REVIEW

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Antigen-restricted γδ T-cell receptors?

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Abstract

After more than two decades of investigation, the biological role of the γδ T-cell receptors (TCRs) remains elusive. In fact, a theory of ligand recognition is still lacking that accounts for their adaptable structure, their peripheral selection, and the observed responses of γδ T cells, which do not require immunization but only include cells sharing germline-encoded components of the TCR. Assuming that all γδ T cells recognize ligands by a common mechanism, we now propose that germline--encoded components of the γδ TCRs provide for the specific recognition of a select set of antigenic determinants (Ags) which appear on the cell surface in various molecular associations. Furthermore, we hypothesize that the adaptivity of the γδ TCRs serves to increase affinity for the molecules with which these Ags associate rather than for the Ags themselves. Here we outline this hypothetical mechanism and discuss its possible implications for thymic selection and potential for complementing known innate and adaptive mechanisms of immune defense.

Key words: T lymphocyte, T-cell receptor, ligand, antigen recognition.

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INTRODUCTION

The immune systems of jawed vertebrates contain three types of adaptive receptors: the B-cell receptors (BCRs) or immunoglobulins, the αβ T-cell receptors (TCRs), and the γδ TCRs (Rast et al. 1997). Lymphocytes expressing these receptors participate in the immune response and play important roles in host defense against pathogens and malignancy, but they are also potentially auto-aggressive. The BCRs and αβ TCRs, expressed by B cells and αβ T cells, are antigen receptors and the mechanisms by which they bind their ligands are well studied and largely understood (Janeway and Travers 1997). Notably, the portion of these receptors that directly interacts with antigen is usually generated via somatic mechanisms of DNA recombination and random nucleotide changes rather than via germline-encoded genetic elements. The γδ TCRs, expressed by γδ T cells, appear to be antigen receptors also, but γδ T cells fail to respond to most of the antigens that stimulate the other two lymphocyte types (Born et al. 1999). There is also evidence that γδ TCRs are instead specific to endogenous ligands, but for the most part it has remained unclear what they recognize and by which mechanism. Here we have approached the question of γδ TCR ligands speculatively. First we start with the assumption that common characteristics for ligand recognition exist for γδ TCRs, as they do for MHC-restricted αβ TCRs that interact with their respective antigens. We feel this is likely because of the evolutionary conservation of the γ and δ genes in all jawed vertebrates so far examined (Litman et al. 2005; Rast et al. 1997) and because mechanisms of variation within TCRs have remained stable in comparison with those for the diversification of the BCRs (Cannon et al. 2004; Litman et al. 2005). Secondly, we stipulate that the mechanism of ligand recognition must account for various experimental observations regarding γδ T--cell responses. For example, available data indicate that γδ T cells, like αβ T cells, recognize ligands expressed on the cell surface (Aydintug et al. 2004; Crowley et al. 1997; Morita et al. 1995; Vidovic et al. 1989). Thirdly, such a mechanism ought to add something to the known ability of the immune system to detect antigens/ligands in order to justify its evolutionary conservation. For example, conventional αβ T cells cannot be activated by antigens/ligands that are too small to crosslink the receptor, hence Ag-presentation. However, such Ags also might appear on the cell surface, bypassing the usual processing and presentation pathways, in associa-

tion with cell-surface molecules other than the specialized antigen-presenting molecules. We envisage that γδ T cells might be able to "see" such antigens in their molecular context because their TCRs carry germline--encoded elements specific for the antigens and they can be selected by molecules that are associated with the antigens (the context).

OBSERVATIONS

Certain key observations constitute clues relevant to the question of TCR-ligand recognition by γδ T cells. First there is the general organization of the γ and δ genes, which resembles that of the TCR α and β and Ig heavy (H) and light (κ and λ) chain genes (Chien et al. 1987; Hayday et al. 1985). Like these, the γ and δ genes consist of multiple gene segments which form functional genes through DNA rearrangement, with the potential of generating many different combinations. Taking into account additional mechanisms such as random nucleotide additions at the junctions between germline-encoded elements and the circumstance that Dδ gene segments can form functional genes in all of their three reading frames, and can be used additively (in tandem), the potential diversity of the γδ TCRs has been estimated to be greater than that of the αβ TCRs and of the immunoglobulins (Davis and Bjorkman 1988). Most of this potential is concentrated in the V-D-J junctional region of the δ genes (Rock et al. 1994). The general organization and mechanism of gene rearrangement of γ and δ genes in rodents and primates are similar, consistent with a similar use of these genes. However, there are species differences with regard to individual germline-encoded gene segments which might lead to differences in the ligands that can be recognized. For example, there are no close homologues between Vγ genes in rodents and primates (Clark et al. 1995) and there is no rodent response to the phosphoantigens that are recognized by primate γδ T cells.

