Development and selection of $\gamma\delta$ T cells

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The $\gamma\delta$ T-cell population, a subpopulation of T cells formed through cell lineages that are independent of the $\alpha\beta$ 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.

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, $\gamma\delta$ TCR, and a novel class of T cells, $\gamma\delta$ T cells [2]. This review covers recent progress in several areas of $\gamma\delta$ T-cell development and selection with major emphasis on the mouse system. The human system is also discussed, but less extensively.

Segregation of $\gamma\delta$ and $\alpha\beta$ T-cell lineages

One interesting issue raised by the discovery of $\gamma\delta$ T cells is their cell lineage relationship with $\alpha\beta$ 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 $\alpha\beta$ T cells, some γ genes are often rearranged in a non-productive (i.e. out-of-frame) form [5]. In contrast, in $\gamma\delta$ thymocytes or peripheral $\gamma\delta$ 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 $\alpha\beta$ and $\gamma\delta$ or 'hybrid' TCRs, such as $\beta\delta$ or $\alpha\gamma$ heterodimers, have not been detected in normal animals [4,6,7]. These observations leave little doubt that the $\alpha\beta$ and $\gamma\delta$ 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 $\gamma\delta$ TCR occurs, and by analogy with the immunoglobulin system, this inhibits further re-

arrangement of any other TCR genes. $\alpha\beta$ 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 $\gamma\delta$ TCR transgenic mice (KN6 Tg mice) constructed with cosmid clones containing functionally rearranged γ and β genes, nearly all $\gamma\delta$ T cells expressed the transgene-encoded $\gamma\delta$ TCR and normal numbers of $\alpha\beta$ T cells were present both in the thymus and in the peripheral lymphoid organs of adult mice [8]. These $\alpha\beta$ 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 $\alpha\beta$ T cells corroborates reports of the silencing of rearranged (both in-frame and out-of-frame) γ genes in the $\alpha\beta$ T cells of normal mice [9], although the $V_2J_2C_2$ γ gene, the protein product of which is rarely expressed on the cell surface, is an exception to this rule [9]. This $\alpha\beta$ T-cell-specific silencing of $C_\gamma 1$-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 $\alpha\beta$ T-cell-specific silencing of γ genes in the segregation of $\alpha\beta$ and $\gamma\delta$ 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 $\alpha\beta$ 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 D_β to J_β) 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.

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 V_β to DJ_β and, eventually $V\alpha$ to J_α; αβ 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 the various locations represent different γδ cell subsets. They express different TCR repertoires, ranging from mono-specific 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 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 $V_\gamma 5$ subset) is disseminated in the epidermis [14] and the other (the $V_\gamma 6$ subset) in the mucosal epithelia of the uterus, vagina and tongue [15]. The $V_\gamma 5$ and the $V_\gamma 6$ subsets appear in two consecutive waves in the fetal and perinatal thymus, respectively [16–18]. A highly diversified γδ T-cell subset (the $V_\gamma 4$ subset) is generated later, in the neonatal and adult thymus [17,19], and exported to the blood and lymphoid organs [20•]. This sublineage expresses predominantly $V_\gamma 4$ and multiple V_δ chains and includes a population of self-reactive cells which express $V_\gamma 1$ and $V_\delta 6$ chains. A fourth γδ cell lineage (the $V_\gamma 7$ 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 $V_\gamma 7$ chain and $V_\delta 4$, $V_\delta 5$, $V_\delta 6$, and $V_\delta 7$ 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 $V_\gamma 1$ and $V_\gamma 4$ rather than $V_\gamma 7$, 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 V_γ and V_δ 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.
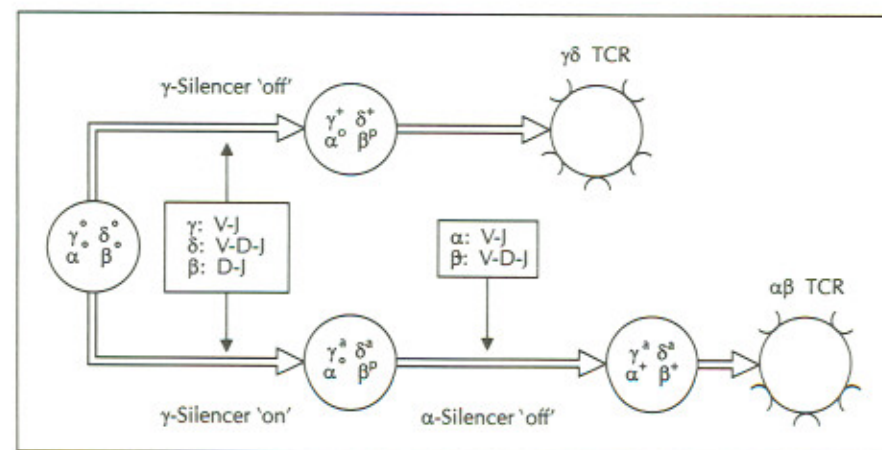
Fig. 1. Silencer model of T-cell lineage segregation. The superscript designations are as follows: o, germline configuration; +, in-frame joining; a, any configuration; p, partial rearrangement. See text for further details.

