GAMMA/DELTA CELLS

Werner Haas

Hoffman-LaRoche, Inc., Nutley, New Jersey 07110

Pablo Pereira and Susumu Tonegawa

Howard Hughes Medical Institute at the Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

KEY WORDS: γδ T cells, T cell subsets, thymic selection, T cell development, nonclassical MHC antigens, heat shock proteins, T cell

Abstract

Before TCR rearrangements, T cell progenitors are committed not only to the αβ and γδ T cell lineage but also to various subsets of both lineages. In the mouse, distinct γδ T cell subsets can develop in the fetal thymus, the adult thymus, or independently of a thymus, probably in intestinal epithelia. The two subsets that develop in the fetal thymus home to and are maintained throughout adult life in the skin and the mucosa of the uterus, vagina, and tongue. They are monospecific. This unusual restriction in receptor repertoires is the result of severe limitations in the generation of diversity in the fetal progenitors of these subsets and the thymic selection. After birth, one γδ T cell subset appears in the blood, spleen, and lymph nodes and one in the intestinal epithelia. The receptor repertoires of these subsets are characterized by the preferential usage of particular Vγ gene segments and extensive junctional diversity. Several murine and human γδ T cell clones have been shown to recognize classical MHC class I and class II proteins or MHC class I-like proteins, and in very few cases the presented peptides are known. We suspect that the various murine γδ T cell subsets interact with different antigen presenting cells which utilize different antigen presenting proteins and reside in different tissues. The function of γδ T cells remains unknown. Preliminary results of experiments
with gene knock out mice which lack either $\alpha\beta$ T cells or $\gamma\delta$ T cells or both suggest that $\gamma\delta$ T cells do not function as helper cells in humoral immune responses but may complement $\alpha\beta$ T cells in the defense against various microorganisms.

**INTRODUCTION**

The long odyssey of immunologists searching for the T cell receptor for antigen (TCR) came to an end in 1984 when clonotypic antibodies were raised against T cell clones, and genes rearranging in T cells but not in B cells were identified and cloned. Because the clonotypic antibodies that were likely to recognize idiotypic determinants of the TCR precipitated an $\alpha\beta$ heterodimer (1–5), it came as a surprise when the third rearranging gene called $\gamma$ was found (6, 7). Antibodies raised against peptides that were synthesized according to the sequence of the $\gamma$ gene revealed a second TCR heterodimer, $\gamma\delta$ (8). The $\delta$ gene was cloned owing to its location within the TCR $\alpha$ locus (9). Further studies showed that cells expressing $\gamma\delta$ heterodimers did indeed exist and represented a new T cell class (10–13). Thus the immunology of $\gamma\delta$ T cells has been progressing in a "reversed direction" i.e. from genes to the TCR and from the TCR to a new class of lymphocytes. At present many laboratories are attempting to elucidate the functions of these cells.

Previous reviews have focused on the structure and organization of $\gamma$ and $\delta$ genes as well as on the development and specificity of $\gamma\delta$ T cells in mice (14–18), human (17–21), and other species (22). In the present review we focus on the specificity and function of $\gamma\delta$ T cells.

**MOUSE $\gamma\delta$ T-CELLS**

*Segregation of $\alpha\beta$ and $\gamma\delta$ Lineages*

In the fetal thymus, rearrangements and surface expression of $\gamma$ and $\delta$ genes precede those of $\alpha$ and $\beta$ genes (23, 24). This and the finding that nonproductive rearrangements of $\gamma$ genes are common in $\alpha\beta$ T cells (25) led to the belief that $\alpha\beta$ T cells are derived from T cell progenitors that failed to express $\gamma\delta$ TCRs (24). If this was correct $\alpha\beta$ T cell development should be impaired in transgenic mice expressing rearranged $\gamma$ and $\delta$ transgenes. However, in the first $\gamma\delta$ TCR transgenic animals $\alpha\beta$ T cells developed normally (26). No transgenic transcripts were found in the $\alpha\beta$ T cells of these mice. Silencing of in-frame and out-of-frame rearranged $\gamma$ genes was also observed in $\alpha\beta$ T cells of normal mice (27). A second set of $\gamma\delta$ TCR transgenic mice was generated with shorter transgenes which
apparently lacked the silencer element (28). In these mice αβ T cell development was severely retarded. These findings suggest that it is the expression of a γ silencer that determines the commitment to the αβ T cell lineage.

A similar silencing element is associated with the TCR α gene (29). Since the γ gene rearranges before the α gene, the α gene silencer cannot be primarily involved in the αβ versus γδ lineage commitment. Perhaps it determines the temporal order of β and α gene rearrangements in cells that have already been committed to the αβ lineage by the previous expression of the γ silencer.

According to another model the deletion of the δ locus by a novel recombination is a prerequisite of αβ T cell development (30, 31). However, the normal development of αβ T cells in γδ TCR transgenic mice is not readily compatible with this proposition.

γδ T Cell Subsets

In mice there are various subsets of γδ T cells, which differ from each other by parameters such as time of appearance in ontogeny, anatomical location, TCR repertoires, and thymus dependence (see Table 1). According to the Vγ segments used preferentially or exclusively, we refer to these subsets as the V5, V6, V4 and V7 subsets. Each subset must express unique adhesion proteins that are responsible for the differences in their migratory behaviour. Two monospecific subsets are disseminated in epithelia, the V5 subset in the epidermis of the skin (32) and the V6 subset in the mucosal surfaces of the uterus, vagina, and tongue (33). They are both derived from the γδ T cells which appear first in the fetal thymus (34–37). Soon after birth most of the γδ T cells in the thymus express the Vγ4 and Vγ1 gene segments and a few of them express Vγ2 and Vγ7 (36, 38, 39). These γ chains pair with many different δ chains, and both chains exhibit great junctional diversity (38, 40, 41). Like αβ T cells V4 subset cells circulate through blood and lymphoid organs such as spleen and lymph nodes (39). It is not clear, however, whether γδ T cells recirculate from blood to lymph in lymph nodes. Indeed a histological study of sheep lymph nodes suggests that γδ T cells do not recirculate from blood to lymph and that they have no functional role in lymph nodes (22, 42).

One subset of γδ T cells is generated outside the thymus, probably somewhere in the intestinal epithelia (43–46). These cells appear during the first weeks of life (43). Unlike thymus-dependent lymphocytes but similar to thymus-independent αβ T cells, most of these cells do not express Thy1 and do express CD8α homodimers (43, 46, 47). Their TCR repertoire is characterized by the predominant usage of Vγ7 and Vγ1 chains, expression of multiple Vδ chains, and high junctional diversity (48–50; P.
<table>
<thead>
<tr>
<th>Subset</th>
<th>Location</th>
<th>TCR usage</th>
<th>Diversity</th>
<th>Characteristics</th>
</tr>
</thead>
</table>
| V5    | Skin                           | V5J1C1-γ, V1D2J1C-δ | None      | Generated in 14-17 day-old fetal thymus  
Generation depends on fetal progenitors and fetal thymus  
Positive selection of monospecific cells in the thymus  
Homing to and maintenance in epidermis  
Recognizes cultured keratinocytes |
| V6    | Vagina, uterus, tongue         | V6J1C1-γ, V1D2J1C-δ | None      | Generated in late fetal and newborn thymus  
Positive selection of monospecific cells in the thymus  
Homing to and maintenance in mucosal epithelia (vagina, uterus, tongue) |
| V1    | Spleen, intestine, skin        | V1J4C4-γ, V6, 4, 5, 7-δ | High     | Abundant in newborn thymus and in spleen 1-2 week after birth  
Autoreactive, spontaneous IL2 production  
Respond also to mycobacterial Hsp60 and Hsp60 peptide 180-196  
Autoreactivity and response to exogenous Hsp is blocked by mAb to γδ TCR, VNR but not class I or II MHC proteins  
Various effects on hemopoiesis in transgenic mice |
| V4    | Blood, lymph nodes, spleen     | V4J1C1-γ, V5, 4, 6, 7-δ | High     | Generated in postnatal thymus  
Major γδ cell population in adult thymus, lymph nodes and spleen |
| V7    | Intestine                      | V7J1C1-γ, V, 4, 5, 6, 7-δ | High     | Highly diversified  
Thymus independent  
Expression of CD8δ homodimers |
| Lung  |                               | Vγ4, Vα6        | High      | 8-20% of resident pulmonary lymphocytes are γδ T cells |
| Liver | V1, 2-γ, Vα6                  |                 | High      | Number increases with age  
Express CD8δ homodimers |
| Mammary gland | V4, 5-γ, Vδ4               |                 | High      | Fourfold increase of number in lactating mammary gland |
Pereira, unpublished observations). In Table 1 we included subset V1 because of its unique autoreactivity even though V1 is probably not a distinct subset, i.e. it probably does not have a distinct progenitor but is rather a population derived from different progenitors but with common properties and Vγ usage. The V1 population has a thymus dependent and independent component.

The γδ T cell populations in the liver, lung, and mammary gland also cannot readily be assigned to one or the other of the above described subsets (51–53). They also may represent mixtures of cells from different subsets.

Human γδ T cell subsets are listed in Table 2. They are described in a later section.

**Heterogeneity of Subset Progenitors**

We assume that all the γδ T cell subsets described above are derived from different progenitor cells that are committed to give rise to distinct sublineages before any TCR gene rearrangements have taken place. This belief is based mainly on two sets of data.

First, γδ T cell subset progenitors differ in their requirements to generate mature progeny. One requires a fetal thymus, one requires an adult thymus, and one does not require any thymus to generate mature progeny. The V7 subset is thymus independent because it is found in the intestines of athymic nude mice and in thymectomized mice that have been lethally irradiated and reconstituted with syngeneic bone marrow cells (43, 46). A fetal thymus is required for the generation of the V5 and V6 subsets. An adult thymus fails to support the generation of the fetal subsets but is sufficient for the generation of the V4 and V1 subset (54, 55).

