Development and selection of γδ T cells

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The γδ T-cell population, a subpopulation of T cells formed through cell lineages that are independent of the αβ T-cell lineage, consists of multiple subsets with distinct receptor repertoires and homing properties. While the cell sublineage is a critical factor in the determination of homing specificity, both cell sublineage and receptor-dependent selection are instrumental in the determination of the functional repertoire.

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Introduction

The search for the genes encoding the antigen receptor of T cells not only revealed genes encoding T-cell receptor (TCR) α and β chains but also led to the identification of a third set of TCR genes, γ [1]. This, in turn, led to the discovery of a second TCR, γδ TCR, and a novel class of T cells, γδ T cells [2]. This review covers recent progress in several areas of γδ T-cell development and selection with major emphasis on the mouse system. The human system is also discussed, but less extensively.

Segregation of γδ and αβ T-cell lineages

One interesting issue raised by the discovery of γδ T cells is their cell lineage relationship with αβ T cells. During thymic ontogeny, rearrangement and cell surface expression of γ and δ chains precedes that of α and β chains [3,4]. In peripheral αβ T cells, some γ genes are often rearranged in a non-productive (i.e. out-of-frame) form [5]. In contrast, in γδ thymocytes or peripheral γδ T cells, β genes almost always occur in an incompletely rearranged DJ form (D, diversity; J, joining), while α genes are not rearranged. In addition, cells bearing both TCR αβ and γδ or ‘hybrid’ TCRs, such as βδ or αγ heterodimers, have not been detected in normal animals [4,6,7]. These observations leave little doubt that the γδ and γδ T-cell lineages are segregated from common progenitor cells prior to the expression of either TCR on the cell surface.

What, then, is the critical event that determines cell lineage segregation? Initially, it seemed that γ and δ gene rearrangement was the primary determinant [4], if the γ and δ gene rearrangements are both productive, then cell-surface expression of γδ TCR occurs, and by analogy with the immunoglobulin system, this inhibits further rearrangement of any other TCR genes. αβ T cells would thus be generated only from those cells that failed to productively rearrange both γ and δ genes. This model, however, was not supported by transgenic mouse experiments. First, in γδ TCR transgenic mice (K/N6 Tg mice) constructed with cosmids clones containing functionally rearranged γ and β genes, nearly all γδ T cells expressed the transgene-encoded γδ TCR and normal numbers of αβ T cells were present both in the thymus and in the peripheral lymphoid organs of adult mice [8]. These αβ T cells retained the apparently normal transgenes γ and δ but their RNA transcripts were undetectable. This transcriptional repression or ‘silencing’ of the transgenic γ gene in αβ T cells corroborates reports of the silencing of rearranged (both in-frame and out-of-frame) γ genes in the αβ T cells of normal mice [9], although the Vβ2γ2 γ gene, the protein product of which is rarely expressed on the cell surface, is an exception to this rule [9]. This αβ T-cell-specific silencing of Cγ1 associated γ genes is mediated by two DNA elements flanking the enhancer element associated with this gene, and by multiple proteins which interact with these DNA elements (S Tonegawa, unpublished data).

The function of αβ T-cell-specific silencing of γ genes in the segregation of αβ and γδ T-cell lineages was strongly suggested by a second set of γ and δ double transgenic mice in which the downstream silencer element was deleted from the transgene construct [10]. In these transgenic mice, the generation of αβ T cells was severely retarded, in contrast to the first set of transgenic mice with the complete γ gene-associated silencers. Thus, it has been proposed (Fig. 1) that independently of the rearrangement of TCR genes, progenitor cells are split into two cell lineages: in one the γ silencer machinery remains inactive while in the other it is activated. The γ and δ gene (and perhaps Dγ to Jγ) rearrangements occur in both lineages of cells but only the γ

Abbreviations

D—diversity; IL—interleukin; J—joining; mAb—monoclonal antibody; MHC—major histocompatibility complex;

PCR—polymerase chain reaction; TCR—T-cell receptor; TL—T leukemia; V—variable.