Table 1. Sublineages of γδ T cells.

Sublineage	Location	TCR usage	Diversity	Characteristics	References
V$_\gamma$5 DEC iEL	Skin	γ: V5JJIC1 (mAb 563) δ: V1D2J1C	None	Generated in 14–17-day-old fetal thymus	[16–18,41]
				Generation depends on fetal progenitors and fetal thymus	[31,32]
				Positive selection of monospecific cells in thymus	[41,42]
				Homing to and maintenance in epidermis	[14,16]
				Recognizes cultured keratinocytes	[44••]
V$_\gamma$6 vut-IEL	Vagina, uterus, tongue	γ: V6J1C1 δ: V1D2J1C	None	Generated in late fetal and newborn thymus	[18]
				Positive selection of monospecific cells in thymus	[41,42]
				Homing to and maintenance in mucosal epithelia (vagina, uterus, tongue)	[15]
V$_\gamma$4	Blood, lymph nodes, spleen	γ: V4J1C1 δ: V2,4,5,6,7	High	Generated in postnatal thymus Major γδ cell population in adult thymus, lymph nodes and spleen	[17,19.20•]
		γ: V1J4C4 δ: V6	High	Appear in spleen 1–2 weeks after birth	[20•]
				Respond to PPD, mycobacterial Hsp65 and self Hsp65; many cells recognize residues 180–196 of Hsp65	[65,66]
				Lack of isotype exclusion in some cells	[67]
				Anti-self response depends on vitronectin receptor	[68]
				Various effects on hemopoiesis in transgenic mice	[65]
V$_\gamma$7 i-IEL	Intestine	γ: V7J1C1 δ: V2,4,5,6	High	Predominant expression of V$_\gamma$7	[25]
				Highly diversified	[25–27]
				Thymus independent	[21,22,23••,24]
				Expression of CD8α homodimers	[23••]
	Lung	γ: V4 δ: V6	High	8–20% of resident pulmonary lymphocytes are γδ T-cells	[29]
	Liver	γ: V1,2 δ: V6	High	Number increaes with age Express CD8α homodimers	[28•]
	Mammary gland	γ: V4,5 δ: V4	High	Fourfold increase of number in lactating mammary gland	[30]
Human V$_\gamma$JV$_\delta$1	Thymus	γ: VIC2 no S-S bridge γ: V1 (mAb δTCS)	High	Predominant in thymus Rare in blood Proportion in blood decreases with age Most cells remain CD45RA	[56••,57,69]
Human V$_\gamma$9 V$_\delta$2	Blood	γ: V9C1 S-S bridge (mAb Tiγa) δ: V3 (mAb V3BB3)	High	Rare in thymus Predominant in blood Proportion in blood increases with age Most cells become CD45RO	[56••,57,69]

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 V$_\gamma$7 subset and the other subsets, since progenitor cells of the former but not the latter can give rise to mature γδ T cells in the absence of a thymus. Evidence from *in vitro* and *in vivo* reconstitution experiments also indicates that the V$_\gamma$5 and V$_\gamma$6 subsets, generated in the fetal and perinatal thymus, are derived from different progenitor cells than the V$_\gamma$4 subset generated in the neonatal and adult thymus. Thus, the progenitors of the adult V$_\gamma$4 subset cannot function as progenitors of the fetal V$_\gamma$5 subset [31,32]. Adult mice lack

both the microenvironment and the progenitor cells required for the generation of the $V_\gamma5$ subset. Analogous observations have been made for the 'early' Lyl$^+$ B-cell sublineage and the 'late' Lyl$^-$ 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 then further modified by antigen-receptor mediated selection (see below).

Does targeting the rearrangement to a specific V_γ gene segment reflect the commitment of a progenitor cell to a particular $\gamma\delta$ T-cell subset? Polymerase chain reaction (PCR)-aided Southern blot analysis of the $V_\gamma7$ 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 $V_\gamma4$ [37–39] and the $V_\gamma5$ 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 $V_\gamma6$ 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 $\gamma\delta$ 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 $\alpha\beta$ T cells in which the various V_γ gene segments may not be rearranged according to the same programs that are established in $\gamma\delta$ T-cell progenitors. In a recent study, we measured the various 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 $\gamma\delta$ T-cell subsets; the $V_\gamma5$ subset appeared first, and the $V_\gamma6$ subset second, followed by the $V_\gamma4$ subset. These data support the targeted rearrangement hypothesis.