### Table 2 Human γδ T cells

<table>
<thead>
<tr>
<th>Subset</th>
<th>Location</th>
<th>TCR usage</th>
<th>Diversity</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vδ1</td>
<td>Thymus</td>
<td>VιC2-γ</td>
<td>High</td>
<td>Predominant in thymus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vδ1</td>
<td></td>
<td>Rare in blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td>most non S-S</td>
<td></td>
<td>Proportion in blood decreases with age</td>
</tr>
<tr>
<td>Vδ2</td>
<td>Blood</td>
<td>V2C1-γ</td>
<td>High</td>
<td>Most cells remain CD45RA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vδ3</td>
<td></td>
<td>Rare in thymus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>most S-S</td>
<td></td>
<td>Predominant in blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Proportion in blood increases with age</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Most cells become CD45RO</td>
</tr>
</tbody>
</table>
Second, γδ T cell subset progenitors are committed to different modes of TCR rearrangements. Rearrangements may be “targeted” to different variable gene segments in different progenitors. Thus, a limited analysis of nonproductive rearrangements in mature cells expressing either Vγ4 (56, 57) or Vγ5 (32) chains revealed a preference for rearrangements of Vγ4 or Vγ5, respectively. Similarly, PCR-aided Southern blot analysis of Cγ1 cells colonizing the intestinal epithelia showed a strong bias for the rearrangement of Vγ7, both in the expressed and in the nonexpressed chromosome (49). Furthermore, in cultures of day−13 fetal liver cells with day−14 fetal thymus stromal cells, the temporal order of Vγ gene rearrangements corresponded to the temporal order of the appearance of the Vγ5 and Vγ6 expressing cells during thymic ontogeny (58). It should be noted that in one study (59), PCR analysis of Vγ gene rearrangements in early fetal thymocytes did not reveal any restricted use of particular Vγ gene segments. However, the data presented in this report are not quantitative. The demonstration that fetal precursors have the potential to rearrange any Vγ gene does not argue against the notion of “targeted rearrangements” i.e. preferential rearrangements of defined Vγ genes. Moreover, the rearrangements observed in this study may have occurred not only in γδ but also in αβ T cell progenitors in which no particular γδ T cell differentiation program is established.

In fetal progenitor cells the rearrangements are not only limited by a restricted usage of variable gene segments but also by the preferential joinings of segments with short sequence homologies and by the lack of N-region additions. The genes that are assembled in fetal progenitor cells appear to encode receptors that mediate “the conservative view of the immune system” with particular recognition of “old” self and microbial antigens. A very similar distinction can be made between fetal and adult subsets in the other two major classes of lymphocytes, B-cells (60–64) and αβ T cells (65–67).

The different modes of rearrangement in different γδ progenitor cells are part of a coordinated differentiation program in which the expression of a particular TCR-repertoire is linked to functional properties such as homing to and maintenance in different peripheral tissues. The homing to different epithelia appears to be independent of the TCR. This has been demonstrated for two γδ T cell subsets in TCR transgenic mice in which T cells expressing “wrong” TCR’s were found in the skin and the intestine (68). However, detailed studies of the lifespan and turnover of γδ T cell populations in normal and γδ TCR transgenic mice are necessary to examine the role of the γδ TCR in the maintenance of the subsets in their “home” tissues. In the case of the fetal subsets, it is conceivable that continuous recognition of a self-antigen is required at least for their main-
tenance in the epithelia of the skin and the mucosae of uterus, vagina, and tongue.

**Thymic Selection**

The finding that the junctional sequences of rearranged Vγ5, Vγ6 and Vδ1 genes in PCR amplified DNA of fetal thymocytes and epidermal γδ T cells showed a limited junctional diversity in nonproductive rearrangements but almost none in productive rearrangements suggested that the accumulation of cells expressing the invariant Vγ5Vδ1 and Vγ6Vδ1 TCRs was due to TCR-mediated positive selection (32, 37). The monospecificity of the fetal subsets also could be the result of molecular constraints at the level of rearrangements and/or assembly of the heterodimeric TCR molecules. Several efforts were made to determine whether the "cellular selection model" or the "molecular constraint model" or both were correct. Receptor-mediated positive selection is best demonstrated by changing or removing the selecting ligand. Thus, several different mouse strains were analyzed in the hope of finding a polymorphism of the putative selecting ligand and consequently a different canonical sequence. The canonical sequences were the same in all strains tested (J. Lafaille, S. Tonegawa, unpublished). In an attempt to artificially alter the selection process Itohara & Tonegawa added antibodies against a constant region of the γδ TCR to fetal thymus organ cultures in which the monospecific fetal γδ T cell subsets are normally generated (58). The addition of the antibodies led to an increase in the frequency of productive Vγ5, Vγ6, and Vδ1 rearrangements with non-canonical junctional sequences. This finding supports the selection model because it shows that the molecular constraint model alone cannot explain the TCR homogeneity of the two fetal subsets.

Recently the same investigators produced mutant mice with a large deletion of the Cδ gene (S. Itohara, P. Mombaerts, J. Lafaille, A. Nelson, A. Farr, S. Tonegawa, submitted). Since these mutant mice do not express γδ TCR on their surface but do undergo Vγ-Jγ and Vδ-D-Jδ rearrangements, they are ideal to address the molecular constraint vs the cellular selection model. Surprisingly the canonical TCR genes that were assembled in mutant mice in the absence of TCR-mediated selection were as homogenous as those assembled in wild type mice. Indeed the preferred joins seem to be generated by using short sequence homologies present at the borders of the gene segments or in the so-called P nucleotides (37). This finding strongly supports the molecular constraint model. It also suggests that the effect of the anti-γδ TCR antibodies on the junctional diversity of the canonical TCRs in the fetal organ cultures was not due to inhibition of positive selection. We assume that the antibody led to the expansion of very rare cells expressing noncanonical TCRs while, for reasons which we
do not understand, it had little or no effect on cells expressing canonical TCRs, which presumably encountered endogenous ligands in the cultures.

Thus the unusual homogeneity of the TCR of the Vγ5 and Vγ6 subsets appears to result from three processes, namely, rearrangement of specific V segments (i.e. targeted rearrangements), rearrangements guided by short homologous regions at the break points (molecular constraint model) and by positive selection (cellular selection model).

The analysis of γδ TCR transgenic mice provided evidence for positive and negative selection of cells belonging to the adult V4 subset. Negative selection was demonstrated in two studies (69, 70). In the first study the γδ TCR transgenes were derived from the KN6 hybridoma which recognizes the T22 gene product (71, 72) expressed by spleen cells from H–2\^b mice but not H–2\^d mice (which carry a nonfunctional T22 gene) (72). The number of cells expressing KN6 TCR was similar in the thymus of transgenic H–2\^b and of H–2\^d mice, but 10 times lower in the spleens of H–2\^d mice. Thymocytes and spleen cells from KN6 TCR ligand-positive H–2\^b mice were anergic in that irradiated H–2\^b spleen cells failed to induce them to produce IL–2 but did induce them to proliferate if exogenous IL–2 was added (69).

In the second study the γδ transgenes were derived from the G8 γδ T cell clone that was obtained from BALB/c nude mice and that is specific for a TL region encoded protein similar if not identical to the KN6 TCR ligand (73, 74). Transgenic TCR expressing cells were found in the intestines of TCR transgenic H–2\^b mice, but not in their peripheral lymphoid organs such as lymph nodes and spleen (70, 75). The intestinal cells were unresponsive to H–2\^b stimulator cells even in the presence of exogenous IL–2. The anergic state was suspected to be followed soon by apoptosis, but this remains to be demonstrated (75).

Evidence for positive selection of γδ T cells was obtained when the two TCR transgenic mice described above were crossed with β2m deficient mice (76, 77). Cells expressing the transgenic TCR at high levels were abundant in the thymus of β2m-deficient H–2\^b and H–2\^d TCR transgenic mice but did not exit to peripheral lymphoid tissues and did not give a strong proliferative response to H–2\^b spleen cells even when exogenous IL–2 was added. The proportion of transgenic TCR expressing thymocytes that are stained with J11d antibodies was nearly 100% in mice with the β2m defect but only 50% in mice without this defect. These findings suggest that the maturation of transgenic γδ TCR expressing cells is arrested in the thymus of β2m deficient mice. J11d appears to be a marker for immature thymocytes not only in the αβ but also in the γδ T cell lineage. After emigration from the thymus the expression of J11d appears to be lost in both lineages (K. A. Kelly, M. Pearse, L. Lefrancois, R. Scollay, unpublished observations).
Not all γδ T cells require positive selection by β2m dependent proteins since no gross abnormalities of γδ T cells were observed in β2m deficient mice that were not transgenic for a particular γδ TCR (78). This finding does not exclude the possibility that most γδ T cells have β2m related specificities and depend on selection by β2m-associated proteins since a few γδ T cell clones with β2m unrelated specificities could expand in the β2m deficient mice to fill up the γδ T cell compartment.

The data obtained with the KN6 TCR transgenic mice and their crosses with the β2m-deficient mice suggest that positive and negative selection are mediated by the recognition of different ligands because only the former is seen in H–2^d^ mice (72). We propose that the positively selecting ligand in H–2^b^ mice is the T22^b^ protein and in H–2^d^ mice the product of the T10 gene, a highly homologous duplicate of the T22 gene. The T22^d^ allele is known to be defective. According to this hypothesis, induction of anergy and activation of mature cells requires recognition of the T22^b^ protein plus a peptide that cannot be presented by the T10 protein.

**Extrathymic Selection of γδ T Cells**

Two groups found that the frequency of cells expressing γ or δ chains with particular sequence motifs varied greatly in different strains of mice. Thus, Sim & Augustin have shown that two TCR sequences named BID and GxYS were expressed by many pulmonary resident lymphocytes from BALB/c mice and BALB.B mice but not from C57BL/6 mice (79–81). The same sequences were also found in (BALB/c × C57BL/6) F1 hybrids and in athymic BALB/c mice. The lack of cells expressing BID TCRs and GxYS TCRs in the lungs of C57Bl/6 mice is not due to a failure of these mice to generate the corresponding γ and δ chain genes, because these genes were found in the thymus of all mouse strains (80). These results suggest that the cells expressing BID TCRs were positively selected by strain specific polymorphic ligands that are encoded outside of the classical H–2 region. The selection can take place in the absence of a thymus.

Lefrancois et al (45) reported that the frequency of lymphocytes expressing the Vδ4 chain in the intestinal γδ T cell population varies from 20% to 50% in different strains of mice. F1 hybrids between Vδ4 high and low expressors were Vδ4 high expressors. The analysis of normal and thymectomized F1 into parent bone marrow chimeras showed that the Vδ4 high expressors were selected by host cells and that a thymus was not required for the selection. Further analysis of recombinant inbred strains and of mice recombinant within H–2 suggested that the Vδ4 high phenotype was controlled by a gene linked to the class II MHC genes and required I-E expression.

In these examples of extrathymic selection it is not clear whether exo-
genous antigens are involved nor whether the selection acts upon immature or mature cells. The most likely explanation seems to us to be antigen driven expansion of mature cells.

Figure 1 gives an overview of $\alpha\beta$ and $\gamma\delta$ T cell development.

$\gamma\delta$ T-CELLS IN OTHER SPECIES

$\gamma\delta$ T cells have been found in all vertebrates examined so far, including humans (12, 13), chickens (82, 83), rats (84, 85), sheep (22), cattle (42).
and pigs (86, 87). (Table 3). A preferential localization to epithelia has been noticed in all these species, but there are differences in the abundance of cells and tissue distribution. Ruminants for example have more γδ T cells than αβ T cells in the blood (22, 42). In human (88, 89) and chicken (83), there seems to be no special γδ T cell population in the epidermis.