Second, the somatic diversity of the γδ TCRs has a biological function, as numerous studies provide evidence for peripheral selection of γδ T cells. These include certain rearrangements, TCR junctional sequences, and TCR V combinations that are overrepresented in normal human development (Hayday 2000; Parker et al. 1990), in human diseases such as multiple sclerosis and polymyositis (Hohlfeld et al. 1991; Shimonkevitz et al. 1993), in non-symptomatic cytomegalovirus infection (Pitard et al. 2008), in normal mice of certain genetic background (Sim and Augustin 1990; Sim and Augustin 1991), and in mice injected with complete Freund's adjuvant or immunized in other ways. In one recent example, mice immunized with collagen/ /CFA expanded γδ T cells expressing Vγ4/δ4 TCRs with highly biased junctional sequences in both TCR chains (Roark et al. 2007). These selectively expanded cells were also distinct in their high frequency of IL-17 expression (>70%), but did not respond to collagen and the ligand they might actually recognize remains unknown.

Third, in apparent contrast to the evidence for clonal selection of γδ T cells, the few antigens that have been found to elicit γδ T-cell responses did not require immunization and involved populations of cells with shared germline-encoded TCR elements (Cady et al. 2000; Constant et al. 1994; O'Brien et al. 1992; Sciammas and Bluestone 1998; Shin et al. 2005; Tanaka et al. 1995). As a caveat, although TCR dependence has been demonstrated in these examples by various methods, only in some cases has direct evidence for TCR-ligand interactions been shown. Typically, the reactivity with these antigens is shared by γδ T cells expressing the same TCR Vs, TCR Js, TCR Ds, or certain combinations of these elements (O'Brien et al. 1991; Shin et al. 2005). For example, we found some time ago that a distinct peptide was recognized by hybridomas representing an entire subset of Vγ1$^+$ γδ T cells derived from non--immunized mice (O'Brien et al. 1992). However, repeated immunization with the same peptide resulted in a prevalence of altered TCRs expressed by the peptide-reactive population of γδ T cells, indicated by changes in CDR3 length and amino-acid composition and by an increased occurrence of identical TCRs (Fu et al. 1993). Polyclonal reactivity is also a characteristic of the γδ T cells in mice that are specific for the inducible T10/T22 molecules (Shin et al. 2005), which are nonclassical MHC class I proteins. This specificity critically depends on the presence of a single TCR-δ D segment in one of the three possible reading frames, producing the TCR δ CDR3 amino-acid sequence WSEGY(E)L, whereas the other TCR gene segments appear to be nonessential and interchangeable for recognition of this ligand (Shin et al. 2005). The WSEGY(E)L-containing γδ TCRs bind to one particular region of T10/T22, to a determinant outside the molecular surface where conventional TCR interactions are expected to take place (Adams et al. 2005). Whether deliberate selection for T10/T22 reactivity might impose additional constraints on the TCR and reduce the diversity of the responding cells is not clear.

The well-studied response of human γδ T cells to the so-called phosphoantigens also appears to require only certain germline-encoded elements (Constant et al. 1994; Tanaka et al. 1995). The phosphoantigens are a class of small molecular moieties characterized by their content of a phosphomonoester group in a distinct molecular configuration (Morita et al. 2001). The responding human γδ T cells express Vγ9Vδ2$^+$ TCRs (also termed Vγ2Vδ2), which contain a distinct Jγ segment, JγP (also termed Jγ1.2). A structural study has revealed a "chemically reasonable binding site" for the phosphoantigens in this TCR (Allison et al. 2001), a positively charged pocket which resembles those of antibodies that bind phosphate-containing antigens (Allison and Garboczi 2001). Both this structural study and functional studies with mutagenized TCRs identified 59Arg of CDR2γ, 109Lys of CDR3γ, and 51Arg of CDR2δ as critical for the antigen response (Yamashita et al. 2003). Although the TCR binding-site for the phosphoantigens

is not fully defined, the amino acids that seem to be essential for antigen recognition are encoded by germline-encoded V or J gene segments, which explains the broad reactivity by polyclonal populations (Allison and Garboczi 2001). It is noteworthy that while γδ T cells from nonhuman primates also respond to phosphoantigens (Wei et al. 2008), rodent γδ T cells do not. In contrast to T10/T22, direct binding between soluble phosphoantigens and the γδ TCR has not been demonstrated.

