, Sondel PM (1990) Human Vγ9/Vδ2 T cells recognize a groEL homolog on Daudi Burkitt's lymphoma cells. Science 250:1269–1273
48. Born W, Hall L, Dallas A, Boymel J, Shinnick T, Young D, Brennan P, O'Brien R (1990) Recognition of a peptide antigen by heat shock reactive $\gamma\delta$ T lymphocytes. Science 249:67–69
49. O'Brien RL, Fu Y-X, Cranfill R, Dallas A, Reardon C, Lang J, Carding SR, Kubo R, Born W (1992) Heat shock protein Hsp-60 reactive $\gamma\delta$ cells: A large, diversified T lymphocyte subset with highly focused specificity. Proc Natl Acad Sci USA 89:4348–4352
50. Fu Y-X, Kersh G, Vollmer M, Kalataradi H, Heyborne K, Reardon C, Miles C, O'Brien R, Born W (1994) Structural requirements for peptides that stimulate a subset of $\gamma\delta$ T cells. J Immunol 152:1578–1588
51. Fu Y-X, Cranfill R, Vollmer M, van der Zee R, O'Brien RL, Born W (1993) In vivo response of murine $\gamma\delta$ T cells to a heat shock protein-derived peptide. Proc Natl Acad Sci USA 90:322–326
52. Kikuchi GE, Roberts K, Shevach EM, Coligan JE (1992) Gene transfer demonstrates that the Vγ1.1Cγ4Vδ6Cδ T cell receptor is essential for autoreactivity. J Immunol 148:1302–1307
53. Wang H, Lee HK, Bukowski JF, Li H, Mariuzza RA, Chen ZW, Nam K-H, Morita CT (2003) Conservation of nonpeptide antigen recognition by rhesus monkey Vgamma2Vdelta2 T cells. J Immunol 170:3696–3706
54. Sarikonda G, Wang H, Puan KJ, Liu XH, Lee HK, Song Y, Distefano MD, Oldfield E, Prestwich GD, Morita CT (2008) Photoaffinity antigens for human gamma delta T cells. J Immunol 181:7738–7750
55. Blond-Elguindi S, Cwirla SE, Dower WJ, Lipshutz RJ, Sprang SR, Sambrook JF, Gething M-JH (1993) Affinity panning of a library of peptides displayed on bacteriophages reveals the binding specificity of BiP. Cell 75:717–728
56. Zhang L, Jasinski JM, Kobayashi M, Davenport B, Johnson K, Davidson H, Nakayama M, Haskins K, Eisenbarth GS (2009) Analysis of T cell receptor beta chains that combine with dominant conserved TRAV5D*04 anti-insulin B:9-23 alpha chains. J Autoimmun 33:42–49
57. Zhang L, Jin N, Nakayama M, O'Brien RL, Eisenbarth GS, Born WK (2010) Gamma delta T cell receptors confer autonomous responsiveness to the insulin-peptide B:9-23. J Autoimmun 34:478–484
58. Abiru N, Wegmann D, Kawasaki E, Gottlieb P, Simone E, Eisenbarth GS (2000) Dual overlapping peptides recognized by insulin peptide B:9–23 T cell receptor AV13S3 T cell clones of the NOD mouse. J Autoimmun 14:231–237
59. Simone E, Daniel D, Schloot N, Gottlieb P, Babu S, Kawasaki E, Wegmann D, Eisenbarth GS (1997) T cell receptor restriction of diabetogenic autoimmune NOD T cells. Proc Natl Acad Sci USA 94:2518–2521
60. Nakayama M, Abiru N, Moriyama H, Babaya N, Liu E, Miao D, Yu L, Wegmann DR, Hutton JC, Elliott JF, Eisenbarth GS (2005) Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice. Nature 435:220–223
61. Mohan JF, Levisetti MG, Calderon B, Herzog JW, Petzold SJ, Unanue ER (2010) Unique autoreactive T cells recognize insulin peptides generated within the islets of Langerhans in autoimmune diabetes. Nat Immunol 4:350–354
62. McMenamin C, Pimm C, McKersey M, Holt PG (1994) Regulation of IgE responses to inhaled antigen in mice by antigen-specific $\gamma\delta$ T cells. Science 265:1869–1871
63. McMenamin C, McKersey M, Kühnlein P, Hünig T, Holt PG (1995) $\gamma\delta$ T cells down-regulate primary IgE responses in rats to inhaled soluble protein antigens. J Immunol 154:4390–4394
64. Ke Y, Pearce K, Lake JP, Ziegler HK, Kapp JA (1997) Gamma delta T lymphocytes regulate the induction and maintenance of oral tolerance. J Immunol 158:3610–3618
65. Wildner G, Hünig T, Thurau SR (1996) Orally induced, peptide-specific γ/δ TCR^+ cells suppress experimental autoimmune uveitis. Eur J Immunol 26:2140–2148

66. Wildner G, Thurau SR, Diedrichs-Mohring M (2004) Gamma–delta T cells as orally induced suppressor cells in rats: in vitro characterization. Ann NY Acad Sci 1029:416–422
67. Kim HT, Nelson EL, Clayberger C, Sanjanwala M, Sklar J, Krensky AM (1995) $\gamma\delta$ T cell recognition of tumor Ig peptide. J Immunol 154:1614–1623
68. Guo Y, Ziegler HK, Safley SA, Niesel DW, Vaidya S, Klimpel GR (1995) Human T-cell recognition of *Listeria monocytogenes*: Recognition of listeriolysin O by TcR$\alpha\beta^+$ and TcR$\gamma\delta^+$ T cells. Infect Immun 63:2288–2294
69. Luccese G, Stufano A, Trost B, Kusalik A, Kanduc D (2007) Peptidology: short amino acid modules in cell biology and immunology. Amino Acids 33:703–707
70. Born W, Cady C, Jones-Carson J, Mukasa A, Lahn M, O'Brien R (1999) Immunoregulatory functions of gammadelta T cells. Adv Immunol 71:77–144