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lineage with inactive silencer machinery has the potential to generate γδ T cells. The surface expression of γδ TCRs prevents, by a feedback mechanism, the completion of β and the initiation of α gene rearrangements, respectively. In the other cell lineage, γδ TCRs will not be expressed on the surface because γ gene expression is blocked at the transcriptional level, and hence the cells may proceed to rearrange Vδ to Dδ and, eventually Vδ to Jδ. γβ T cells are generated from this subpopulation upon productive rearrangements of both α and β genes. It is expected that further analysis of the γ gene silencer machinery will help to explain the molecular events underlying the lineage segregation.

It was proposed that a similar silencing element associated with the α gene also affected T-cell development [11]. However, it is unlikely that this silencing element directly affects the segregation of the αβ and γδ T-cell lineages because α gene rearrangement is delayed and therefore there is no need to silence the α gene rearrangement at this point of development. It is more likely that the α gene silencer regulates α gene rearrangements relative to β gene rearrangements after the lineage determination is made.

According to another model of αβ T-cell lineage segregation, deletion of the δ locus by a novel recombination determines the commitment to the αβ T-cell lineage [12]. Indeed, there is evidence of deletions in some pro-thymocytes along the αβ T-cell lineage [13**]. However, this model is not compatible with the normal development of αβ T cells that carry rearranged δ transgenes (see above). It may be that multiple mechanisms, including both the γ gene silencing and the δ locus deletion, ensure that each lineage expresses only one type of TCR.

**γδ T-cell subsets**

γδ T cells are not only present in the blood and lymphoid organs such as spleen and lymph nodes but are also disseminated in various tissues, mainly in close association with the epithelial layers that cover the internal and external surface of the body. The γδ cell populations at various locations represent different γδ T-cell subsets. They express different TCR repertoires, ranging from monospecific to highly diversified, and differ with regard to their appearance in ontogeny and to thymus dependence. Subset-specific surface markers are not yet available and no clear differences have yet been described in functional properties such as cytolytic activity and lymphokine production.

The two γδ T-cell subsets that appear first in ontogeny are most unusual in that their TCRs show no diversity [14,15] even though they are encoded by rearranged γ and δ genes. One (the Vγ5 subset) is disseminated in the epithelium [14] and the other (the Vγ6 subset) in the mucosal epithelia of the uterus, vagina and tongue [15]. The Vγ5 and the Vγ6 subsets appear in two consecutive waves in the fetal and perinatal thymus, respectively [16–18]. A highly diversified γδ T-cell subset (the Vγ4 subset) is generated later, in the neonatal and adult thymus [17,19], and exported to the blood and lymphoid organs [20**]. This subset expresses predominantly Vγ4 and multiple Vδ chains and includes a population of self-reactive cells which express Vγ1 and Vγ6 chains. A fourth γδ T cell lineage (the Vγ7 subset) is thymus-independent [21**,22,23**,24], resides mainly in the epithelial layers of the intestine and expresses Lyt2 (CD8α) homodimers [23**] and predominantly the Vγ7 chain and Vγ4, Vγ5, Vγ6, and Vγ7 chains which exhibit extensive junctional diversity [24–27].

Table 1 lists these and other γδ T-cell populations, including three which cannot readily be assigned to one or the other subset. The γδ T cells in the liver probably belong to the intestinal lineage since they express CD8 homodimers [28**]. However, they preferentially express Vγ1 and Vγ4 rather than Vγ7, which predominates in intestinal γδ T cells. The γδ cells that have been isolated from the lung [29] and the lactating mammary gland [30] express multiple Vγ and Vδ chains and exhibit extensive junctional diversity. At least some γδ T cells in the lung are thymus-independent (see below). It is conceivable that the blood and some organs such as lymph nodes, spleen, lung or mammary gland contain γδ cells of different sublineages in different proportions.

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**Fig. 1.** Silencer model of T-cell lineage segregation. The superscript designations are as follows: o, germline configuration; †, in-frame joining; a, any configuration; p, partial rearrangement. See text for further details.
Table 1. Sublineages of γδ T cells.