Fourth, there is considerable experimental evidence to support the notion that γδ T cells recognize ligands displayed on the cell surface. This is obviously the case with ligands that are expressed as transmembrane cell--surface molecules, such as Qa-1^b (Vidovic et al. 1989), T10/22 (Crowley et al. 1997), or MICA/B (Groh et al. 1998), but it is also suggested in the case of the phosphoantigens, where cell contact was found to be a requirement for the responses (Morita et al. 1995; Sarikonda et al. 2008). However, phosphoantigens do not stably associate with the cell surface and cannot be pulsed on to antigen-presenting cells (APCs) (Espinosa et al. 2001; Morita et al. 1995). To overcome this problem, which may be due to low affinity, Morita and coworkers have generated photoaffinity analogs of phosphoantigens, which are recognized by γδ T cells even after UV-crosslinking them to the APC surface (Sarikonda et al. 2008). Their study now shows that a broad range of hematopoietic and non-hematopoietic cell types can present these antigens and it rules out any requirement for classical MHC class I and II, β2--microglobulin, or CD1 molecules. Confirming earlier studies with xenogeneic cells (Green et al. 2004), the murine cell lines in this study also did not function as APCs for human γδ T cells (Sarikonda et al. 2008). Whether only one or several molecules can present phosphoantigens remains to be determined.

More generally, the finding that soluble multimerized (sm) γδ TCRs stain cells specifically and selectively indicates that ligands for the γδ TCRs tend to be present on the cell surfaces. This was first shown with smTCRs derived from the T22-specific KN6 hybridoma and murine γδ T cells of unknown ligand specificity (Aydintug et al. 2008; Aydintug et al. 2004), but more recently also with a tetramerized TCR representing a macaque-derived $V\gamma9V\delta2^+$ γδ T cell, which stained the surface of cells treated with phosphoantigen (Wei et al. 2008). Interestingly, as was found with human γδ T cells, the binding of the tetramerized macaque γδ TCR required matched (primate) cells to present phosphoantigen. This rules out the possibility that the requirement for closely matched APCs merely reflects a cellular need for accessory molecules and instead suggests that only primates express the necessary presenting molecule for the phosphoantigens (Wei et al. 2008) or that the responding γδ T cells are positively selected at some point to co-recognize a presenting molecule or molecular context before they encounter stimulating phosphoantigens peripherally.

Indeed, there is evidence for thymic selection of γδ T cells, albeit coming from a different corner. The murine $V\gamma5\delta1^+$ cells, which recognize a ligand expressed on autologous keratinocytes, require positive selection in the thymus, involving a newly identified gene expressed in skin and thymus (skint1) that encodes an Ig-like transmembrane protein (Boyden et al. 2008). The T10/T22-rective γδ T cells in mice also appear able to undergo TCR-ligand interactions in the thymus because ligand-experienced and -inexperienced cells were found to express different cytokines (Jensen et al. 2008). Although T10/T22 expression is not required for the generation of γδ T cells with T10/T22-reactive TCRs (Schweighoffer and Fowlkes 1996), selection on T10/T22 may be necessary for the development of a distinct functional profile. Evidence for negative selection of γδ T cells in the thymus has also been presented, involving mouse models with transgenic TCRs. For example, γδ T cells expressing the T10/$T22^b$ reactive TCR from the G8 cell line are negatively selected in the thymus of T10/$T22^b$-expressing mice (Dent et al. 1993), and this is also true for mice transgenic for the lower--affinity T10/T22 reactive TCR derived from the thymic hybridoma KN6 (Bonneville et al. 1990). Transgenic T cells expressing either the G8- or KN6-derived TCRs are produced abundantly from thymuses of $T10^d$--expressing mice, however. The G8 TCR was recently shown to have a lower affinity for $T10^d$ than for either $T10^b$ or $T22^b$, however, which may explain this difference (Adams et al. 2008). If the T10/T22-reactive cells are typical, negative selection for γδ TCRs then resembles that for αβ TCRs, i.e. TCRs with low affinity for self are tolerated whereas those with high affinity are eliminated.