Human γδ T cells have been studied extensively with regard to TCR repertoire and putative sublineages. Rearrangements at the human TCR γ and δ loci also appear to occur in a developmentally ordered fashion (90, 91). The γδ TCR repertoire that is initially generated in the fetal thymus is small because of the targeting of rearrangements to a limited number of variable gene segments and because of very limited junctional diversity. In the thymuses of 8.5- to 15-week-old human embryos, rearrangements involve joinings of Vδ2 to Dδ3 and of Vγ1.8 or Vγ9 to the Jγ1 cluster (90, 91). The cells which express these TCR chains may be referred to as the Vδ2 subset. From 4 to 6 months after birth, rearrangements involve joinings of other Vδ segments, in particular Vδ1 to Dδ1 and Dδ2 and joinings of upstream Vγ gene segments in the Vγ1 family including Vγ2, 3, 5 and 8 to the Jγ2 cluster (90, 91). The cells which express these TCR chains may be referred to as the Vδ1 subset. The TCR chains of this subset exhibit extensive junctional diversity.

The two human γδ T cell subsets can be distinguished by monoclonal antibodies such as δTCS1 which recognizes Vδ1Jδ1 and Vδ1Jδ2 but not Vδ1Jδ3 (92), BB3 which recognizes Vδ2 (93) or TiγA which recognizes Vγ9 (94). In the postnatal thymus the Vδ2 subset represents about 15% and the Vδ1 subset about 80% of all γδ T cells (95, 96). These proportions of γδ TCR expressing thymocytes remain relatively constant throughout adult life (96). In the blood, however, the Vδ2 subset increases with age from about 25% in cord blood to more than 70% in the blood of most adults. The Vδ1 subset decreases from about 50% in cord blood to less

| Table 3 | Distribution of γδ T cells (% of lymphocytes in each organ). Data compiled from: humans (88, 89); mice (33, 36); rat (84, 85); chicken (82, 83); sheep (22) and cattle (42) |
|-------|--------|--------|--------|--------|--------|--------|
|       | Humans | Mice   | Rat    | Chicken| Sheep  | Cattle |
| Blood | 0.5–16 | 0.5–2  | 2      | 15     | 15–50  | 15–40  |
| Thymus| 0.5–1.5| 0.5–1.5| 10     | 1–4    | 1–5    |        |
| Spleen | 2–30   | 0.5–2  | 2      | 25     | 5–7    |        |
| Lymph node | 5      | 0.5–3  | 4      |        | 1–6    | 1–3    |
| Intestine | 10     | 50     |        | +      |        | +      |
| Skin (epidermis) | -      | +      |        | -/+    | +      |        |
| Other epithelia |        | +      |        |        |        | +      |
| (tongue, etc.) |        |        |        |        |        | +      |
than 30% in the blood of adults (95–99). Most Vδ2 subset cells become positive for CD45RO, a probable marker for memory cells, while most Vδ1 subset cells remain CD45RO negative (100–102). The accumulation of CD45RO positive Vδ2 cells in the blood is thought to be the result of stimulation of mature cells by common ligands for Vδ2/Vγ9 TCRs, many of which are suspected to be superantigens (96). Selection of the predominant γδ T cell subset in adult human blood by superantigens is consistent with the extensive junctional diversity of their TCR (103).

**γδ T-CELL SPECIFICITY**

**Self-Antigens**

The murine V5 subset recognizes cultured keratinocytes or fibroblasts treated with tryptic digests of keratinocytes (104). Since the third complementarity determining regions of the canonical TCR of the V6 and V5 subset are identical one might speculate on the basis of current models of TCR/antigen/MHC protein interactions that the two TCRs recognize the same endogenous peptide in the context of different tissue specific peptide presenting proteins. The presenting proteins appear not to be classical MHC proteins.

The V1 population also appears to recognize an endogenous antigen that is expressed by lymphocytes and probably other hemopoietic cells. Since most autoreactive cells of the V1 subset also recognize heat shock proteins they will be described below.

Cultured human γδ T cells often lyse autologous target cells. However, this killing does not involve the γδ TCR. In most cases it is due to IL–2 induced promiscuous killing activity that has also been observed with many IL–2 dependent αβ T cell clones. The biological significance of this undiscriminating lytic activity in vitro is questionable.

**Classical MHC Proteins**

A few murine and human γδ T cell clones have been shown to be specific for class I and class II proteins (Table 4). MHC class I and class II protein specific γδ T cell clones were obtained from nude mice after immunization or repeated in vitro stimulation with allogeneic spleen cells (74, 105, 106). The specificity of these clones was unusual in that it was broadly cross-reactive for the products of different alleles. Several human γδ T cell clones were shown to recognize HLA-A2, HLA-A24, HLA-DR7, HLA-DR3 (107–110) or HLA DQA1/DQB1 heterodimers (111).

Recognition of MHC proteins by γδ T cells probably involves recognition of presented peptides. In one recent study four human clones have been shown to recognize HLA DRw53 and tetanus toxin peptide 1235–
<table>
<thead>
<tr>
<th>Origin of $\gamma\delta$ T cells</th>
<th>Specificity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4$^+$ CD8$^-$ $\gamma\delta$ T cell line obtained from draining lymph node cells of C57Bl/10 mice 7 days after immunization with B10.BR spleen cells in CFA in footpaw</td>
<td>H-2 D$^b$ (+ peptide?)</td>
<td>Bluestone et al (1988) <em>J. Exp. Med.</em> 168, 1899</td>
</tr>
<tr>
<td>Lymph node cells from C57Bl/10 nude mice were repeatedly restimulated in vitro with B10.BR spleen cells, cloned and fused with BW5147 thymoma cells; hybridoma LBK.5F3</td>
<td>E$^b$, E$^b$, E$^c$, E$^c$ (+ peptide?)</td>
<td>Matis et al (1989) <em>Science</em> 245, 746</td>
</tr>
<tr>
<td>$\gamma\delta$ T cell clones were obtained from cultures containing purified CD4$^+$ CD8$^-$ PBL from healthy donors and allogeneic stimulator cells; Clone 40.1(V$\delta$1)</td>
<td>HLA-A24 (+ peptide?) lysis of P815 cells transfected with A24 cDNA</td>
<td>Ciccone et al (1989) <em>Eur. J. Immunol.</em> 19, 1267</td>
</tr>
<tr>
<td>$\gamma\delta$ T cell clones were obtained from cultures containing purified CD4$^+$ CD8$^-$ PBL from healthy donors and allogeneic stimulator cells; Clones ES-204 (V$\delta$3) and ES-443 (V$\delta$1)</td>
<td>HLA-A2 (+ peptide?) recognition of P815 cells transfected with A2 cDNA</td>
<td>Spits et al (1990) <em>J. Immunol.</em> 144, 4156</td>
</tr>
<tr>
<td>$\gamma\delta$ T cell clones were obtained from cultures containing purified CD4$^+$ CD8$^-$ PBL from healthy donors and allogeneic stimulator cells after repeated restimulations and cell separations</td>
<td>HLA-DR1 (0501)/HLA DQB1 (0301) cis or trans encoded heterodimer (+ peptide?)</td>
<td>Bosnes et al (1990) <em>Eur. J. Immunol.</em> 20, 1429</td>
</tr>
<tr>
<td>Two donor specific $\gamma\delta$ T cell clones from mitogen stimulated PBL of a patient with a HLA mismatched kidney graft; clone 21 and 40</td>
<td>HLA-DQw6</td>
<td>Vandekerckhove et al (1990) <em>J. Immunol.</em> 144, 1288</td>
</tr>
<tr>
<td>Four $\gamma\delta$ T cell clones isolated from cultures containing the synovial fluid of T cells of rheumatoid arthritis patients that were repeatedly restimulated with AP-MT in the presence of autologous PBMC</td>
<td>HLA-DRw53+ tetanus toxin peptide (1235–1246); also reactive to AP-MT without DR restriction</td>
<td>Holoshits et al (1992) <em>J. Clin. Invest.</em> 89, 308</td>
</tr>
<tr>
<td>$\gamma\delta$ T cells were isolated from blood and clones were established by stimulation with irradiated allogeneic PBMC, EBV-B cell line, PHA and IL2; clone N2A11</td>
<td>HLA-DR7 (+ peptide?)</td>
<td>Jitsukawa et al (1988) <em>Eur. J. Immunol.</em> 18, 1671</td>
</tr>
</tbody>
</table>
1246 (112). These examples of γδ T cell specificity for classical MHC proteins are exceptions. In many cases γδ T cell responses could not be inhibited by antibodies against the classical MHC proteins that serve as restriction elements for αβ T cells. Moreover, large numbers of murine γδ T cell hybridomas failed to recognize classical H–2 proteins (113), and the vast majority of human γδ T cell clones that were activated in limiting dilution cultures were not specific for the HLA proteins of the stimulator cells (114). Clearly, γδ T cells do not have the bias for classical class I or class II MHC proteins that is characteristic for αβ T cells.

**MHC-Like Proteins**

TL region encoded proteins are not only expressed by thymocytes and leukemic T cells but also by epithelial cells in the intestine. The T3b gene of C57BL/6 mice and the T3d and T18d genes of BALB/c mice are highly expressed in the epithelium of the small intestine (115). A T3b product-specific antibody binds to columnar epithelial cells (116) which are in close contact with intestinal γδ T cells (117). Two other TL region genes of C57BL/6 mice, T9b and T21b are also expressed almost exclusively by intestinal epithelial cells (116). The various murine γδ T cell subsets may possibly recognize antigens that are presented by different tissue-specific TL region–encoded proteins (72, 118). However, thus far only a very few murine γδ T cell clones have been shown to recognize TL region–encoded proteins (Table 5). The hybridoma KN6 mentioned previously is specific for the T22b gene product (72, 119). TL region–encoded proteins may serve as antigen-presenting molecules. Indeed, recently Imani et al (120, 121) have been able to show the binding of two peptides to the T23b (Qa–1b) protein and one hybridoma recognizes the T23b protein and one of these peptides, namely the synthetic copolymer Glu: Tyr (122).