Homing of $\gamma\delta$ 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 $\gamma\delta$ cell subsets. They generated TCR-transgenic mice encoding either the TCR of the $V_\gamma5$

subset that normally resides in the skin, or the TCR of the $V_\gamma4$ subset that normally resides in the blood and lymphoid organs. In these mice, $\gamma\delta$ cells expressing the 'wrong' 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 $\gamma\delta$ 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 $\gamma\delta$ cell subset. However, this is not the case for the two major $\alpha\beta$ 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 $\gamma\delta$ sublineage differentiation is TCR-independent does not imply that $\gamma\delta$ cell sublineage development cannot be modified by interactions between maturing cells and TCR ligands. Evidence for TCR-mediated thymic selection of $\gamma\delta$ 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 $\gamma\delta$ 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 $V_\gamma5$, $V_\gamma6$ and $V_\delta1$ genes in PCR-amplified DNA of fetal thymocytes and epidermal $\gamma\delta$ 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 $V_\gamma5V_\delta1$ and $V_\gamma6V_\delta1$ 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 $\gamma\delta$ T-cell subsets can be generated in fetal organ cultures. When they added antibodies against a constant region of the $\gamma\delta$ TCR to such cultures they observed an increase in the frequency of productive rearrangements of $V_\gamma5$, $V_\gamma6$ and $V_\delta1$ 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 $V_\gamma5$ to $J_\gamma1$ 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 tryptic 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 $V_\gamma 4$ 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-2^b) mouse; it expresses a TCR that is typical for the $V_\gamma 4$ sublineage and recognizes the T27^b gene product, presumably as an autologous peptide-presenting molecule [47]. Interleukin (IL)-2 production is induced in KN6 TCR expressing H-2^d cells by spleen cells from H-2^b mice (ligand-positive) but not by spleen cells from H-2^d mice (ligand-negative), which carry a non-funtional 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-2^b 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.

Pereira et al. [46••] showed that the development of cells expressing KN6 TCRs depended on positive selection by a protein with β-microglobulin-dependent expression, by crossing the KN6 TCR transgenic mice with β$_2$-microglobulin-deficient mice. Spleens of β$_2$-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 αβ cell lineage) showed that the proportion of cells expressing KN6 TCR that were stained with anti-J11d mAb was about 50% in β$_2$-microglobulin-positive mice but almost 100% in β$_2$-microglobulin-negative mice. Hence it was suggested that cells expressing KN6 TCR fail to mature in β$_2$-microglobulin-deficient mice. This finding was

further supported by functional studies. Purified γδ thymocytes from β$_2$-microglobulin-deficient KN6 TCR transgenic mice failed to proliferate in response to H-2^b spleen cells in the absence of exogenous IL-2 and responded only very poorly in the presence of exogenous IL-2. The unresponsive cells from β$_2$-microglobulin-negative, ligand-negative H-2^d mice resembled the unresponsive cells of β$_2$-microglobulin-positive, ligand-positive H-2^b 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 leukemia (TL) region product in β$_2$-microglobulin-positive and β$_2$-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-2^d mice [45,46••]. This finding can be interpreted as follows. Positive selection of cells expressing KN6 TCR can be mediated in H-2^b mice by the T27^b protein and in H-2^d mice by the product of the T10 gene, a highly homologous duplicate of the T27^b gene. The induction of anergy and activation of mature cells requires recognition of a T27^b protein-associated endogenous peptide that cannot be presented by the T10^d protein.

As expected from previous studies with αβ TCR transgenic mice [50] the development of CD8$^+$ αβ cells is impaired in β$_2$-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 β$_2$-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 β$_2$-microglobulin-dependent proteins. In the latter case all γδ T cells in the β$_2$-microglobulin-deficient mice would be derived from β$_2$-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 $V_\gamma 4$ sublineage 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 $V_\delta 4$ were observed in the spleens of different mouse strains