<table>
<thead>
<tr>
<th>Sublineage</th>
<th>Location</th>
<th>TCR usage</th>
<th>Diversity</th>
<th>Characteristics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vγ5</td>
<td>Skin</td>
<td>γ: V5JIC1</td>
<td>None</td>
<td>Generated in 14–17-day-old fetal thymus</td>
<td>[16–18,41]</td>
</tr>
<tr>
<td>DEC</td>
<td>(mAb 563)</td>
<td>δ: V1D2J1C</td>
<td></td>
<td>Generation depends on fetal progenitors and fetal thymus</td>
<td>[31,32]</td>
</tr>
<tr>
<td>iEL</td>
<td></td>
<td></td>
<td></td>
<td>Positive selection of monospecific cells in thymus</td>
<td>[41,42]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Homing to and maintenance in epidermis</td>
<td>[14,16]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Recognizes cultured keratinocytes</td>
<td>[44••]</td>
</tr>
<tr>
<td>Vγ6</td>
<td>Vagina, uterus, tongue</td>
<td>γ: V6JIC1</td>
<td>None</td>
<td>Generated in late fetal and newborn thymus</td>
<td>[18]</td>
</tr>
<tr>
<td>vut-EL</td>
<td>δ: V1D2J1C</td>
<td></td>
<td></td>
<td>Positive selection of monospecific cells in thymus</td>
<td>[41,42]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Homing to and maintenance in mucosal epithelia (vagina, uterus, tongue)</td>
<td>[15]</td>
</tr>
<tr>
<td>Vγ4</td>
<td>Blood, lymph nodes, spleen</td>
<td>γ: V4JIC1</td>
<td>High</td>
<td>Generated in postnatal thymus</td>
<td>[17,19,20•]</td>
</tr>
<tr>
<td></td>
<td>δ: V2,5,6,7</td>
<td></td>
<td></td>
<td>Major γδ cell population in adult thymus, lymph nodes and spleen</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>γ: V1JIC4</td>
<td>High</td>
<td>Appear in spleen 1–2 weeks after birth</td>
<td>[20•]</td>
</tr>
<tr>
<td></td>
<td>δ: V6</td>
<td></td>
<td></td>
<td>Respond to PPD, mycobacterial Hsp65 and self Hsp65; many cells recognize residues 180–196 of Hsp65</td>
<td>[65,66]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lack of isotype exclusion in some cells</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anti-self response depends on vitronectin receptor</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Various effects on hemopoiesis in transgenic mice</td>
<td>[65]</td>
</tr>
<tr>
<td>Vγ7</td>
<td>Intestine</td>
<td>γ: V7JIC1</td>
<td>High</td>
<td>Predominant expression of Vγ7</td>
<td>[25]</td>
</tr>
<tr>
<td>i-EL</td>
<td>δ: V2,5,6,7</td>
<td></td>
<td></td>
<td>Highly diversified</td>
<td>[25–27]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thymus independent</td>
<td>[21,22,23**24]</td>
</tr>
<tr>
<td>Lung</td>
<td>γ: V4</td>
<td>High</td>
<td>8–20% of resident pulmonary lymphocytes are γδ T-cells</td>
<td>[29]</td>
<td></td>
</tr>
<tr>
<td>δ: V6</td>
<td></td>
<td></td>
<td></td>
<td>Number increases with age</td>
<td>[28•]</td>
</tr>
<tr>
<td>Liver</td>
<td>γ: V1,2</td>
<td>High</td>
<td></td>
<td>Express CD8α homodimers</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>δ: V6</td>
<td></td>
<td></td>
<td>Fourfold increase of number in lactating mammary gland</td>
<td></td>
</tr>
<tr>
<td>Mammary gland</td>
<td>γ: V4,5</td>
<td>High</td>
<td></td>
<td>Predominant in thymus</td>
<td>[56••,57,69]</td>
</tr>
<tr>
<td></td>
<td>δ: V4</td>
<td></td>
<td></td>
<td>Rare in blood</td>
<td></td>
</tr>
<tr>
<td>Human Vγ1Vγ6</td>
<td>Thymus</td>
<td>γ: V1C2</td>
<td>High</td>
<td>Proportion in blood decreases with age</td>
<td>[56••,57,69]</td>
</tr>
<tr>
<td></td>
<td>no S-S bridge</td>
<td></td>
<td></td>
<td>Most cells remain CD45RA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>γ: V1 (mAb STCS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Vγ9Vγ2</td>
<td>Blood</td>
<td>γ: V9C1</td>
<td>High</td>
<td>Predominant in thymus</td>
<td>[56••,57,69]</td>
</tr>
<tr>
<td></td>
<td>S-S bridge (mAb Tira)</td>
<td></td>
<td></td>
<td>Rare in thymus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>δ: V3 (mAb V38B3)</td>
<td></td>
<td></td>
<td>Predominant in blood</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Proportion in blood increases with age</td>
<td></td>
</tr>
</tbody>
</table>

mAb, monoclonal antibody; TCR, T-cell receptor; T cells are murine except where specified.