The human MHC class I family includes at least 15 loci other than the classical transplantation antigens HLA-A, B, and C (123), many of which may be located telomeric of the HLA-A locus (124). Some of these genes encode class I-like proteins which may be the human equivalents of the murine TL region encoded proteins (125). Recognition of these proteins by γδ T cells has not been described. However, a hint for the recognition of human MHC class I-like proteins was obtained when γδ T cells from peripheral blood were stimulated with a HLA loss variant cell line (126). Cytolytic T cells were generated in this culture that specifically lysed the HLA loss variant cells. The killing was inhibited by anti-γδ TCR antibodies and by antibodies against HLA-B and C even though HLA-B and C proteins were not expressed. A similar inhibition pattern was also shown for a γδ T cell clone, the specificity of which could not be mapped to classical class I MHC genes (127). These findings were interpreted to mean
**Table 5** Recognition of MHC like proteins by γδ T cells

<table>
<thead>
<tr>
<th>Origin of γδ T cells</th>
<th>Specificity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>γδ T cell clones obtained from draining lymph node cells of Balb/c nude mice 7 days after immunization with B10.BR spleen cells in CFA in foot pad; clones FY and G8</td>
<td>TLa&lt;sup&gt;a&lt;/sup&gt; (+ peptide?)</td>
<td>Bluestone et al (1988) <em>J. Exp. Med.</em> 168, 1899</td>
</tr>
<tr>
<td>γδ T cell hybridoma obtained by fusion of CD4&lt;sup&gt;+&lt;/sup&gt; CD8&lt;sup&gt;-&lt;/sup&gt; thymocytes of adult C57BL/6 mice with BW 5417</td>
<td>H-2 T22&lt;sup&gt;b&lt;/sup&gt; (+ peptide?)</td>
<td>Bonneville et al (1989) <em>PNAS</em> 86, 5928</td>
</tr>
<tr>
<td>γδ T cell hybridoma obtained by fusion of BW5417 with draining lymph node cells from DBA/2 mice immunized 7 days previously with the synthetic copolymer Glu:Tyr (GT)</td>
<td>H-2 T23&lt;sup&gt;b&lt;/sup&gt;(Qa-1&lt;sup&gt;b&lt;/sup&gt;, 37)+GT</td>
<td>Itoh et al (1990) <em>Cell</em> 62, 549</td>
</tr>
<tr>
<td>γδ T cell line (IPD2), Vγ9/Vδ1 obtained from immunodeficient patient</td>
<td>CD1c (+ peptide?) lysis of human rhabdomyosarcoma cell line transfected with CD1c cDNA</td>
<td>Vidovic et al (1989) <em>Nature</em> 340, 646</td>
</tr>
<tr>
<td>γδ T cell line (J287), Vγ3 or 4/Vδ1 isolated from blood of healthy donor</td>
<td>CD1c (+ peptide?) (rare specificity)</td>
<td>Kronenberg, pers. commun.</td>
</tr>
<tr>
<td>γδ T cell clones were obtained from PBL of two healthy donors (E and G) stimulated with allogeneic B-LCL</td>
<td>T cell target 1 (TCT.1 = Blast-1 = CD48) (+ peptide?) CD48 is a member of Ig superfamily and is encoded in the same band of chromosome 1 as the CD1 gene cluster</td>
<td>Porcelli et al (1989) <em>Nature</em> 341, 447</td>
</tr>
<tr>
<td>One clone isolated from PBL after stimulation with allogeneic B-LCL and IL-4</td>
<td>Class I like MHC antigen</td>
<td>Faure et al (1990) <em>Eur. J. Immunol.</em> 20, 703</td>
</tr>
<tr>
<td>Two clones isolated from PBL after stimulation with HLA class I loss variants</td>
<td>MHC class I like protein reactive with anti-HLA B and HLA-C antibodies (+ peptide?)</td>
<td>Mami-Chouaib et al (1990) <em>J. Exp. Med.</em> 172, 1071</td>
</tr>
</tbody>
</table>
that the γδ T cells recognized a peptide presented by MHC class I like proteins that crossreact with antibodies against HLA-B and C proteins.

Two human γδ T cell clones were found to recognize CD1c (128, 129), which is encoded by one gene of a cluster of five closely related MHC class I-like genes on chromosome 1 (130). However, the frequency of CD1 protein-specific γδ T cells appears to be very low and in the same order of magnitude as the frequency of CD1 protein-specific αβ T cells (129).

Several human γδ T cell clones recognize a cell surface protein referred to as T cell target antigen 1 (TCP1 or CD48), a member of the Ig superfamily (131–133) encoded by a gene that is located in the same band of chromosome 1 as the CD1 gene cluster (132). CD48 may be an antigen presenting protein or a ligand for a surface protein involved in γδ T cell activation.

We favor the view that γδ T cells recognize peptides and perhaps other small molecules such as carbohydrates in association with nonpolymorphic antigen presenting proteins. So far there are only a few well-documented cases of γδ T cell specificities for such proteins. In contrast αβ T cells specific for allogeneic MHC proteins are readily detectable even in very small αβ T cell population samples. The high alloreactivity of αβ T cells is due to crossreactions of αβ TCRs with a + 1 and b or a + 1 and b + 2, where letters are products of MHC alleles and numbers are presented antigens. Such cross-reactions are not expected for TCRs which recognize nonpolymorphic antigen presenting proteins.

**Mycobacteria and Heat Shock Proteins**

Both murine (Table 6) and human (Table 7) γδ T cells mount strong proliferative responses to killed mycobacteria in the presence of antigen presenting cells. Initially a few γδ T cell lines and clones were obtained from the blood of a BCG immune donor (134), blood or biopsy material from a lepromin skin test of patients with tuberculoid leprosy (135), or synovial fluid of rheumatoid arthritis patients (136). Several of these lines responded to recombinant mycobacterial heat shock proteins. It soon became clear that γδ T cells from healthy donors with negative tuberculin tests and no history of mycobacterial infections also vigorously responded to killed mycobacteria or mycobacterial extracts in the presence of antigen presenting cells and that mycobacterial heat shock proteins were not the major stimulating components (132–139). Every second γδ T cell in the circulating blood of some donors responded to killed mycobacteria, but only a few of the mycobacteria reactive clones also responded to PPD or mycobacterial Hsp65 (137). The major γδ T cell stimulatory components of mycobacteria were found in a small molecular weight fraction (2–10 kd) of extracts, were resistant to proteolytic enzymes, and were shown to
<table>
<thead>
<tr>
<th>Origin of γδ T cells</th>
<th>Specificity</th>
<th>References</th>
</tr>
</thead>
</table>
| B10.A mice were immunized with M.t. in limb; draining lymph node cells were analyzed and restimulated in vitro | *in vivo*: increase in γδ T cells in draining lymph nodes from 1.5% to 10.4% after immunization  
*in vitro*: proliferation and IL-2 production in response to M.t.; not blocked by anti-class II MHC antibodies | Janis et al (1989) *Science* 244, 713 |
| BALB/c mice were immunized with aerosol containing M.t. antigen of tuberculin (PPD) or with CFA at the base of the tail | *in vivo*: increase in γδ T cells in draining lymph nodes  
Rajasekar et al (1990) *PNAS* 87, 1767 |
| Thymocytes of B10 newborn mice were fused with BW 5417 | Autoreactive γδ T cell hybridomas (IL-2 production)  
Additional response to PPD and less well to Hsp65 (BCG) in the presence of spleen cells  
25 of 26 Vγ1/Vδ6 hybridomas react with PPD  
Most PPD reactive hybridomas respond to Hsp65 peptide (180–196)  
No inhibition by anti-class I or II MHC antibodies | O’Brien et al (1989) *Cell* 57, 667 |
| Spleen cells from adult B10 mice were fused with BW5417 | 10–20% of hybridomas respond to Hsp60 M.t. and Hsp60 M.t. peptide (180–196); all Vγ1/Vδ6; extensive junctional diversity of both TCR chains  
All Vγ1/Vδ6 cells are also autoreactive | O’Brien et al (1992) *PNAS* 89: 4348 |
<table>
<thead>
<tr>
<th>Origin of γδ T cells</th>
<th>Specificity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>γδ T cell line (GD) was established from PBL of a BCG immune donor after stimulation with PPD and autologous PBMC</td>
<td>Autologous APC + PPD or + rHsp60 (M.t.)</td>
<td>Hargewoin et al (1989) <em>Nature</em> 340, 309</td>
</tr>
<tr>
<td></td>
<td>Allogeneic APC work less well</td>
<td></td>
</tr>
<tr>
<td>Four γδ T cell clones were isolated from cultures, containing the synovial fluid T cells of rheumatoid arthritis patients that were repeatedly restimulated with AP-MT</td>
<td>Autologous or allogeneic APC + AP-MT or + purified HSP64 (M. bovis)</td>
<td>Holoshitz et al (1989) <em>Nature</em> 339, 226</td>
</tr>
<tr>
<td>PBMC (line 1) or skin biopsy cells (line 2) of patients with tuberculoid leprosy were stimulated with M. leprae cell wall antigen and IL2 in the presence of partially HLA matched allogeneic PBMC as APC; long term γδ T cell lines were established after depletion of γβ T cells</td>
<td>Autologous APC + M. leprae PPD (line 1 and 2) or M. leprae cell wall (line 1 and 2) or rHsp65 (BCG) (line 1)*</td>
<td>Modlin et al (1989) <em>Nature</em> 338, 544</td>
</tr>
<tr>
<td></td>
<td>rHsp18 (M. leprae) (line 1)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tetanus toxin (line 8)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*very weak response</td>
<td></td>
</tr>
<tr>
<td>PBL from donors with negative tuberculin test were stimulated with killed M.t. and PBMC as APC in bulk and limiting dilution cultures</td>
<td>Autologous APC + killed M.t. recognized by 1 in 2 to 23 γδ T cells in the blood; only very few of them recognize PPD or Hsp65</td>
<td>Kabelitz et al (1990) <em>J. Exp. Med.</em> 171, 667</td>
</tr>
<tr>
<td>PBL from healthy donors were stimulated with various preparations from mycobacterial lysates and PBMC as APC in bulk and limiting dilution cultures</td>
<td>Autologous APC + protolytic digest (2–10k) of mycobacterial lysates recognized by 1 in 50 to 100 γδ T cells</td>
<td>Pfeffer et al (1990) <em>Eur. J. Immunol.</em> 20, 1175</td>
</tr>
<tr>
<td>PBL of 22 PPD positive, 2 PPD negative donor and cord blood from 4 neonates were stimulated with killed M.t. and PBMC as APC</td>
<td>Autologous APC + killed M.t.</td>
<td>Panchamoery et al (1991) <em>J. Immunol.</em> 147, 3360</td>
</tr>
<tr>
<td></td>
<td>All responding γδ T cells use Vγ9/Vδ2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vδ2 chains show extensive junctional diversity</td>
<td></td>
</tr>
</tbody>
</table>
| PBMC and pleural fluid cells of patients with tuberculous pleuritis were stimulated with killed M.t. | Autologous APC+ killed M.t.  
All responding γδ T cells use Vγ9/Vδ2  
147, 3353 |
| Large panel of Vγ9/Vδ2 T cell clones from blood | Autologous APC+ killed M.t.  
Recognized by almost all Vγ9/Vδ2 cells  
All M.t. specific clones also recognize MOLT-4 cells, some recognize also Listeria and E. coli  
173, 1311 |
| T cells from peripheral blood of 5 PPD positive and 3 PPD negative healthy donors were cultured with monocytes either infected with live M.t. or pulsed with killed M.t. | Monocytes infected with M.t. induced expansion of γδ T cells from all but one donor  
87, 729 |
| γδ T cells were obtained from peritoneal cavity of mice infected 3 days previously with Listeria monocytogenes | CD4− CD8− γδ T cells produce IFNγ and macrophage chemotactic factor in response to PPD or Hsp65 and irradiated syngeneic spleen cells | Hiromatsu et al (1992) *J. Exp. Med.*  
173, 49 |
| γδ T cells were obtained from the lung of mice primed intranasally with influenza virus and challenged intranasally | γδ T cells obtained 7 days after challenge express mRNA for IL-2, IL-10, IFNγ, IFNβ, GM-CSF; the frequency of cells producing IL-10 was higher in γδ than in αβ T cells | Eichelberger (1991) *J. Immunol.*  
147, 2069 |
| γδ T cell subsets were isolated from intestinal IEL | CD8+ γδ IEL produce either IFNγ or IL-5 or both; stimulation by anti-γδ TCR or anti-CD8 mAb result in enhanced production of these lymphokines | Taguchi et al (1991) *J. Immunol.*  
147, 3736 |
| Splenic and intestinal γδ T cells were stimulated with anti-CD3 mab | Produce IL-2, IL-3, IFNγ, GM-CSF, IFNα, TGFβ (no IL-4) | Bluestone et al (1991) *Immunol. Rev.*  
120, 5 |
bind to some lectins (138, 139). The chemical nature of this material remains to be elucidated. It also remains to be worked out whether the ligand that is recognized by mycobacteria reactive γδ T cells is of mycobacterial origin or whether it is an endogenous ligand that is induced by mycobacterial components in antigen presenting host cells. Havril et al (140) found that γδ T cells from human blood responded not only to monocytes exposed to dead mycobacteria or PPD but even better to monocytes that were infected with live mycobacteria. This interesting observation needs to be confirmed.