[20•]. In intestinal γδ T-cell populations, expression of the V$_\delta$4 high phenotype was linked to particular class II MHC alleles (dominant in crosses of V$_\delta$4 high and low phenotype), was not dependent on the thymus and was determined by host cells in F1 to parent bone marrow chimeras [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 thymuses 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 8.5–15-week-old human embryos involve the joining of V$_\delta$2 to D$_\delta$3 and of V$_\gamma$8 or V$_\gamma$9 to the J$_\gamma$1 cluster [55]. The cells which express these TCR chains may be referred to as the V$_\gamma$9V$_\delta$2 subset. There is no evidence for selection of monospecific sublineages in the human fetal thymus. From four to six months after birth rearrangements involve the joining of other V$_\delta$ segments, in particular V$_\delta$1 to D$_\delta$1 and D$_\delta$2 and the joining of upstream V$_\gamma$ gene segments in the V$_\gamma$I family including V$_\gamma$2, V$_\gamma$3, V$_\gamma$5 and V$_\gamma$8 to the J$_\gamma$2 cluster [55]. The cells that express these TCR chains may be referred to as the V$_\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$IV$_\delta$2 can be distinguished by monoclonal antibodies such as δTCS1 which recognizes V$_\delta$1J$_\delta$1 and V$_\delta$1J$_\delta$2 but not V$_\delta$1J$_\delta$3, BB3 which recognizes V$_\delta$2, and TiγA which recognizes V$_\gamma$9. In the postnatal thymus the V$_\gamma$9 V$_\delta$2 subset represents about 15% and the V$_\gamma$I V$_\delta$1 subset about 80% of all γδ and T cells [56••,57]. These proportions of thymocytes expressing γδ TCR remain relatively constant throughout adult life. In the blood, however, the V$_\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$IV$_\delta$1 subset from about 50% to less than 30%. Most V$_\gamma$9V$_\delta$2 cells become CD45RO$^+$, a probable marker for memory cells, while most V$_\gamma$IV$_\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$2 TCRs, such as the superantigen staphylococcal enterotoxin A [58] and unidentified components of mycobacteria [59••,60••], *Plasmodium falciparum* [61•] or cell lines such as Molt4 [59••] and Daudi [62,63], which may express superantigens. Selection of the predominant V$_\delta$ 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.

Acknowledgements

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

1. SAITO H, KRANZ DM, TAKAGAKI Y, HAYDAY AC, EISEN HN, TONEGAWA S: Complete Primary Structure of a Heterodimeric T-cell Receptor Deduced from cDNA Sequences. *Nature* 1984, 309:757–762.

2. BRENNER MB, MCLEAN J, DIALYNAS DP, STROMINGER JL, SMITH JA, OWEN FL, SEIDMAN JG, IP S, ROSEN F, KRANGEL MS: Identification of a Putative Second T-cell Receptor. *Nature* 1986, 322:145–149.

3. RAULET DH, GARMAN RD, SAITO HY, TONEGAWA S: Developmental Regulation of T-cell Receptor Gene Expression. *Nature* 1985, 314:103–107.

4. PARDOLL DM, FOWLKES BJ, BLUESTONE JA, KRUISBECK A, MALOY WL, COLIGAN JE, SCHWARTZ RH: Differential Expression of Two Distinct T-cell Receptors During Thymocyte Development. *Nature* 1987, 326:79–81.

5. HEILIG JS, TONEGAWA S: T-cell γ Gene is Allelically but not Isotypically Excluded and is not Required in Known Functional T-cell Subsets. *Proc Natl Acad Sci USA* 1987, 84:8070–8074.

6. ITOHARA S, NAKANISHI N, KANAGAWA O, KUBO R, TONEGAWA S: Monoclonal Antibodies Specific to Native Murine T-cell Receptor γδ: Analysis of γδ T-cells in Thymic Ontogeny and Peripheral Lymphoid Organs. *Proc Natl Acad Sci USA* 1989, 86:5094–5098.

7. HOCHSTENBACH F, BRENNER MB: T-cell Receptor delta -chain can Substitute for α to Form a βδ Heterodimer. *Nature* 1989, 340:562–565.

8. ISHIDA I, VERBECK S, BONNEVILLE M, ITOHARA S, BERNS A, TONEGAWA S: T-cell Receptor γδ and γ Transgenic Mice Suggest a Role of a γ Gene Silencer in the Generation of αβ T-cells. *Proc Natl Acad Sci USA* 1990, 87:3067–3071.

9. HEILIG JS, TONEGAWA S: Diversity of Murine Gamma Genes and Expression in Fetal and Adult T Lymphocytes. *Nature* 1986, 322:836–840.

10. BONNEVILLE M, ISHIDA I, MOMBAERTS P, KATSUKI M, VERBEEK S, BERNS A, TONEGAWA S: Blockade of αβ T-cell Development by TCRγδ Transgenes. *Nature* 1989, 342:931–932.

11. WINOTO A, BALTIMORE D: αβ Lineage-specific Expression of the γ T-cell Receptor Gene by Nearby Silencers. *Cell* 1989, 59:649–655.