TCR-independent steps in γδ T-cell subset development

Do the various γδ T-cell subsets represent distinct sublineages that are derived from different TCR-negative progenitor cells? This is almost certainly true for the intestinal Vγ7 subset and the other subsets, since progenitor cells of the former but not the latter can given rise to mature γδ T cells in the absence of a thymus. Evidence from in vitro and in vivo reconstitution experiments also indicates that the Vγ5 and Vγ6 subsets, generated in the fetal and perinatal thymus, are derived from different progenitor cells than the Vγ4 subset generated in the neonatal and adult thymus. Thus, the progenitors of the adult Vγ4 subset cannot function as progenitors of the fetal Vγ5 subset [31,32]. Adult mice lack...
both the microenvironment and the progenitor cells required for the generation of the V_{5} subset. Analogous observations have been made for the 'early' V_{1}+ B-cell sublineage and the 'late' V_{2}+ B-cell sublineage [33-34]. The antigen receptors of the 'early' sublineages exhibit limited diversity that is largely determined by germline sequences, and recognize common bacterial antigens and self antigens; the non-randomness of their antigen receptor repertoires seems to be due to a more primitive rearrangement mechanism, perhaps requiring fewer regulatory proteins than 'late' sublineages but relying on sequence homologies at the ends of the recombined gene segments [35-36]. The antigen receptors of the 'late' sublineages exhibit extensive diversity, particularly in the junctional regions and are able to recognize a wide range of antigens in order to counteract the high genetic flexibility of microbial pathogens. The antigen-receptor repertoires of both types of sublineages are further modified by antigen-receptor mediated selection (see below).

Does targeting the rearrangement to a specific V_{i} gene segment reflect the commitment of a progenitor cell to a particular γδ T-cell subset? Polymerase chain reaction (PCR)-aided Southern blot analysis of the V_{7} subset colonizing the intestine indicates that this is the case [27]. It has been suggested that this may also be true for the progenitor cells of the V_{4} [37-39] and the V_{5} subsets [14], by limited analysis of non-productive rearrangements, although more data are required before a firm conclusion can be drawn. No analogous data are available for the V_{6} subset. In one study, PCR analysis indicated that there was no good correlation between RNA expression of rearranged V_{γ} gene segments and their cell-surface expression during the course of fetal thymic development, suggesting that targeted rearrangement is not the primary determinant for the sequential appearance of γδ T-cell subsets [39]. However, interpretation of this type of analysis is complicated, for example, by the fact that some of the γ mRNA comes from the progenitors of γδ T cells in which the various V_{γ} gene segments may not be rearranged according to the same programs that are established in γδ T-cell progenitors. In a recent study, we measured the rearranged V_{γ} gene segments in the descendants of 13-day-old fetal liver cells that were supplied to the culture of 14 day-old fetal thymuses (S Itohara and S Tonegawa, unpublished data). We found that the order of the V_{γ} rearrangements corresponded to the order of the appearance of the γδ T-cell subsets; the V_{5} subset appeared first, and the V_{6} subset second, followed by the V_{4} subset. These data support the targeted rearrangement hypothesis.

Homing of γδ T-cell subsets to and their maintenance in particular peripheral tissues also suggests that differentially targeted rearrangement in the progenitor cells does occur and is part of a coordinated differentiation program that links a particular TCR repertoire to particular functional properties. Bonneville et al. [40] demonstrated the TCR-independence of these functional properties for at least two γδ cell subsets. They generated TCR-transgenic mice encoding either the TCR of the V_{5} subset that normally resides in the skin, or the TCR of the V_{4} subset that normally resides in the blood and lymphoid organs. In these mice, γδ cells expressing the TCR were found in the intestine and the skin. It is interesting that cells in the intestinal epithelia which expressed the transgenic 'skin' TCR did acquire the CD8 marker; that is normally expressed by γδ cells in the intestine but not the skin. [40]. This suggests that the expression of CD8 is not dependent on the TCR in the intestinal γδ cell subset. However, this is not the case for the two major γδ cell subsets, CD4+ and CD8+ cells, whose generation depends on the TCR interaction with MHC class II and class I molecules, respectively.