The human γδ T cells that respond to mycobacteria all use Vγ9 and Vδ2 chains, both of which exhibit considerable junctional diversity (141–144). This suggests that mycobacteria contain or induce a superantigen for human Vγ9/Vδ2 cells.

In mice γδ T cells accumulated in the draining lymph nodes a few days after immunization with killed mycobacteria in the limb. In vitro these γδ T cells responded to killed mycobacteria with proliferation and IL–2 production (145). The response was not blocked by antibodies against class II MHC proteins of the host (145). As in humans a high frequency of mycobacteria reactive γδ T cells was also found in mice. Thus many hybridomas obtained by fusion of BW5147 thymoma cells with thymocytes from newborn mice or spleen cells from adult mice were found to produce IL–2 in response to purified protein derivative (PPD) from mycobacteria (113, 146). Most PPD reactive hybridomas also responded to spleen cells pulsed with recombinant mycobacterial Hsp65 or peptide 180–196 from mycobacterial Hsp65, and less well to the corresponding peptide of murine Hsp63 that is identical in sequence with the corresponding chinese hamster and human Hsp65 peptides (113, 147). The reactivity of these hybridomas with Hsp is not readily demonstrable because of a very high level of spontaneous IL–2 production. The response to the exogenous antigens can only be seen when the hybridoma cells are cultured at low density and in the presence of spleen cells. The spontaneous production as well as the response to exogenous antigen is inhibitable by antibodies against the γδ TCR and against the vitronectin receptor but not against MHC class I or class II proteins (113, 148, 149). The endogenous ligand that is responsible for the autoreactivity of the V1 population remains to be defined. It may or may not be a Hsp. So far hybridomas were used to study the specificity of the V1 cells. The autoreactivity of the hybridomas is revealed only in the presence of ligands for the vitronectin receptor (VNR). It is not known whether freshly isolated V1 cells are also autoreactive and whether they express VNRs.

All Hsp responsive γδ T cell hybridomas were derived from V1 cells and use Vγ1 and Vδ6 TCR chains, both exhibiting extensive junctional diversity
(146, 150, 151). These findings suggest that mycobacterial Hsp peptide 180–196 is a superantigen for V1 cells. The same peptide is recognized by two rat αβ T cell clones one transferring experimental acute encephalitis (EAE) and one protecting against EAE (152).

Superantigens

Staphylococcal enterotoxin A (SEA) is a superantigen for both αβ T cells and γδ T cells (153–155). SEA coated cells are lysed by all human γδ T cells expressing Vγ9 chains. In contrast to αβ T cells the γδ T cells do not proliferate in vitro in response to SEA coated cells. SEA binds on the T cell site to Vγ9 and Vβ and on the antigen presenting site to a non-polymorphic region of MHC class II proteins.

Many of the γδ T cell stimulating cells or agents such as Daudi cells, Molt4 cells, microbial extracts, or heat shock proteins are also suspected to represent or contain superantigens. This suspicion is based on the finding that the TCRs of the responding cells are composed of δ and/or γ chains that use the same variable region gene segments but exhibit extensive junctional diversity. However, the molecular nature of putative superantigens for γδ T cells and their interactions with presenting molecules, as well as with the γδ TCR, remain to be elucidated.

γδ T-Cell Function

γδ T cells have not been noticed by cellular immunologists in innumerable studies of humoral and cell mediated immune responses. It was the discovery of rearranging genes other than Ig and TCRαβ genes rather than the observation of a new function that led to their discovery. Extensive analysis of these cells over the last several years revealed only a few phenotypic differences from αβ T cells.

First, γδ T cells do share many cell surface proteins with αβ T cells such as CD2, CD3, CD4, CD5, CD7, CD8, CD11b, CD16, CD25, CD28 or CD45, although the frequency of cells expressing a particular protein and the level of expression vary widely not only between αβ T cells and γδ T cells but also between subsets of γδ T cells and γδ T cells of different species (88, 100–102, 156–162). Besides the γδ TCR, a protein named T19 (also referred to as WC1) is the only unique surface protein of γδ T cells known so far. T19 was discovered by Mackay et al at the surface of γδ T cells from sheep (163). Anti-T19 antibodies stain γδ T cells from other ruminants but not from mice or humans. Recently WC1 cDNA was cloned. It encodes a transmembrane protein of 1436 amino acids with a large extracellular domain that contains 11 repeats that are typical for a family of proteins which includes CD5, CD6, the scavenger receptor, and probably additional WC1 like proteins (164).
Second, like αβ T cells, γδ T cells can be stimulated to secrete many lymphokines. The production of different combinations of lymphokines by different γδ T cell clones has been noticed by several authors (Table 8) (39, 90, 165–176). We do not yet know whether functionally distinct γδ T cell subsets analogous to the TH1 and TH2 subsets of the αβ T cell lineage exist.

Third, γδ T cells resemble αβ T cells, NK cells and lymphokine activated killer cells (LAK cells) in that they can lyse target cells (177–179) and express the same granule mediators of cytotoxicity such as perforin and serine esterase 1 and 2 (180–182). Cytolytic activity is upregulated by IL-2 as in the other cytolytic cell types mentioned above. Freshly isolated intestinal γδ T cells from some but not all mouse strains resemble NK cells in that they appear to constitutively express cytolytic activity (180, 183, 184; H. Ishikawa, Y. Li, A. Abeliovich, S. Yamamoto, S. H. E. Kaufmann, S. Tonegawa, submitted).

It is interesting that γδ T cells isolated from murine skin and human blood share with NK cells the expression of Fc-receptors (murine FcyRα and human FcyRIII also named CD16) which mediate antibody dependent cellular cytotoxicity (ADCC) (185, 186). Because the homogeneous TCR on epidermal γδ T cells in mice severely restricts antigen recognition, the FcR on these cells may broaden their scope for antigen recognition via aggregated IgG (185).

Like all other lymphocytes, γδ T cells are under strict control of their antigen receptors. Ligand binding to γδ TCRs leads to transmission of signal 1 by the CD3 complex (177), which appears to have the same subunit composition as the CD3 complex in αβ T cells (186a). One or more additional signals mediated by other T cell surface antigens must accompany signal 1 to induce a response such as high affinity IL-2 receptor expression, proliferation, cytolytic activity, or secretion of lymphokines (187–189). The functional role of the various proteins at the γδ T cell surface remains to be elucidated.

While most mature αβ T cell express either CD4 or CD8, most γδ T cells lack both markers (13, 36, 88, 161, 179). However a few γδ T cells do express either CD8 (36, 88, 89) or CD4 (88, 167, 168, 190, 191), and most intestinal γδ T cells express CD8α homodimers (46, 192). Analysis of human γδ T cell clones indicated that CD4 γδ T cells resemble CD4 αβ T cells in that activated cells produce lymphokines at high levels but express little or no cytolytic activity (167, 168). The reverse is true for CD8 positive αβ and γδ T cells. We conclude that γδ T cells and αβ T cells do use the same “tools.”

γδ T cell research has emerged from molecular studies and remains heavily dominated by molecular studies. At the present time functional
studies in vivo are only beginning to address the putative role of γδ T cells in the defense against infections or in various pathological immune responses. The classical method to define the function of a particular cell type is to see what happens if it is eliminated. Recent studies investigate mice (193) and rats (194) depleted of αβ T cells by treatment from birth on with anti-αβ TCR antibodies. The spleens and lymph nodes of these animals contained normal numbers of γδ T cells. A few αβ T cells expressed the αβ TCR at 5 to 10 times lower levels than αβ T cells from untreated mice. αβ T cell–deficient mice were also obtained from embryonal stem cells (ES cells) in which the α or β TCR locus was disrupted by homologous gene recombination (195, 196). These αβ TCR knock out mice have no αβ T cells at all but normal numbers of γδ T cells. Initial results obtained with αβ T cell–depleted mice and rats and with αβ TCR knock out mice are described in the following sections, together with observations that were made in normal mice and in patients with various immunological disorders.

Role of γδ T-Cells in Humoral Immune Responses

γδ T cells have been found to induce Ig secretion in B cell lines (197) and to induce autoantibody production in blood cells of patients with lupus erythematosus (198). A small fraction of human γδ T cells that express the CD4 marker could provide help for antibody responses in vitro (167, 168). The latter study showed that the γδ TCR was not involved in the interaction with B cells (168). The biological significance of this in vitro observation is questionable. Indeed no antibody responses to T cell–dependent antigens were obtained in αβ T cell–depleted mice and rats (193, 194) nor in αβ TCR knock out mice (196; P. Mombaerts, J. Iacomini, S. Tonegawa, unpublished), while antibody responses to type I and type II T cell–independent antigens were the same as in normal mice. These findings suggest that γδ T cells do not normally function as helper cells for B cells. Whether they can suppress B cell responses remains to be seen.