Fifth, and of comparatively minor significance, γδ T cells often respond faster than αβ T cells, especially in infectious diseases (Hiromatsu et al. 1992). It is not clear whether the γδ TCR-ligand interactions, due to intrinsic properties of the γδ TCR (Hayes and Love 2002), determine response kinetics, but it stands to reason that polyclonally initiated responses would have an edge over clonal responses, at least at the onset. More importantly perhaps, studies in mycobacteria-infected nonhuman primates indicate that secondary responses of the γδ T cells are more rapid and vigorous than the primary responses (Shen et al. 2002). Since these animals also contain αβ T cells, which are known to make memory responses, it is difficult to rule out that the γδ T-cell response during the second exposure is accelerated by external influences. Alternatively, γδ T-cell responders might have been expanded and perhaps selected during the first exposure, which would result in an increased secondary response.

ANTIGEN-RESTRICTED LIGAND RECOGNITION?

It appears that conventional mechanisms of ligand recognition cannot account for the combined observa-

tions listed above. In particular, if in fact somatically generated components of the γδ TCR determine antigen recognition, it would not be evident why such somatically adaptive receptors should only mediate recognition of a select number of antigens. Therefore it seems more likely that antigen recognition is determined by the finite number of germline-encoded TCR elements, including individual portions of the V, D, and J segments of the γ and δ chains and combinations of such portions. Examples include the Dδ sequence SYGEL, which largely determines reactivity with T10/T22 (Shin et al. 2005), and the less well-defined germline-encoded binding site, defined by a combination of Vγ-, Jγ-, and Vδ-derived residues, that determines reactivity with the phosphoantigens (Allison et al. 2001; Yamashita et al. 2003). These TCR elements might have evolved to recognize antigens that are indicative of infectious diseases and of certain critical states of the host organism, such as endogenous cell stress/death (Janeway et al. 1988), drastic developmental tissue changes (Heyborne et al. 1992; Reardon et al. 1990), and malignancy (Fisch et al. 1990; Kabelitz et al. 2007). Presently known antigens that fit this general description include not only the so-called phosphoantigens (exogenous and endogenous), the ESAT-6 antigen of mycobacteria and perhaps other proteins of the ESAT-6 family (Brodin et al. 2004; Li and Wu 2008), cardiolipin (Born et al. 2003), heat-shock proteins and derived peptides (Born et al. 1990), insulin (Harrison et al. 1996), and herpes simplex glycoprotein I (Johnson et al. 1992), but also determinants of the inducible MICA/B (Groh et al. 1998; Wu et al. 2002) and T10/T22 molecules (Crowley et al. 2000). These molecular moieties appear to be recognized by all γδ T cells that share certain germline-encoded recognition elements and

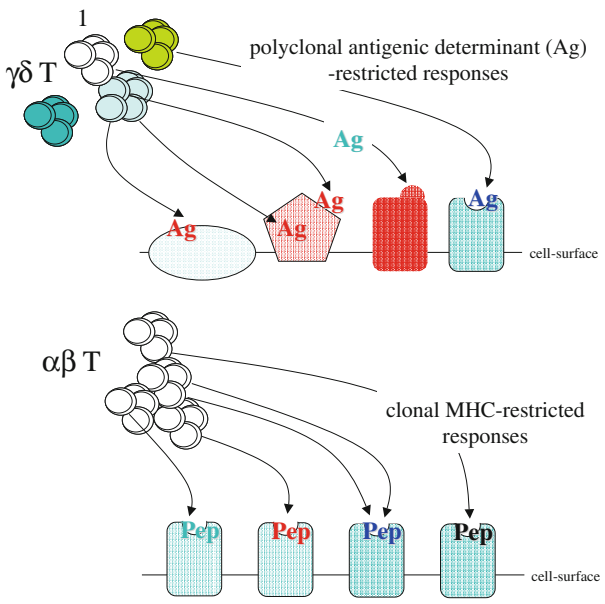

Fig. 1. Hypothetical mechanism of polyclonal antigen-restricted γδ T-cell responses – comparison with MHC-restricted antigen responses of αβ T cells.

