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Development and selection of γδ T cells

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Summary: Two main lineages of T cells develop in the thymus: those that express the αβ T-cell receptor (TCR) and those that express the γδ TCR. Whereas the development, selection, and peripheral localization of newly differentiated αβ T cells are understood in some detail, these processes are less well characterized in γδ T cells. This review describes research carried out in this laboratory and others, which addresses several key aspects of γδ T-cell development, including the decision of precursor cells to differentiate into the γδ versus αβ lineage, the ordered differentiation over the course of ontogeny of functional γδ T-cell subsets expressing distinct TCR structures, programming of ordered Vγ gene rearrangement in the thymus, including a molecular switch that ensures appropriate Vγ rearrangements at the appropriate stage of development, positive selection in the thymus of γδ T cells destined for the epidermis, and the acquisition by developing γδ T cells of cues that determine their correct localization in the periphery. This research suggests a coordination of molecularly programmed events and cellular selection, which enables specialization of the thymus for production of distinct T-cell subsets at different stages of development.

Keywords: *γδT cells, T-cell development, T-cell receptors, T-cell receptor gene rearrangement, tissue localization*

Introduction

Unlike conventional αβ T cells, which reside primarily in secondary lymphoid organs and play a central role in adaptive immune responses, many γδ T cells reside in epithelial layers of tissues underlying internal and external surfaces of the body, such as the skin, intestinal epithelium, lung, and tongue, where they function as a first line of defense (1–4). In these locations, the diversity of the repertoire of T-cell receptor (TCR) genes is much more limited than that observed in αβ T cells or γδ T cells that reside in secondary lymphoid organs (3). Furthermore, the repertoire of expressed variable region genes differs strikingly in the various anatomical locations. It is believed that the highly restricted TCRs expressed by different subsets of γδ T cells enable them to recognize ligands that are specifically expressed in infected, diseased, or stressed cells in those anatomical sites (3, 4). Early T-cell development in the thymus is characterized

by progressive waves of differentiation of distinct γδ subsets characterized by expression of specific Vγ and Vδ gene segments. The underlying processes presumably evolved to produce functionally distinct sets of γδ T cells in an organized fashion.

A major focus of our research on γδ T cells over the years has been the molecular and cellular processes that dictate the highly organized differentiation of γδ cells and γδ subsets in mice. Therefore, we have addressed the regulation of Vγ gene rearrangements in one of the clusters of murine TCRγ genes, the Cγ1 cluster (*Fig. 1*). All the V genes in this cluster rearrange to a single Jγ gene segment (Jγ1), yet occur in a strikingly ordered fashion over the course of ontogeny (*Fig. 1*). Thus, this cluster includes Vγ genes expressed by cells that arise at the fetal stage (Vγ3 and Vγ4) and at the adult stage (Vγ2 and Vγ5), using the nomenclature described by Garman *et al.* (5). We chose this cluster because its complex developmental regulation was accompanied by a compact size (approximately 50 kb) and relatively simple composition (four Vγ genes and one Jγ and Cγ gene), suggesting that it would be amenable to molecular analysis. In this review article, we describe findings from our laboratory and others, which have led to a greater understanding of many of these underlying processes. In addition, another focus of the laboratory has been the decision made by precursor cells at the adult stage of T-cell development to differentiate along the αβ versus γδ lineage. Overall, the findings show that programmed differentiation events and cellular selection processes work together in a systematic fashion to produce functional γδ (and αβ) T-cell sets.