Role of γδ T-Cells in Graft Rejection

The vigorous rejection of grafts from MHC-mismatched donors is due to the high frequency of αβ T cells that recognize allogeneic MHC proteins. As pointed out above γδ T cell populations do not seem to contain many cells that recognize allogeneic MHC proteins or any other polymorphic proteins. No proliferative response was observed in mixed leukocyte cultures containing responder cells from αβ T cell–depleted mice or rats and stimulator cells from MHC disparate strains (193, 194). Even when exogenous IL–2 was added, no response to the allogeneic stimulator cell was seen. Moreover αβ T cell depleted rats failed to reject skin grafts from MHC-disparate donors (194).
<table>
<thead>
<tr>
<th>Origin of γδ T cell</th>
<th>Species</th>
<th>Lymphokine</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Various T cell subpopulations were obtained from blood and stimulated with PPD or PHA</td>
<td>Human</td>
<td>γδ T cell clones were heterogeneous with regard to lymphokine production, high production of IL-4, IL-5 (clone HD 109), IFNγ (clone IPD2.4) IL-2, IL-4 IFNγ (clone LG.C6) correlation seen only for IL-2 and TBFα Only CD4 γδ T cell clones produce high levels of IL-2 and GM-CSF</td>
<td>Morita et al (1991) <em>Eur. J. Immunol.</em> 21, 2999</td>
</tr>
<tr>
<td>Various γδ T cell clones were established from blood by stimulation with irradiated Jy cells and IL-2 or IL-4</td>
<td>Human</td>
<td>IFNγ and GM-CSF production High by CD4⁺ CD28⁺ CD11b⁻ γδ T cells Low by CD8⁺ CD28⁺⁻ CD11b⁺ γδ T cells</td>
<td>Spits et al (1991) <em>J. Immunol.</em> 147, 1180</td>
</tr>
<tr>
<td>HLA-A2 specific γδ T cell clones obtained stimulation of CD4⁻ CD8⁻ PBL with irradiated Jy cells and IL-2 or IL-4</td>
<td>Human</td>
<td>γδ clones product IL-2, IFNγ, GM-CSF Only some produce IL-4</td>
<td>Spits et al (1990) <em>J. Immunol.</em> 144, 4150</td>
</tr>
<tr>
<td>γδ T cells were purified from blood of patients with schistosomiasis and carcinoma of the urinary bladder</td>
<td>Human</td>
<td>γδ T cells produce high levels of BCGF and BCDF but are deficient in IL-2 production</td>
<td>Raziuddin et al (1992) <em>Eur. J. Immunol.</em> 22, 309</td>
</tr>
<tr>
<td>Freshly isolated blood γδ T cells were stimulated with anti-CD3 or anti-γδ TCR mab</td>
<td>Human</td>
<td>IL-2, TNF, IFNγ production</td>
<td>Mingari et al (1987) <em>Int. J. Cancer</em> 40, 495</td>
</tr>
<tr>
<td>γδ T cell clones were stimulated with anti-CD3 or lectin</td>
<td>Human</td>
<td>IL-2, IL-4, IL-5, TNFα GM-CSF, IFNγ production IL-2 and IL-4 production low or undetectable in most clones</td>
<td>Porcelli et al (1991) <em>Immunol. Rev.</em> 120, 137</td>
</tr>
</tbody>
</table>
| γδ T cells were obtained from early fetal (8–15 weeks) thymi and postnatal thymi (4–6 months) | Human | Early thymic γδ T cells produce IL-4, IL-5, GM-CSF, IFNγ
Late thymic γδ T cells produce GM-CSF and IFNγ | Krangel et al (1990) *J. Exp. Med.* 172, 847 |
| γδ T cell clones were isolated from fetal liver and thymus (14 weeks) | Human | Produce:
- IL-2, IL-4, IFNγ production (clone 12)
| γδ T cells were obtained from peritoneal cavity of mice infected 3 days previously with Listeria monocytogenes | Mouse | CD4^+ CD8^- γδ T cells produce IFNγ and macrophage chemotactic factor in response to PPD or Hsp65 and irradiated syngeneic spleen cells | Hiromatsu et al (1992) *J. Exp. Med.* 173, 49 |
| γδ T cells were obtained from the lung of mice primed intranasally with influenza virus and challenged intranasally | Mouse | γδ T cells obtained 7 days after challenge express mRNA for IL-2, IL-10, IFNγ, IFNβ, GM-CSF; the frequency of cells producing IL-10 was higher in γδ than in αβ T cells | Eichelberger (1991) *J. Immunol.* 147, 2069 |
| γδ T cell subsets were isolated from intestinal IEL | Mouse | CD8^- γδ IEL produce either IFNγ or IL-5 or both; stimulation by anti-γδ TCR or anti-CD8 mab result in enhanced of these lymphokines | Taguchi et al (1991) *J. Immunol.* 147, 3736 |
| Splenic and intestinal γδ T cells were stimulated with anti-CD3 mab | Mouse | IL-2, IL-3, IFNγ, GM-CSF, IFNβ, TGFβ production; no IL-4 production | Bluestone et al (1991) *Immunol. Rev.* 120, 5 |
About one third of all lymphocytes that were isolated from endomyocardial biopsies of human heart allografts more than 1 year after transplantation were \( \gamma \delta \) T cells, while earlier biopsies contained less \( \gamma \delta \) T cells (199). The biopsy derived \( \gamma \delta \) T cells were not specific for the donor cells. They were suspected to downregulate immune responses to allogeneic cells.

\( \gamma \delta \) T cells have also been studied in patients receiving bone marrow or fetal liver and thymus transplants for treatment of immunodeficiency or neoplastic diseases (200–204). The immune system of most reconstituted patients consists of a complex mixture of host and donor cells and often does not function well for many months after transplantation. Some patients had elevated numbers of circulating \( \gamma \delta \) T cells early after the transplantation presumably because \( \gamma \delta \) T cells that were present in the graft expanded in the host (200–204). Antidonor reactivity of \( \gamma \delta \) T cells has been implicated in the poor function of the immune system in some cases (200). In other cases \( \gamma \delta \) T cells from transplanted patients did not show any reactivity to donor or host cells (203).

**Role of \( \gamma \delta \) T-Cells in Infectious Diseases**

In vitro responses of \( \gamma \delta \) T cells to microrganism and microbial compounds and related in vivo observations in humans and mice are summarized in Table 9 (bacteria), Table 10 (viruses), and Table 11 (parasites).

\( \gamma \delta \) T cells accumulate in the draining lymph nodes of mice infected in the footpad with mycobacteria (145), in the lungs of mice infected intranasally with influenza virus (175, 205), in the peritoneal cavity of mice infected with *Listeria monocytogenes* (174, 206), in the hepatic granulomas of Schistosome infected mice (207), and in the skin lesions of patients with the tuberculous form of leprosy or with cutaneous leishmaniasis (135, 208). Elevated numbers of \( \gamma \delta \) T cells have been noticed in the spleens of mice infected with *Trypanosoma cruzi* and *Plasmodium chabaudi* (209), in the blood of patients during the acute and convalescent phases of malaria infections (210, 211). In the acute phase of Epstein Barr virus (EBV) infection, the number of circulating V\( \delta \)2 cells was increased (212) while in vitro EBV transformed cells mainly stimulated V\( \delta \)1 (213). Elevated numbers of circulating \( \gamma \delta \) T cells were in the blood of HIV infected patients with AIDS (214–216). Two of 35 T cell clones obtained from cells in the cerebrospinal fluid of patients with measles virus mediated subacute, sclerosing panencephalitis were \( \gamma \delta \) T cells (217).

Interestingly little evidence suggests that the expansion or accumulation of \( \gamma \delta \) T cell clones in the infected tissues is due to the recognition of microbial antigens. A single \( \gamma \delta \) T cell clone specific for a viral protein was isolated from the draining lymph node of a mouse infected in the footpad.
Table 9  Bacterial infections

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Species</th>
<th>Observations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacteria</td>
<td>Mouse</td>
<td>Increase in number of γδ T cells in draining lymph nodes of mice immunized with M.t. in limb</td>
<td>Janis et al (1989) Science 244, 713</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>γδ T cells accumulate granulomatous skin lesions of patients with leprosy (reversal reaction and positive lepromin skin test) increased number of γδ T cells in tuberculous lymphadenitis</td>
<td>Modlin et al (1989) Nature 338, 544</td>
</tr>
<tr>
<td>Listeria monocytogenes (L.m.)</td>
<td>Mouse</td>
<td>Increased number of γδ T cells in peritoneal cavity of mice 3 days after intraperitoneal infection with L.m. The early appearing γδ T cells proliferate and secrete IFNγ and macrophage chemotactic factor in response to PPD from M.t. or Hsp65 from M. bovis but not to killed Listeria Mice depleted of αβ T cells by mAb treatment show resistance of early stage of infection only Treatment with anti-γδ TCR mAb leads to enhanced L.m. multiplication at an early stage of infection</td>
<td>Falini et al (1989) J. Immunol. 143, 2480</td>
</tr>
<tr>
<td>Various bacteria</td>
<td>Human</td>
<td>γδ T cells from blood of healthy donors Proliferation induced by killed mycobacteria, group A streptococci; staphylococcus aureus or Listeria monocytogenes Killing of antigen pulsed target cells reveals recognition of shared and nonshared microbial components A large number of different bacteria, especially gram negative bacteria induce proliferation of γδ T cells from adult blood or cord blood</td>
<td>Hiromatsu et al (1992) J. Exp. Med. 175, 49</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>Human</td>
<td>All Vγ9 expressing cells lyse target cells pulsed with staphylococcal enterotoxin (SEA)</td>
<td>Kaufmann et al pers. commun.</td>
</tr>
<tr>
<td>Virus</td>
<td>Species</td>
<td>Observations</td>
<td>References</td>
</tr>
<tr>
<td>------------------------</td>
<td>---------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------------------------------</td>
</tr>
</tbody>
</table>
| Influenza virus        | Mice    | Number of γδ T cells is increased in lung lavage cells 7 to 10 days after intranasal infection  
γδ T cells may provide protective cover of the lung during the time that tissue repair is proceeding through the secretion of  
cytokines (IFNγ, IFNβ, GM-CSF) in response to hsp expressing macrophages  
Alternatively γδ T cells are passively recruited from blood and play no active part in the disease process | Carding et al (1990) *J. Exp. Med.* 172, 1225  
| Herpes simplex virus (HSV-1) | Mice    | A γδ T cell clone was isolated from the draining lymph node of a mouse infected with HSV-1 in the foot pad; biological significance? | Johnson et al (1992) *J. Immunol.* 148, 983 |
| Epstein Barr virus (EBV) | Human  | Elevated number of Vγ9/Vδ2 cells in blood in acute phase of EBV infection  
Numbers remain high for 4 weeks  
| Human immunodeficiency virus (HIV) | Human  | Increased numbers of Vδ1 cells in blood of some HIV infected patients; increase is most marked in patients with AIDS  
Vδ1 increase may be due to diminished retention within the thymus that is damaged by HIV infection or to stimulation by activated autologous B cells | de Paoli et al (1991) *Clin. Exp. Immunol.* 83, 187  
| Measles virus          | Human   | Subacute sclerosing panencephalitis (SSPE) is due to an immune response against measles virus in the central nervous system; 2 of 35 T cell clones from cerebrospinal fluid of a patient with SSPE were γδ T cells; no proliferative response to measles virus infected cells was obtained in vitro | Ang et al (1987) *J. Exp. Med.* 165, 1453 |
Table 11  Parasitic infections