12. DE VILLARTAY JP, COHEN DL: Gene Regulation Within the TCR-γ/δ Locus by Specific Deletion of the TCR-δ Cluster. *Res Immunol* 1990, 141:618–623.

13. DE VILLARTAY JP, MOSSALAYI D, DE CHASSEVAL R, DALLOUL A,
•   DEBRE P: The Differentiation of Human Pro-thymocytes Along the TCR-α/β Pathway *in vitro* is Accompanied by the Site-specific Deletion of the TCR-δ Locus. *Int Immunol* 1991, 3:1301–1305.
Shows that some of the cultured immature thymocytes differentiating along the αβ T-cell lineage lose the TCR δ locus by a specific rearrangement event.

14. ASARNOW DM, KUZIEL WA, BONYHADI M, TIGELAAR RE, TUCKER PW, ALLISON JP: Limited Diversity of γ/δ Antigen Receptor Genes of Thy-1+ Dendritic Epidermal Cells. *Cell* 1988, 55:837–847.

15. ITOHARA S, FARRY AG, LAFAILLE JJ, BONNEVILLE M, TAKAGAKI Y, HAAS W, TONEGAWA S: Homing of a γ/δ Thymocyte Subset with Homogenous T-cell Receptors to Mucosal Epithelial. *Nature* 1990, 343:754–757.

16. HARVAN WL, ALLISON JP: Developmentally Ordered Appearance of Thymocytes Expressing Different T-cell Antigen Receptors. *Nature* 1988, 335:443–445.

17. ITOHARA S, NAKANISHI N, KANAGAWA O, KUBO R, TONEGAWA S: Monoclonal Antibodies Specific to Native Murine T-cell Receptor γ/δ: analysis of γ/δ T-cells During Thymic Ontogeny and in Peripheral Lymphoid Organs. *Proc Natl Acad Sci USA* 1989, 86:5094–5098.

18. ITO K, BONNEVILLE M, TAKAGAKI Y, NAKANISHI N, KANAGAWA O, KRECKO E, TONEGAWA S: Different γδ T-cell Receptors are Expressed on Thymocytes at Different Stages of Development. *Proc Natl Acad Sci USA* 1989, 86:631–635.

19. TAKAGAKI Y, NAKANISHI N, ISHIDA I, KANAGAWA O, TONEGAWA S: T-cell Receptor + γ and − δ Genes Preferentially Utilized by Adult Thymocytes for the Surface Expression. *J Immunol* 1989, 142:2112–2121.

20. BLUESTONE JA, CRON RQ, BARRETT TA, HOULDEN B, SPERLING
•   AI, DENT A, HEDRICK S, RELLAHAN B, MATIS LA: Repertoire Development and Ligand Specificity of Murine TCR γδ Cells. *Immunol Rev* 1991, 120:5–33.
Review with particular emphasis on γδ T-cells of the V,4 sublineage in the adult thymus and peripheral lymphoid organs such as lymph nodes and spleen.

21. BANDEIRA A, ITOHARA S, BONNEVILLE M, BURLEN-DEFRANOUX O,
••   MOTA-SANTOS T, COUTINHO A, TONEGAWA S: Extrathymic Origin of Intestinal Intraepithelial Lymphocytes Bearing T-cell Antigen Receptor γδ. *Proc Natl Acad Sci USA* 1991, 88:43–47.
Intestinal γδ T-cells were generated in lethally irradiated mice, with and without a thymus, after reconstitution with fetal liver cells.

22. MOSLEY RL, STYRE D, KLEIN JR: Differentiation and Functional Maturation of Bone Marrow-derived Intestinal Epithelial T-cells Expressing Membrane T-cell Receptor in Athymic Radiation Chimeras. *J Immunol* 1990, 145:1369–1375.

23. GUY-GRAND D, MALASSIS-SERIS M, BRIOTTET C, VASSALLI P: Cyto-
••   toxic Differentiation of Mouse Gut Thymodependent and Independent Intraepithelial T Lymphocytes is Induced Locally. Correlation Between Functional Assays, Presence of Perforin and Granzyme Transcripts and Cytoplasmic Granules. *J Exp Med* 1991, 173:1549–1552.
CD8 homodimers were found on the surface of thymus-independent γδ and αβ T-cells isolated from mouse intestinal epithelia.

24. LEFRANCOIS L, LECORRE R, MAYO J, BLUESTONE JA, GOODMAN T: Extrathymic Selection of TCR γδ+ Cells by Class II Major Histocompatibility Complex Molecules. *Cell* 1990, 63:333–340.

25. BONNEVILLE M, JANEWAY CA JR, ITO K, HASER W, ISHIDA I, NAKANISHI N, TONEGAWA S: Intestinal Intraepithelial Lymphocytes are a Distinct Set of γδ T-cells. *Nature* 1988, 336:479–481.