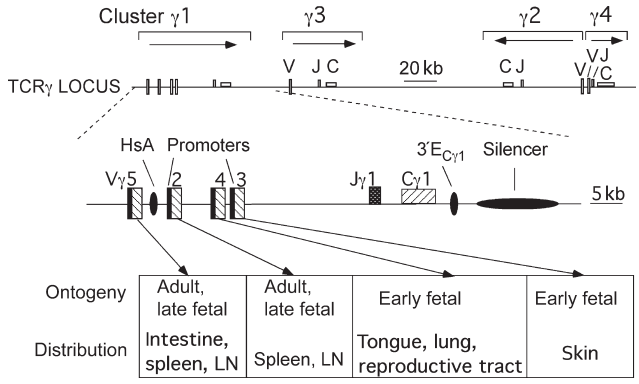

***Fig. 1.* Organization of mouse TCRγ genes and *cis*-acting regulatory elements, the developmental pattern of Vγ gene rearrangements, and the peripheral localization of specific subsets of γδ T cells defined by Vγ expression.** The locus consists of three functional clusters (γ1, γ2, and γ4) and a non-functional one (γ3). The γ1 cluster, explored in this review in detail, is expanded to show specific gene segments and regulatory elements (2, 4, 16).

Overview of γδ T-cell development in mice

Role of TCR expression in commitment of thymocytes to differentiate into αβ T cells versus γδ T cells

γδ T cells and αβ T cells arise from a common progenitor cell in the thymus. The molecular events leading to the lineage decision of developing $CD4^-CD8^-$ thymocytes to differentiate into γδ T cells versus αβ cells have not been fully resolved. It is clear that the decision does not reflect a specialization in TCR gene rearrangements, such that one subset of cells rearranges TCR γ and δ genes while another rearranges TCRβ genes, the latter event being necessary for expression of the pre-TCR that is important for early stages of αβ T-cell development. Indeed, studies show that complete rearrangements of TCR γ, δ, and β genes are present in both mature αβ and γδ T cells. Furthermore, complete rearrangements (V–D–J or V–J) occur at about the same time in T-cell development (6, 7).

Most TCR gene rearrangements result in joining of the V and the J segments in the incorrect translational reading frame and are therefore non-productive, so the fact that a cell makes a particular type of rearrangement is not an indication that it can ever express a corresponding TCR chain. Approximately 33% of complete rearrangements are productive. The randomness underlying the creation of a productive TCR gene rearrangement has been envisaged as one plausible determinant of lineage in this instance. Indeed, we and others reported evidence that developing T cells that express a functional γδ TCR are generally excluded from the αβ lineage (8–10). Whereas these data are at least partially consistent with the notion that the expressed TCR dictates the alternative lineage fate decision of the cell (αβ versus γδ), they do not prove this hypothesis. The data are also consistent with the possibility that the expressed TCR simply enables the survival and further differentiation of cells that already made a lineage decision consistent with the identity of the expressed TCR. For example, progenitor cells may differentiate into αβ- or γδ-committed populations before TCR gene rearrangement occurs in development. Further successful differentiation may only occur in the subset of each population that subsequently succeeds in productively rearranging and expressing a TCR that matches its predetermined lineage.

A study from our group provided evidence for the notion that lineage determination to some extent is independent of the type of TCR expressed (11). At the double-negative 2 (DN2) stage of thymocyte development (phenotype $CD3^-CD4^-CD8^-CD25^+CD44^{hi}$), at which stage complete TCR gene rearrangements are not yet made in the great majority of cells, cells that express high or low levels of the interleukin-7

receptor (IL-7R) on the cell surface can be distinguished. Analysis of the developmental potential of these cells, based on intrathymic injection experiments, showed that the IL-7R^{hi} DN2 cells were more likely to differentiate into γδ T cells, whereas IL-7R^{low} cells were more likely to differentiate into αβ T cells (11). These data suggested that some DN2 cells exhibit a bias in their lineage potential at a stage before TCRs are ever expressed on the cell surface. Recent clonal analysis of the potential of DN2 cells in a Notch ligand-dependent cell culture system arrived at a similar conclusion (12).