**TCR-dependent selection in the thymus**

The fact that the initial step of the γδ sublineage differentiation is TCR-independent does not imply that γδ cell sublineage development cannot be modified by interactions between maturing cells and TCR ligands. Evidence for TCR-mediated thymic selection of γδ cells has been obtained by two approaches, one using anti-TCR antibodies to interfere with normal development in vitro and the other relying on mice that express transgenic γδ TCR with specificity for ligands that are expressed by some but not all strains.

The first of these approaches was based on the findings of Arsanow et al. [14] and Lafaille et al. [41] that the junctional sequences of rearranged V_{2}, V_{6}, and V_{4} genes in PCR-amplified DNA of fetal thymocytes and epidermal γδ cells showed a limited junctional diversity in unproductive rearrangements but almost none in productive rearrangements; this suggested that the accumulation of cells expressing the invariant V_{5}V_{γ}1 and V_{6}V_{γ}1 TCRs was due to TCR-mediated positive selection. To prove that this was indeed the case, Itohara and Tonegawa [42] designed an experiment that took advantage of the fact that the two monospecific γδ T-cell subsets can be generated in fetal organ cultures. When they added antibodies against a constant region of the γδ TCR to such cultures they observed an increase in the frequency of productive rearrangements of V_{5}, V_{6}, and V_{8} with non-canonical junctional sequences. The antibody either mediated positive selection of cells expressing non-canonical TCRs or, more likely, prevented the positive selection of cells expressing canonical TCRs. Whatever the correct explanation might be, the experiment showed that non-canonical TCRs can be generated in the progenitor cells of these two monospecific subsets, i.e. there is a diverse population from which the cells expressing the invariant receptors can be selected. The detection of an increased level of non-canonical, productive V_{5} to J_{1,1} junctional sequences in late fetal liver supports this conclusion [43].

Since the canonical receptors of these two subsets are identical in the region that corresponds to the third complementarity-determining region of the antibody chains, one might speculate, on the basis of current models of TCR-antigen/MHC protein interactions,
that the two γδ T-cell receptors recognize the same endogenous peptide antigen in the context of different, tissue-specific peptide-presenting protein (that is not MHC). The selecting ligands must be expressed in the fetal and perinatal thymus and should be inducible in the epidermis and mucosal epithelia by insults which induce a γδ T-cell response. Indeed, recent experiments by Allison’s group [44] have shown that cells expressing the invariant ‘skin’ γδ TCR recognize an endogenous ligand that is inducible in keratinocytes, and that is expressed by fibroblasts treated with trypsin digests of keratinocytes. Expression of the presenting protein by fibroblasts is not in favour of, but is also not incompatible with, our hypothesis of tissue-specific presenting proteins for a common peptide.

Evidence for positive and negative thymic selection of γδ T cells from the Vγ4 lineage has come from experiments with transgenic mice expressing the rearranged γ and δ genes of the KN6 hybridoma [45,46]. This hybridoma was derived from a thymocyte from a C57BL/6 (H-2b) mouse; it expresses a TCR that is typical for the Vγ4 sublineage and recognizes the T27b gene product, presumably as an autologous peptide-presenting molecule [47]. Interleukin (IL)-2 production is induced in KN6 TCR expressing H-2d cells by spleen cells from H-2d mice (ligand-positive) but not by spleen cells from H-2d mice (ligand-negative), which carries a non-functional T27 gene. Ligand-positive and ligand-negative TCR transgenic mice had similar numbers of thymocytes expressing KN6 TCR but the ligand-negative mice had approximately 10 times fewer spleen cells expressing KN6 TCR. Thymocytes and spleen cells from ligand-positive mice that expressed KN6 TCR failed to produce IL-2 in response to H-2b stimulator cells. However, they proliferated in response to these stimulator cells if exogenous IL-2 was added to the cultures [45]. Another group also found this type of anergy in the thymocytes of similar γδ TCR transgenic mice [48]. Since these investigators did not find any transgenic TCR expressing cells in the spleen of ligand-positive mice they suggested that the anergic state was followed by apoptosis. Whether this is indeed the case remains to be demonstrated.