26. KYES S, CARFEW E, CARDING SR, JANEWAY CA JR, HAYDAY A: Diversity in T-cell Receptor γ Gene Usage in Intestinal Epithelium. *Proc Natl Acad Sci USA* 1989, 86:5527–5531.

27. TAKAGAKI Y, DECLOUX A, BONNEVILLE M, TONEGAWA S: Diversity of γδ T-cell Receptors on Murine Intestinal Intraepithelial Lymphocytes. *Nature* 1989, 339:712–714.

28. OHTEKI T, ABO T, SEKI S, KOBATA T, YAGITA H, OKUMURA
•   K, KUMAGAI K: Predominant Appearance of γδ T Lymphocytes in the Liver of Mice After Birth. *Eur J Immunol* 1991, 21:1733–1740.
Up to 23% of CD3+ cells in the liver of 4-week-old mice of various strains were γδ T-cells, most of which expressed CD8.

29. AUGUSTIN A, KUNO RT, SIM GK: Resident Pulmonary Lymphocytes Expressing the γδ T-cell Receptor. *Nature* 1989, 340:239–241.

30. REARDON C, LEFRANCOIS L, FARR A, KUBO R, O'BRIEN R, BORN W: Expression of γ/δ T-cell Receptors on Lymphocytes from the Lactating Mammary Gland. *J Exp Med* 1990, 172:1263–1266.

31. HAVRAN WL, ALLISON JP: Origin of Thy-1+ Dendritic Epidermal Cells of Adult Mice from Fetal Thymic Precursors. *Nature* 1990, 344:68–70.

32. IKUTA K, KINA T, MACNEIL I, UCHIDA N, PEAULT B, CHIEN Y, WEISSMAN IL: A Developmental Switch in Thymic Lymphocyte Maturation Potential Occurs at the Level of Hematopoietic Stem Cells. *Cell* 1990, 62:863–874.

33. KIPPS TJ: The CD5 B-cell. *Adv Immunol* 1989, 47:117–185.

34. FORSTER I, RAJEWSKY K: Expansion and Functional Activity of Ly-1+ B Cells Upon Transfer of Peritoneal Cells into Allotype-congenic Newborn Mice. *Eur J Immunol* 1987, 17:521–528.

35. GU H, FORSTER I, RAJEWSKY K: Sequence Homologies, N Sequence Insertion and J_H Gene Utilization in $V_H D J_H$ Joining: Implications for the Joining Mechanism and the Ontogenetic Timing of Lyl B Cell and B-CLL Progenitor Generation. *EMBO J* 1990, 9:2133–2140.

36. JEONG HD, TEALE JM: Contribution of the CD5+ B Cell to D-proximal V_H Family Expression Early in Ontogeny. *J Immunol* 1990, 145:2725–2729.

37.   KRANZ DM, SAITO H, HELLER M, TAKAGAHI Y, HASS W, EISEN HN, TONEGAWA S: Limited Diversion of the Rearranged T-cell γ Gene. *Nature* 1985, 313:752–755.

38.   KORMAN AJ, MARUSIC-GALESIC S, SPENCER D, KRUISBECK A, RAULET DH: Predominnat Variable Region Gene Usage by δ/γ T Cell Receptor-bearing Cells in the Adult Thymus. *J Exp Med* 1988, 168:1021–1040.

39.   CARDING SR, KYES S, JENKINSON EJ, KINGSTON R, BOTTOMLY K, OWEN JT, HAYDAY AC: Developmentally Regulated Fetal Thymic and Extrathymic T-cell Receptor γδ Gene Expression. *Genes Dev* 1990, 4:1304–1315.

40.   BONNEVILLE M, ITOHARA S, KRECKO EG, MOMBAERTS P, ISHIDA I, KATSUKI M, BERNS A, FARR AG, JANEWAY CA JR, TONEGAWA S: Transgenic Mice Demonstrate that Epithelial Homing of γδ T Cells is Determined by Cell Lineages Independent of T Cell Receptor Specificity. *J Exp Med* 1990, 171:1015–1026.

41.   LAFAILLE JL, DECLOUX A, BONNEVILLE M, TAKAGAKI Y, TONEGAWA S: Junctional Sequences of T Cell Receptor γδ Genes: Implications for γδ T Cell Lineages and for a Novel Intermediate of V-(D)-J Joining. *Cell* 1989, 59:857–870.

42.   ITOHARA S, TONEGAWA S: Selection of γδ T Cells with Canonical T-cell Antigen Receptors in Fetal Thymus. *Proc Natl Acad Sci USA* 1990, 87:7935–7938.