Other recent studies have focused on whether lineage determination might result from differences in the quality or strength of the signal propagated by the pre-TCR versus the γδ TCR (13–15). These studies provided strong evidence that the strength of signal delivered by the TCR in developing T cells has a major impact on the outcome of the process. The γδ TCR appears to provide stronger signals that promote γδ TCR development, whereas weaker signals associated with pre-TCR signaling promote the development of αβ T cells (14, 15). The effects of TCR signal strength are not necessarily incompatible with the aforementioned TCR-independent bias in lineage potential. Effective rescue of γδ-committed precursors may simply require a stronger TCR signal, whereas rescue of αβ-committed precursors may require a weaker TCR signal. Alternatively, it was suggested that the bias in lineage potential, which arises early in DN2 cell differentiation may be reversible in the case of a sufficiently strong instructive signal at a later stage in the process (11). Success in identifying the molecular signals responsible for lineage determination should ultimately resolve this issue in a definitive fashion.

Differentiation of subsets of γδ T cells

Many tissue-specific γδ T cells express different specific subsets of TCRs with little or no diversity (2, 4, 16, 17). At one extreme, nearly all γδ T cells in the epidermal epithelium express an identical ('canonical') γδ TCR composed of Vγ3–Jγ1Cγ1 and Vδ1–Dδ2–Jδ2Cδ chains with identical junctional sequences (18). These skin γδ T cells are usually called dendritic epidermal T cells (DETCs). Similarly, γδ T cells in the tongue, lung, and reproductive tract epithelium express a canonical Vγ4–Jγ1Cγ1/Vδ1–Dδ2–Jδ2Cδ TCR (19). In a less exclusive situation, the epithelium of the small intestine contains both γδ and αβ T cells (20, 21) and the γδ T cells predominantly express Vγ5$^+$ and Vγ1.1$^+$ Vγ chains, but with diverse junctional sequences (22). Finally, in secondary lymphoid organs, where γδ T cells represent only a small fraction of total T cells, γδ TCRs predominantly include Vγ2, Vγ1.1, and Vγ1.2 V regions, although with extensive, even dramatic, junctional diversity (22, 23).

The different subsets of γδ T cells arise in the thymus at different stages of ontogeny. Vγ3$^+$ T cells are exclusively generated in the early fetal thymus, where they are the first T cells detected in ontogeny, around day 13 of gestation. From the fetal thymus, these γδ T cells migrate to the epidermal epithelium, where they expand locally to reach adult numbers and are normally maintained there for the life of the animal (18, 24). Vγ4$^+$ T cells are also produced in the early fetal thymus and they migrate to their epithelial destinations, such as the reproductive tract, tongue, and lung. Vγ3 and Vγ4 TCRs display a high frequency of invariant canonical junctional sequences in the γ chains due, in large part, to the absence in fetal thymocytes of terminal deoxynucleotidyl transferase (TdT), which is necessary for the addition of N-nucleotides at V–J junctions, coupled with a preference for rearrangement at the sites of microhomologies at the ends of the Vγ and Jγ gene segments (25, 26). Similar mechanisms have been invoked to account for canonical junctional sequences in the Vδ1 rearrangements that encode the dominant TCRδ chain expressed by both Vγ3$^+$ and Vγ4$^+$ T cells (27). Positive selection during development in the thymus may also play a role in allowing only those cells with the canonical junctional sequences to mature.

Vγ3$^+$ and Vγ4$^+$ T cells are not generated in the adult thymus. Instead, the adult thymus switches to the production of γδ T cells expressing Vγ2, Vγ1.1, and Vγ1.2 gene segments with highly diverse junctional sequences, which localize to the secondary lymphoid organs on exiting the thymus (1, 28–30). Adult Vγ5$^+$ cells localize to the intestinal epithelium as well as secondary lymphoid organs (22). There are also reports suggesting that some intestinal epithelial Vγ5$^+$ γδ T cells develop extrathymically (31, 32).

The sequential generation of specific subsets of γδ T cells at different stages of ontogeny is a fixed developmental program that cannot be easily reordered. For example, disruption of the generation of γδ T cells in the early fetal thymus by injecting anti-γδ-TCR antibody into pregnant mothers results in the absence of DETCs in adult mice (3). Furthermore, DETCs cannot be replaced with bone marrow stem cells in adult mice, following full-body irradiation and reconstitution (33).