Perretta et al. [46,47] showed that the development of cells expressing KN6 TCRs depended on positive selection by a protein with β2-microglobulin dependent expression, by crossing the KN6 TCR transgenic mice with β2-microglobulin-deficient mice. Spleens of β2-microglobulin-deficient, ligand-positive and ligand-negative TCR transgenic mice contained very few cells expressing KN6 TCRs, although large numbers of these cells were found in the thymuses of both types of mice. In an interesting experiment, double staining with a KN6 TCR clonotypic monoclonal antibody (mAb) and mAbs against J11d (a marker for immature thymocytes in the γδ cell lineage) showed that the proportion of cells expressing KN6 TCR that were stained with anti-J11d mAb was about 50% in β2-microglobulin-positive mice but almost 100% in β2-microglobulin-negative mice. Hence it was suggested that cells expressing KN6 TCR fail to mature in β2-microglobulin-deficient mice. This finding was further supported by functional studies. Purified γδ thymocytes from β2-microglobulin-deficient KN6 TCR transgenic mice failed to proliferate in response to H-2b spleen cells in the absence of exogenous IL-2 and responded only very poorly in the presence of exogenous IL-2. The unresponsive thymocytes from β2-microglobulin-negative, ligand-negative H-2d mice resembled the unresponsive cells of β2-microglobulin-positive, ligand-positive H-2d mice but differed from them in that they expressed the transgenic TCR at much higher levels [46]. Essentially the same results were obtained by Wells et al. [49] who studied the fate of cells expressing a transgenic TCR with specificity for an undefined T cells (T) region product in β2-microglobulin-positive and β2-microglobulin-negative mice. Curiously, the TCR transgenes that were used in this study came from a T-cell clone that was derived from nude mice. One has to assume, therefore, that the selection that was observed by these investigators in the thymus can also take place extrathymically.

The ligands that mediate the positive and negative selection of cells expressing KN6 TCR are not known. However, it can be predicted that positive and negative selection are mediated by recognition of different ligands, since only the former is seen in H-2b mice [45,46]. This finding can be interpreted as follows. Positive selection of cells expressing KN6 TCR can be mediated in H-2b mice by the T27 protein and in H-2d mice by the product of the T10 gene, a highly homologous duplicate of the T27 gene. The induction of anergy and activation of mature cells requires recognition of a T27 protein-associated endogenous peptide that cannot be presented by the T10 protein.

As expected from previous studies with αβ TCR transgenic mice [50] the development of CD8+ αβ cells is impaired in β2-microglobulin-deficient mice, which do not express MHC class I proteins [51]. In contrast, no gross abnormalities of γδ T cells were noticed in these mice. This could mean either that most γδ T cells do not depend on selection by a β2-microglobulin-dependent protein or that the majority of γδ T cells depend (as do cells expressing KN6 TCRs described above) on selection by recognition of β2-microglobulin-dependent proteins. In the latter case all γδ T cells in the β2-microglobulin-deficient mice would be derived from β2-microglobulin-unrelated γδ T cells which proliferate to fill up the γδ T-cell compartment. We favor the latter possibility and suggest that positive selection of Vγ4 lineage cells, which recognize foreign antigens, resembles the positive selection of αβ T cells, in that cells with highly diversified antigen receptors are selected by the recognition of ligands that are presumably much less diverse. In contrast, positive selection of the monospecific γδ T cells in the fetal thymus is probably mediated by recognition of the same ligands that activate their mature progeny in the periphery.