43.   KYES S, PAO W, HAYDAY A: Influence of Site Expression on
  •   the Fetal γδ T-cell Receptor Repertoire. *Proc Natl Acad Sci USA* 1991, 88:7830–7833.
Shows that the late fetal and newborn liver contains an augmented level of non-canonical, in frame-joined $V_\gamma 5 J_\gamma 1$ mRNA° (germline) sequences, than that of adult liver, supporting the notion that the canonical sequence is generated by cellular selection.

44.   HAVRAN WL, CHIEN YH, ALLISON JP: Recognition of Self Anti-
  ••   gens by Skin-derived T Cells with Invariant γδ Antigen Receptors. *Science* 1991, 252:1430–1432.
Shows that cells expressing the invariant TCR of the Vγ5 sublineage recognize cultured keratinocytes or fibroblasts treated with tryptic digests of cultured keratinocytes. Most importantly, this shows that the invariant Vγ5 TCR recognizes both a presenting protein and a peptide.

45.   BONNEVILLE M, ISHIDA I, ITOHARA S, VERBEEK S, BERNS A, KANAGAWA O, HAAS W, TONEGAWA S: Self-tolerance to Transgenic γδ T Cells by Intrathymic Inactivation. *Nature* 1990, 344:163–165.

46.   PEREIRA P, ZIJLSTRA M, MCMASTER J, LORING JM, JAENISCH R,
  ••   TONEGAWA S: Blockade of Transgenic γδ T Cell Development in β2-microglobulin Deficient Mice. *EMBO J* 1992, 11:25–31.
Demonstration of positive selection of transgenic γδ T cells in the thymus by a β2-dependent protein.

47.   ITO K, VAN KAER L, BONNEVILLE M, HAU S, MURPHY DB, TONEGAWA S: Recognition of the Product of a Novel MCH TL Region Gene (27b) by a Mouse γδ T Cell Receptor. *Cell* 1990, 62:549–556.

48.   DENT AL, MATIS LA, HOOSHMAND F, WIDACKI SM, BLUESTONE JA, HEDRICK SM: Self-reactive γδ T Cells are Eliminated in the Thymus. *Nature* 1990, 343:714–719.

49.   WELLS FB, GAHM SJ, HEDRICK SM, BLUESTONE JA, DENT A, MATIS
  ••   LA: Requirement for Positive Selection of γδ Receptor Bearing T Cells. *Science* 1991, 253:903–905.
B₂-microglobulin-deficient mice were crossed with transgenic mice expressing γδ TCR with specificity for an undefined TL region gene product. Cells expressing this transgenic TCR fail to mature in β₂-microglobulin-deficient mice.

50.   VON BOEHMER H: Development Biology of T Cells in T Cell Receptor Transgenic Mice. *Annu Rev Immunol* 1990, 8:531–556.

51.   ZIJLSTRA M, BIX M, SIMISTER NE, LORING JM, RAULET DH, JAENISCH R: β2-microglobulin Deficient Mice Lack CD4⁻8⁺ Cytolytic T Cells. *Nature* 1990, 344:742–746.

52.   SIM GK, AUGUSTIN A: Dominantly Inherited Expression of BID, an Invariant Undiversified T Cell Receptor δ Chain. *Cell* 1990, 61:397–405.

53.   SIM GK, AUGUSTIN A: Dominant Expression of the T Cell
  •   Receptor BALB Invariant δ (BID) Chain in Resident Pulmonary Lymphocytes is Due to Selection. *Eur J Immunol* 1991, 21:859–861.
Demonstration of cells expressing BID TCRs in the thymus of C57B1/6 embryos but not in the lung or lymph nodes of adult C57B1/b mice. This is an example of extrathymic γδ TCR-dependent selection.

54.   SIM GK, AUGUSTIN A: Extrathymic Positive Selection of γδ T Cells. *J Immunol* 1991, 146:2439–2445.

55.   KRANGEL MS, YSSEL H, BROCKLEHURST C, SPITS H: A Distinct Wave of Human T Cell Receptor γ/δ Lymphocytes in the Early Fetal Thymus: Evidence for Controlled Gene Rearrangements and Cytokine Production. *J Exp Med* 1990, 172:847–859.

56.   PORCELLI S, BRENNER B, BAND H: Biology of the Human γδ
  ••   T Cell Receptor. *Immunol Rev* 1991, 120:137–183.
Comprehensive review on human γδ T cells including sections on molecular aspects of γδ TCR receptor repertoires, specificity and function.