<table>
<thead>
<tr>
<th>Parasites</th>
<th>Species</th>
<th>Observations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Plasmodium falciparum</em></td>
<td>Human</td>
<td>Increase in number of γδ T cells in blood during acute and convalescent phases of malaria infections</td>
<td>Roussilhon et al (1990) <em>J. Inf. Dis.</em> 162, 283</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>Outgrowth of Vγ9 cells from peripheral blood stimulated in vitro with merozoites, schizont lysate or whole parasitized red blood cells</td>
<td>Ho et al (1990) <em>Immunol. Letters</em> 25, 139</td>
</tr>
<tr>
<td>Cutaneous Leishmaniasis</td>
<td>Human</td>
<td>Accumulation of γδ T cells in granulomatous skin lesions</td>
<td>Behr &amp; Dubois (1992) <em>Int. Immunol.</em> 4, 361</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>In hepatic granulomas of schistosome infected mice activated γδ T cells are present and express high levels of IgA and IgM FcR and low levels of IgG FcR; ADCC by γδ T cells?</td>
<td>Raziuddin et al (1992) <em>Eur. J. Immunol.</em> 22, 309</td>
</tr>
</tbody>
</table>
with *Herpes simplex* virus (218). Recently Flynn found γδ T cells specific for various trypanosome proteins in the blood of cattle following infection with *trypanosoma congoense*. Interestingly such responses were observed in West African N'Dama cattle which recover from trypanosoma infections but not in Boran cattle which succumbed to the infection (N. Flynn, personal communication).

We are not aware of any other case in which γδ T cells were unequivocally shown to recognize and respond to defined microbial antigens in infected humans or animals. The γδ T cells in the lungs of mice infected with influenza virus appear to recognize host cell components rather than viral antigens (175). Similarly the γδ T cells that accumulate in the peritoneal cavity of mice 3 days after infection with *Listeria monocytogenes* proliferate and secrete IFNγ and macrophage chemotactic factor in response to putative mycobacterial superantigens but not to listerial antigens (174). Mice depleted of αβ T cells by mab treatment show resistance to the infection by *Listeria monocytogenes* within the first few days after infection but cannot control the infection later (S. H. Kaufmann, P. Mombaerts, unpublished observation). This finding is consistent with the well-established protective role of *Listeria monocytogenes*-specific αβ T cells. γδ T cells appear to have a crucial function early after infection at a time when there is no protection by specific αβ T cells. An exaggerated multiplication of Listeria microorganisms is seen in normal mice and αβ TCR knock out mice that were treated with anti-γδ TCR antibodies (S. H. Kaufmann, P. Mombaerts, unpublished observation). These findings are the first indication for complementary functions of αβ and γδ T cells. The protection by γδ T cells is not Listeria specific. It is fast but incomplete. For survival αβ T cells must recognize and respond to listerial antigens and mount an immune response that eliminates the pathogen. Recently similar observations were made in a mouse model of malaria (R. Nussenzweig, personal communication).

**Immune Surveillance Against Cancer by γδ T-Cells**

γδ T cells have been suspected to have a surveillance function against tumors. The following observations (summarized in Table 12) are cited to support this idea:

First, IL–2 activated γδ T cells can kill many different tumor cells. The molecular basis of the distinction between normal cells and tumor cells by the lymphokine activated γδ T cell cells is not known.

Second, a subset of human γδ T cells appears to recognize a superantigen on the surface of some Burkitt lymphoma cells (219–223). Another subset recognizes Epstein Barr virus transformed B cells (213).

Third, some γδ T cells that were isolated from patients with Burkitt
lymphomas or acute lymphoblastic leukemias (ALL) in complete remission were shown specifically to recognize autologous tumor cells (224).

Fourth, γδ T cell lines could be established from populations of tumor infiltrating lymphocytes (TIL) (225–227). The γδ TIL isolated from lung tumors lysed autologous tumor cells, and this lysis was inhibited by anti-class I MHC antibodies (227). No evidence for specific lytic activity was obtained with γδ TIL that were isolated from Wilms tumors, melanomas, and sarcomas (225). These observations are interesting but far from being convincing evidence for a role of γδ T cells in the defense against tumors.

**Pathological Immune Responses by γδ T-Cell**

γδ T cells have been suspected to contribute to pathological immune responses in several different diseases (Table 13). The synovial fluid from patients with rheumatoid arthritis was found by many investigators to contain γδ T cells of the V1 subset that represents a minor proportion of circulating γδ T cells in most healthy individuals (136, 228–238). Vδ1 cells also accumulate in the intestinal lesions of patients with coeliac disease (239–243) and were found increased in the blood of a patient with type I autoimmune polyglandular syndrome that was associated with aplastic anemia (244). Vδ2 cells were found in 10 of 10 brain autopsies from multiple sclerosis patients (238) and the number of Vδ2 cells was elevated in the blood of a patient with atopic dermatitis (245).

The analysis of junctional regions of TCR chains from γδ T cells of patients with rheumatoid arthritis (232, 236–238), multiple sclerosis (242) or coeliac disease (243) did not reveal any extensive expansion of particular γδ T cell clones. However large γδ T cell clones were found in the blood and the bronchoalveolar lavage of some patients with pulmonary sarcoidosis (246, 247).

These reports are interesting but do not document a pathogenic or protective role of γδ T cells.

**γδ T-Cells in Immunodeficiency Diseases**

Brenner et al were the first to identify peripheral blood T cells which were CD3 positive but αβ TCR negative (8). While the initial attempts to expand these cells from the blood of normal donors failed, since the cultures were quickly overgrown by αβ T cells, these investigators succeeded in obtaining two CD3⁺, αβ TCR⁻ lines from one patient with bare lymphocyte syndrome (IDP1) and one patient with ectodermal dysplasia syndrome (IDP2) respectively. These were the first cells from which CD3 cross-linked γδ TCRs were precipitated (8). The two TCR chains of both lines were non-disulphide linked. Later several additional lines and clones expressing either non-disulfide linked or disulfide linked γδ TCRs were obtained from
<table>
<thead>
<tr>
<th>Cancer</th>
<th>Species</th>
<th>Observations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cell lymphoma</td>
<td>Mouse</td>
<td>$\gamma\delta$ T cells proliferate in response to CD5 positive B cell lymphomas and induce Ig secretion</td>
<td>Sperling &amp; Wortis (1989) <em>Int. Immunol.</em> 1, 434</td>
</tr>
<tr>
<td>Burkitt's lymphoma and EBV transformed B-LCL</td>
<td>Human</td>
<td>$\gamma\delta$ T cells from children with Burkitt lymphoma lyse autologous tumor cells; the $\gamma\delta$ T cell tumor cell interaction involves the $\gamma\delta$ TCR and surface Ig of the tumor</td>
<td>Wright et al (1989) <em>J. Exp. Med.</em> 169, 1557</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Daudi Burkitt lymphoma cells induce selective growth of V$<em>{\gamma9}/V</em>\delta2$ T cells; EBV transformed B-LCL induce growth of V$_{\gamma1}$ T cells</td>
<td>Hacker et al (1992) <em>EJI. Submitted</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Many of 356 $\gamma\delta$ T cell clones lyse Daudi but not Raji Burkitt lymphoma cells while NK cell lyse both targets</td>
<td>Fisch et al (1990) <em>J. Exp. Med.</em> 171, 1567</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All $\gamma\delta$ T cell clones which lyse Daudi cells express V$<em>{\gamma9}$ V$</em>\delta2$ TCR; the V$_\delta2$ chains show extensive juncional diversity</td>
<td>Sturm et al (1990) <em>J. Immunol.</em> 145, 3202</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Freshly isolated V$<em>{\gamma9}/V</em>\delta2$ T cells from blood proliferate in response to Daudi cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lysis of Daudi cells by $\gamma\delta$ T cells is inhibited by a rabbit antiserum against mammalian Hsp50</td>
<td>Fisch et al (1990) <em>Science</em> 250, 1269</td>
</tr>
<tr>
<td>Condition</td>
<td>Species</td>
<td>Description</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Lung carcinoma</td>
<td>Human</td>
<td>The majority of freshly isolated tumor infiltrating lymphocytes from 2 patients were γδ T cells; 100% and 40% respectively were Vδ1⁺; the γδ T cells lysed autologous tumor cells; lysis was inhibited by anti-class I MHC antibodies</td>
<td>Fisch et al (1990) <em>J. Immunol.</em> 148, 2315</td>
</tr>
<tr>
<td>Langerhans cell histiocytosis</td>
<td>Human</td>
<td>γδ T cells are increased in number in the dermal and epidermal infiltrate in close association with Langerhans cells</td>
<td>Alaibac (1992) <em>Int. J. Dermatol.</em> 31, 157</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia (ALL)</td>
<td>Human</td>
<td>84 γδ T cell clones were isolated from blood of 3 ALL patients after complete remission was achieved; all clones express cytolytic activity but only 10 clones lysed autologous leukemia cells</td>
<td>Bensussan et al (1989) <em>Blood</em> 73, 2077</td>
</tr>
<tr>
<td>Disease</td>
<td>Species</td>
<td>Observations</td>
<td>References</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Rheumatoid arthritis (RA) | Human   | 10 of 202 T cell clones from synovial fluid of 4 juvenile arthritis patients are probably γδ T cells  
γδ T cells are enriched in synovial fluid as compared to blood in some patients with RA  
Coordinate expansion of γδ T cells and CD5+ B cells in blood of patients with RA and primary Sjogren’s syndrome  
4 γδ T cell clones isolated from synovial fluid of a patient with RA respond to acetone precipitable fraction of M.t.  
γδ T cells in synovial fluid of RA patients preferentially use Vδ1  
Vδ1 chains have diverse junctional sequences  
Vδ1+, CD69+, CD25+ γδ T cells in synovial compartment of 6 juvenile RA patients  
Lower numbers of γδ T cells in blood of RA patients  
No consistent increase of γδ T cells in synovial fluid or tissues of RA patients  
Increased number of γδ T cells in synovial fluid of RA patients show extensive junctional diversity except in one patient  
| Multiple sclerosis (MS)   | Human   | Identification of γδ T cells in MS brain lesions by immunohistochemical techniques  
Evidence for clonal expansion of γδ T cells in MS brain lesions γ and δ transcripts were found in 12 of 12 MS brains but only in 1 of 10 control brains junctional diversity of Vδ2 chains was limited |                                                                                                                                 |
<table>
<thead>
<tr>
<th>Condition</th>
<th>Cell Type</th>
<th>Description</th>
<th>Reference(s)</th>
</tr>
</thead>
</table>
| Coeliac disease        | Human     | Elevated number of $\gamma\delta$ T cells ($V\gamma1^+$) in intestinal epithelia of patients with coeliac disease | Spencer et al (1989) *Eur. J. Immunol.* 19, 1335  
| Kikuchi's lymphadenitis| Human     | Hyperimmune response to unknown antigen massive $\gamma\delta$ T cell infiltrates | Falini et al (1989) *J. Immunol.* 143, 2480 |
| Lupus nephritis        | Human     | 7 of 59 autoantibody inducing TH lines obtained from blood were $\gamma\delta$ T cells | Rajagopalan et al (1990) *PNAS* 87, 7020 |
| Discoid chronic lupus erythematosus | Human | $\gamma\delta$ T cells accumulate in dermis and basal keratinocyte layer of epidermis, frequently surrounding damaged keratinocytes | Platzer et al (1990) 20th Annual Meetg. Eur. Society of Dermatological Research |
| Type I Autoimmune polyglandular syndrome | Human | Increase in $V\delta1^+$ cells in blood associated with aplastic anemia | Hara et al (1990) *Blood* 75, 941 |
| Pulmonary sarcoidosis  | Human     | 7 of 20 patients have elevated $\gamma\delta$ T cells in blood and bronchoalveolar lavage from lower respiratory tract  
patients with different primary immunodeficiency diseases such as partial DiGeorge syndrome, common variable immunodeficiency (248), or Wiskott Aldrich syndrome (249). The studies published so far on γδ T cells from patients with primary immunodeficiency syndromes are rather fragmentary, however.