they elicit polyclonal responses in non-immunized hosts, reminiscent of the so-called superantigens (Herman et al. 1991). We will refer to them as antigenic determinants (Ags). For a given recognition element in the γδ TCRs there might be an entire set of structurally related Ags (e.g. like the phosphoantigens) (Hayday 2000) or only one or two (e.g. the determinants recognized on T10/T22) (Adams et al. 2005). Affinities between Ags and the corresponding recognition element of the TCR are likely to be low, preventing inappropriate responses. In fact, it seems possible that portions of the TCR that are not part of the recognition element might counteract binding interactions with a given Ag. We would expect that γδ T cells recognize the Ags as cell-surface-bound molecules. The various phosphoantigens, for example, might appear on the cell surface after having been shed by extracellular pathogens or after being induced from the cell's interior. We envisage that because they are not processed and presented by dedicated MHC molecules, Ags are likely to enter associations with various cell-surface molecules (Fig. 1), mainly depending on the physicochemical properties of the individual Ag and the cell/tissue surfaces involved. If, as we suspect, peptides can also be Ags, conventional processing and presentation might be a special case in this general scenario. Another special case might be autologous cell-surface-expressed molecules, which contain as an integral part specific determinants that are recognized as Ags. In this case, for Ag expression to change, these molecules would have to be expressed at variable levels. T10/T22 (Crowley et al. 1997), the product of skint1 (Boyden et al. 2008), and perhaps also MICA/B (Groh et al. 1998) might be such examples.

If Ags mainly interact with germline-encoded portions of the γδ TCRs, then the adaptive potential of the TCRs is free to serve a different purpose. We suspect that it acts to fine-tune the γδ T cell responses by enabling interactions with molecules to which the Ags bind (the context). Such context interactions might enhance or reduce the overall TCR-ligand affinity and determine whether or not cells respond or if they are positively or negatively selected. For example, in the case of a small Ag such as a phosphoantigen, which might bind to several different molecules on the cell surface, an initially polyclonal response of γδ T cells expressing low-affinity receptors might evolve under ligand selection into an oligoclonal response of γδ T cells with higher-affinity receptors, not because of a change in their ability to bind the Ag, but because of the increased affinity of these TCRs for the context. By this mechanism the γδ T-cell response might become increasingly tissue and cell specific, and indirectly also more sensitive in the presence of diminishing Ag concentrations. In the case of Ags with fixed molecular contexts (T10/T22 determinants) (Adams et al. 2005), such selection might already have taken place in the thymus (Jensen et al. 2008), and whether or not γδ T-cell responses are triggered in the periphery might depend here instead on whether peripheral expression of

T10/T22 molecules has been induced (Chien et al. 1996). Hence, the responses of γδ T cells to T10/T22 and the like might be specific because no other molecules contain similar Ags or because the γδ T cells have been selected to recognize the molecular context together with the Ag.

Because their TCRs are generated by random mechanisms, developing γδ T cells must be negatively selected to ensure self tolerance. Presumably, most of the strongly autoreactive clones are already silenced in the thymus. When Ags or their homologues are already present in the thymus (IPP, T10/T22), γδ T cells with intermediate affinities for "Ag plus context" might also be positively selected in a manner not unlike that for αβ T cells, although this does not seem to be required for γδ T-cell thymic maturation (Schweighoffer and Fowlkes 1996).

In Fig. 1 we compare the hypothetical Ag-restricted responses of γδ T cells to the conventional MHC--restricted responses of αβ T cells. Accordingly, the antigen collection available for recognition by the αβ T cells is limited by the properties of the specialized antigen-presenting molecules and antigens are only recognized in their association. In contrast, the smaller though structurally more diverse collection of Ags recognized by the γδ T cells is limited by the germline-encoded elements of the γδ TCRs, but may be recognized in various cell-surface associations/contexts.

TESTS

The hypothetical mechanism outlined above invites testable predictions.

- Eliminating TCR germline elements associated with an Ag response should eliminate the response to that Ag with no possibility for selecting alternative TCRs with specificity to the same Ag.
- Transplanted germline elements of the γδ TCR should enable Ag recognition. Experiments of this type have already been carried out successfully (Adams et al. 2008; Wang et al. 2008).
- A single γδ TCR might carry more than one Ag specificity.
- The same Ag provided in different defined contexts (e.g. firmly associated with different proteins) should select γδ T cells that share germline TCR elements, but differ in somatically generated components of their TCRs.
- Lowering the dose of an Ag should lead to selection of γδ T cells bearing somatically changed TCR components while continuing to share the Ag-specific germline element.
- If the T10/T22 molecules provide both the Ag and the context, it might be possible to transplant the Ag portion on to a different protein and still elicit a γδ T-cell response, especially among γδ T cells derived from T10/T22-deficient mice.

CONCLUDING REMARKS

In this essay we described a potential mechanism of ligand recognition by γδ T cells that is consistent with available experimental observations in primates and rodents and that could potentially broaden the immune--response repertoire. However, this mechanism is purely speculative. By putting it up for discussion, we hope to stimulate experiments that will test it, confirm or refute it, and perhaps replace it with a better one.

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