57.   CASORATI G, DELIBERO G, LANZAVECCHIA A, MIGONE N: Molecular Analysis of Human γδ Clones from Thymus and Peripheral Blood. *J Exp Med* 1989, 170:1521–1535.

58.   RUST CJJ, VERRECK F, VICTOR H, KONING F: Specific Recognition of Staphylococcal Enterotoxin A by Human T Cells Bearing Receptors with the $V_\gamma 9$ Region. *Nature* 1990, 346:572–574.

59.   DE LIBERO G, CASORATI G, GIACHINO C, CARBONARA C, MIGONE
  ••   N, MATZINGER P, LANZAVECCHIA A: Selection by Two Powerful Antigens May Account for the Presence of the Major Population of Human γ/δ T Cells. *J Exp Med* 1991, 173:1311–1322.
Demonstration that most cells expressing $V_\gamma 9 V_\gamma 2$ recognize a surface component of Molt4 cells, which acts as a superantigen, and that these cells include a subset which recognizes antigen presenting cells pulsed with mycobacteria tuberculosis or other bacteria.

60.   KABELITZ D, BENDER A, PROSPERO T, WESSELBORG S, JANSSEN O,
  ••   PECHHOLD K: The Primary Response of Human γ/δ⁺ T Cells to Mycobacterium Tuberculosis is Restricted to $V_\gamma 9$-bearing Cells. *J Exp Med* 1991, 173:1331–1338.
Demonstration of selective proliferation of cells expressing $V_\gamma 9$ $V_\delta 2$ TCRs in cultures of CD4⁻CD8⁻ thymocytes stimulated with mycobacteria tuberculosis. The response is inhibited by anti-Vγ9 mAb and abolished by selective depletion of Vγ9 positive cells.

61.   GOERLICH R, HACKER G, PFEFFER K, HEEG K, WAGNER H:
  •   Plasmodium falciparum Merozites primarily Stimulate the $V_\gamma 9$ Subset of Human γ/δ T Cells. *Eur J Immunol* 1991, 21:2613–2616.
1/150 to 1/300 T cells from blood proliferate in response to *Plasmodium falciparum* microzoites. The responding cells express Vγ9.

62.   STURM E, BRAAKMAN E, FISCH P, VREUGDENHIL RJ, SONDEL P, BOLHUIS RLH: Human Vγ9Vδ2 T Cell Receptor-γδ Lymphocytes Show Specificity to Daudi Burkitt's Lymphoma Cells. *J Immunol* 1990, 145:3202–3208.

63. FISCH P, MALKOVSKY M, BRAAKMAN E, STURM E, BOLHUIS RLH, PRIEVE A, SOSMAN JA, LAM VA, SONDEL PM: γ/δ T Cell Clones and Natural Killer Cell Clones Mediate Distinct Patterns of Non-major Histocompatibility Complex-restricted Cytolysis. *J Exp Med* 1990, 171:1567–1579.

64. TAMURA N, HOLROYD KJ, BANKS T, KIRBY M, OKAYAMA H, CRYSTAL RG: Diversity in Junctional Sequences Associated with the Common Human Vγ9 and Vδ2 Gene Segments in Normal Blood and Lung Compared with the Limited Diversity in a Granulomatous Disease. *J Exp Med* 1990, 172:169–181.

65. FERRICK DA, SYDORA B, WALLACE V, GAMMELL-HORI L, KRONENBERG M, MAK TW: Self-reactive γδ T Lymphocytes: Implications for T-cell Ontogeny and Reactivity. *Immunol Rev* 1991, 120:51–69.

66. BORN W, HALL L, DALLAS A, BOYMEL J, SHINNICK T, YOUNG D, BRENNAN P, O'BRIEN R: Recognition of a Peptide Antigen by Heat Shock-reactive γδ T Lymphocytes. *Science* 1990, 249:67–69.

67. KONING F, YOKOYAMA WM, MOLOY WL, STRINGE G, McCONNELL TJ, COHEN DI, SHEVACH EM, COLLIGAN JE: Expression of Cγ4 Cell Receptors and Lack of Istype Exclusion by Dendritic Epidermal T Cell Lines. *J Immunol* 1988, 141:2057–2062.

68. ROBERTS K, YOKOYAMA WM, KEHN PJ, SHEVACH EM: The Vitonectin Receptor Serves as an Accessory Molecule for the Activation of a Subset of γ/δ T Cells. *J Exp Med* 1991, 173:1231–1240.

69. PARKER CM, GROH V, BAND H, PORCELLI SA, MORITA C, FABBI M, GLASS D, STROMINGER JI, BRENNER MB: Evidence for Extrathymic Changes in the T Cell Receptor γδ Repertoire. *J Exp Med* 1990, 171:1597–1612.

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