**Extrathymic TCR-dependent selection**

Differences in the proportion of γδ T cells expressing Vγ4 were observed in the spleens of different mouse strains
In intestinal γδ T-cell populations, expression of the V\(\gamma\)4 high phenotype was linked to particular class II MHC alleles (dominant in crosses of V\(\gamma\)4 high and low phenotype), was not dependent on the thymus and was determined by host cells in FL to parent bone marrow chimerae [24]. In pulmonary γδ T cells from BALB/c but not BALB/b or B6 mice, two types of TCR sequences referred to as BID and GxYs were repeatedly found [52,53,54]. These sequences were also found in nude BALB/c but not nude B6 mice. However, B6 mice were able to generate BID and GxYs since BID was found in the fetal B6 thymus and GxYs in the adult B6 thymus [54]. In these examples of extrathymic selection it is not clear whether exogenous antigens are involved nor whether immature cells were selected. The most likely explanation seems to us to be antigen-driven expansion of mature cells.

Human γδ T cells

Human γδ cells have been extensively studied for their TCR repertoire and putative sublineages. As in mice, rearrangements at the human TCR γ and δ loci also occur in a developmentally ordered fashion [55]. The γδ TCR repertoires that are initially generated in the fetal thymus of mice and humans are small because rearrangements are targeted to a very limited number of variable gene segments and because junctional diversity is very limited. Rearrangements in the thymuses of 8-15-week-old human embryos involve the joining of V\(\gamma\)2 to D\(\delta\)3 and of V\(\delta\)3 or V\(\delta\)9 to the J\(\delta\)1 cluster [55]. The cells which express these TCR chains may be referred to as the V\(\gamma\)9V\(\delta\)2 subset. There is no evidence for selection of monospecific lineages in the human fetal thymus. From four to six months after birth rearrangements involve the joining of other V\(\gamma\)2 segments, in particular V\(\gamma\)1 to D\(\delta\)1 and D\(\delta\)2 and the joining of upstream V\(\delta\) gene segments in the V\(\delta\) family including V\(\delta\)2, V\(\delta\)3, V\(\delta\)5 and V\(\delta\)8 to the J\(\delta\)2 cluster [55]. The cells that express these TCR chains may be referred to as the V\(\gamma\)1V\(\delta\)1 subset. The TCR chains of this subset exhibit extensive junctional diversity.

The two major human γδ T cell subsets, V\(\gamma\)9V\(\delta\)2 and V\(\gamma\)4V\(\delta\)2 can be distinguished by monoclonal antibodies such as 8TC1 which recognizes V\(\gamma\)1V\(\delta\)1 and V\(\gamma\)1V\(\delta\)2 but not V\(\gamma\)4V\(\delta\)3, B3 which recognizes V\(\gamma\)2, and TiyA which recognizes V\(\gamma\)9. In the postnatal thymus the V\(\gamma\)9V\(\delta\)2 subset represents about 15% and the V\(\gamma\)1V\(\delta\)1 subset about 80% of all γδ T cells. These proportions of thymocytes expressing γδ TCR remain relatively constant throughout adult life. In the blood, however, the V\(\gamma\)9V\(\delta\)2 subset increases with age from about 25% in cord blood to more than 70% in the blood of most adults. There is a corresponding decrease in the V\(\gamma\)1V\(\delta\)1 subset from about 50% to less than 30%. The majority of V\(\gamma\)9V\(\delta\)2 cells become CD45RO\(^+\), a probable marker for memory cells, while most V\(\gamma\)4V\(\delta\)1 subset cells remain CD45RO\(^-\). The accumulation of CD45RO\(^+\) V\(\gamma\)9V\(\delta\)2 cells in the blood is thought to be the result of stimulation of mature cells by common ligands for V\(\gamma\)9V\(\delta\)2 TCRs, such as the superantigen staphylococcal enterotoxin A [58] and unidentified components of mycobacteria [59,60], Plasmodium falciparum [61] or cell lines such as Molli [59] and Daudi [62,63], which may express superantigens. Selection of the predominant V\(\gamma\)9 T-cell subset in adult human blood by superantigens is consistent with the extensive junctional diversity of their TCRs. Expansion of antigen-specific γδ T-cell clones has also been reported. For example, γδ T-cell subsets with very limited diversity were observed in the blood and bronchoalveolar lavage of some patients with sarcoidosis [65]. The data from two patients were particularly striking. Sequencing of mRNA obtained from their blood revealed identical junctions in 84% and 54% of V\(\gamma\)9 transcripts. In one case, 67% of V\(\gamma\)9 transcripts from bronchoalveolar lavage showed the same junctional sequence and this was identical to the predominant sequence in the blood.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

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