Elevated numbers of γδ T cell were also found in the blood of patients with Down's syndrome who are more prone to autoimmune diseases, have a greatly increased susceptibility to viral and bacterial infections, and a 10- to 20-fold higher incidence of childhood leukemia (250). At least 5 of 10 patients with ataxia-teleangiectasia had an increased ratio of γδ T cells to αβ T cells. This finding is thought to reflect both a recombinational defect that interferes with Ig and TCR gene rearrangements and an inability to repair damage to the DNA (251).

CONCLUDING REMARKS

We do believe that γδ T cells are as important for defense against microbes as B cells and αβ T cells. There are many reasons why their function has not yet been recognized. One is certainly the preconception about T cells that is derived from our knowledge of αβ T cells. Most investigators searching for γδ T cell functions designed their experiments as if γδ T cells were just other αβ T cells. It was assumed that γδ T cells recognize peptides presented by MHC or MHC-like proteins on the surface of macrophages or dendritic cells. Indeed, spleen cells appear to be appropriate antigen presenting cells, MHC and MHC like proteins are recognized at least by some γδ T cells, and γδ T cells responded to antigen recognition with proliferation, lymphokine production, expression of cytolytic activity or anergy—just like αβ T cells. We do suspect that the observed responses were either mediated by rare γδ T cells that are not representative for entire γδ T cell subsets, or the responses were directed to superantigens that activate large fractions of cells irrespective of the junctional diversity of their receptors. The great diversity of γδ T cells must be crucial for their function. Physiologically relevant responses of γδ T cells may be directed to peptides with posttranslational modifications that occur only in microbes or even to nonpeptidic antigens such as carbohydrates that are presented by novel antigen presenting proteins which coevolved with Vγ and Vδ gene segments. We suspect that there is a diverse set of antigen presenting cells that utilize different antigen presenting proteins in different tissues. This possibility has not been explored extensively. The analysis of these cells and of their mode of antigen presentation may be the key to the understanding of the unique functions of γδ T cells.
ACKNOWLEDGMENTS

For helpful suggestions and/or for providing unpublished data, we thank C. Bernard, R. L. H. Bohluis, M. Bonneville, J. Cihak, J. E. Coligan, G. De Libero, T. Hünig, J. Jacomini, F. Imani, C. Janeway, Jr., D. Kabelitz, K. A. Kelly, M. Kronenberg, B. Kyewski, C. Mackay, R. L. O'Brien, T. H. Rabbits, R. Rajasekar, K. Shortman, H. Spits, E. Sturmi, and J. E. de Vries. W. Haas is supported by Hoffman La Roche, P. Pereira by the Leukemia Society of America, and S. Tonegawa by Howard Hughes Medical Institute and grants from NIH.

Literature Cited

15. Allison, J. P., Havran, W. L. 1991. The immunobiology of T cells with in-


42. Mackay, C. R., Heim, W. R. 1989. A large proportion of bovine T cells express the γδ T cell receptor and show a distinct tissue distribution and surface phenotype. Int. Immunol. 1: 540–45


86. Hirt, W., Saalmuller, A., Reddehase,


\(\gamma \delta\) expressing T cell lines recognize MHC-controlled elements on auto-
logous EBV-LCL that are HLA-A, B, -C, -DR, -DQ, or -DP. J. Immunol.
145: 36-45

\(\gamma \delta\) cytotoxic T lymphocyte clones involves secretion of Na-benzylxoyacetoxycarbonyl-L-
llysine thiobenzyl ester serine esterase and influx of Ca\(^{2+}\) ions. J. Immunol.
143: 1506-11

128. Porcelli, S., Brenner, M. B., Green-
341: 447-50

129. Faure, F., Jitsukawa, S., Miossec, C., Harnois, M., Hercend, T. 1990. CD1c as a target recognition structure for human T lymphocytes: analysis with peripheral blood 
\(\gamma \delta\) cells. Eur. J. Immunol. 20: 703-6


major histocompatibility complex restricted T lymphocytes. J. Exp. Med.
172: 1071-82

132. Del Porto, P., Mami-Chouaib, F., Bru-
neau, J. M., Jitsukawa, S., Dumas, J., Harnois, M., Hercend, T. 1991. TCT.1, a target molecule for 
\(\gamma \delta\) T cells is encoded by an immunoglobulin superfami-
ly gene (Blast-1) located in the CD1 region of human chromosome 1.
J. Exp. Med. 173: 1339-44

133. Mami-Chouaib, F., Del Porto, P., Delorme, D., Hercend, T. 1991. Further evidence for a \(\gamma \delta\) T cell receptor-

134. Haregewoin, A., Soman, G., Hom, R. C., Finberg, R. W. 1989. Human \(\gamma \delta\) T cells respond to mycobacterial heat-

1989. Lymphocytes bearing antigen-
specific \(\gamma \delta\) T cell receptors accumulate in human infectious disease lesions. Nature
338: 544-48

136. Holoshitz, J., Koning, F., Coligan, J. E., De Bruyn, J., Strober, S. 1989. Isolation of CD4\(^-\)CD8\(^-\) mycobacteria-
reactive T lymphocyte clones from rheumatoid arthritis synovial fluid.
Nature 339: 226-29

137. Kabelitz, D., Bender, A., Schon-
delmaier, S., Schoel, B., Kaufmann, S. H. E. 1990. A large fraction of human
peripheral blood \(\gamma \delta\) T cells is activated by Mycobacterium tuberculosis but not by its 65-kD heat shock protein. J. Exp.
Med. 171: 667-79

mary responses of human T cells to mycobacteria: a frequent set of \(\gamma \delta\) T
20: 1175-79

protease-resistant mycobacterial ligand specifically activates Vg9\(^+\) human \(\gamma \delta\) T

140. Havlir, D. V., Ellner, J. J., Chevrenak,
K. A., Boom, W. H. 1991. Selective expansion of human \(\gamma \delta\) T cells by mono-
87: 729-33

173: 1331-38

predominance of the T cell receptor V\(\gamma\)2/V\(\delta\)2 subset in human mycobacteria-
147: 3360-69

143. Ohmen, J. D., Barnes, P. F., Uyemura,
\(\gamma \delta\) T cells reactive to Mycobacterium tuberculosis are encoded by specific V
147: 3353-59

144. De Libero, G., Casorati, G., Giachino,
C., Carbonara, C., Migone, N., Mat-
zinger, P., Lanavecchia, A. 1991. Selection by two powerful antigens may account for the presence of the major population of human peripheral 
\(\gamma \delta\) T cells. J. Exp. Med. 173: 1311-22


186. van de Griend, R. J., Tax, J. W. J. M., van Krimpen, B. A., Vreudenhil, R.


203. Bacchetta, R., Vandekerckhove, B. A. E., Touraine, J. L., Bigler, M.,


222. Fisch, P., Malkovsky, M., Kovats, S., Sturm, E., Braakman, E., Klein, B. S., Voss, S. D., Morrissey, L. W., DeMars, W., Welch, W. J., Bolhuis, R. L. H.,
Sondal, P. M. 1990. Recognition by human V_{y9}^{+}/\delta8^{+} T-cells of a Gro1 homolog on Daudi Burkitt's lymphoma cells. Science 250: 1269–73


Alaia, M. 1992. \(\gamma\delta\) T-lymphocytes: Relevance of the current studies to dermatology. Int. J. Dermatol. 31: 157–59


De Maria, A., Malnati, M., Moretta, A., Pende, D., Bottino, C., Casorati, G., Cottafava, F., Melioli, G., Mingari, M. C., Migone, N., Romagnani, S., Moretta, L. 1987. CD3\(^+\)4\(^-\)8\(^-\) WT31 \(\gamma\delta\) T-cell receptor \(\gamma\delta\) cells and other unusual phenotypes are frequently detected among spontaneously interleukin 2-responsive 2-responsive T lymphocytes present in the joint fluid in juvenile rheumatoid arthritis. A clonal analysis. Eur. J. Immunol. 17: 1815–19


Trejos-Siewacz, L. K., Calabrese, A., Smart, C. J., Oakes, J. J., Howdle, P. D., Crabtree, J. E., Losowsky, M. S., Lancaster, F., Boylston, A. W. 1991. \(\gamma\delta\) T cell receptor-positive cells of the human gastrointestinal mucosa: occurrence and V region gene expression in Helicobacter pylori-associated gastritis,