Programmed rearrangement of TCR Vγ gene segments

Evidence that ordered Vγ gene rearrangement is a programmed process

The appearance of cells with different Vγ gene rearrangements at different stages of development could reflect a developmental program that controls the rearrangement machinery or could be

because of a cellular selection process that promotes the survival of different γδ subsets at different stages of development, or both. Several lines of evidence showed that developmentally regulated Vγ gene segment recombination is a developmentally programmed process. Initially, scrutiny of Vγ gene rearrangements showed that non-productive Vγ gene rearrangements in cell lines were usually of the same type as the productive rearrangements (16). Because non-productive rearrangements cannot be subject to cellular selection processes, this finding suggested that developing γδ T cells are programmed to choose specific Vγ gene segments for rearrangement. Consistent with an intrinsic program of Vγ gene rearrangement, we later found that germline transcription of Vγ genes correlates with their rearrangement pattern. Germline transcription is usually correlated with greater accessibility of genes in chromatin to recombinases, suggesting a possible link between gene accessibility and rearrangement in this system (34).

As a more direct test of whether the ordered pattern of TCRγ gene rearrangement occurs in the absence of cellular selection, we generated several lines of transgenic mice that harbored a 39-kb genomic transgene (called γB) that spans most of the unrearranged TCRγ Cγ1 cluster. Each of the three Vγ genes in the transgene (Vγ2, Vγ4, and Vγ3) contained a frameshift mutation that prevented functional expression of the corresponding V region (35). Despite the fact that the transgene could not encode intact TCRγ chains that might influence the selection of cells in which they occurred, rearrangements of the Vγ genes in the reporter transgene, like rearrangements of the endogenous Vγ genes, appeared at the appropriate stage in thymic development. In a different but related approach, it was shown that a targeted deletion of the TCRδ gene, which abrogates the formation of functional γδ TCRs (although not protein expression of TCRγ genes), did not disrupt the normal developmental pattern of Vγ or Vδ gene rearrangements (27). These data provided compelling evidence that programmed rearrangement of Vγ genes is a major determinant of the ordered generation of different subsets of γδ T cells at different stages of ontogeny.

Regulation of programmed Vγ gene rearrangement

The finding that Vγ and Vδ rearrangement patterns are programmed independent of cellular selection led us to investigate the underlying molecular mechanisms. As possible determining factors of the rearrangement patterns, we assessed both the role of *cis*-acting regulatory elements in the genes and the location of the V genes within the cluster. Both these factors have been proposed or implicated as determinants of rearrangement patterns in studies of other rearranging genes.

Over the years, we and others have identified and characterized several *cis*-acting elements in the TCRγ Cγ1 cluster, which play unique and overlapping roles in regulating the Vγ gene rearrangement process as well as transcription and expression of TCRγ genes (*Fig. 1*). The roles and properties of some of these elements are described in more detail and in different contexts later in this review, but their role in ordered rearrangement is addressed first. The elements include the promoter regions of each Vγ gene, two enhancer-like elements (3′-ECγ1 and HsA) that cooperate to regulate transcription of the locus, and a putative silencer element that prevents TCRγ expression in αβ T cells.

Vγ gene promoter regions determine the rearrangement pattern in the adult thymus

Numerous studies have shown that the accessibility of immunoglobulin (Ig) and TCR genes is a critical determinant of rearrangement patterns during development. Accessibility refers to whether chromatin is in a sufficiently open configuration for recombinase to access the recombination signal sequences (RSSs) that flank the gene. Accessibility usually correlates with transcription of the unrearranged genes (germline transcription). As already mentioned, early studies found a correlation between germline transcription and rearrangement of individual Vγ genes in the adult thymus. Specifically, germline transcription from the unrearranged Vγ3 gene was strongly suppressed at this stage, while germline transcription from the unrearranged Vγ2 gene remained high, suggesting greater accessibility of the Vγ2 gene. Subsequent studies of adult thymocytes substantiated the conclusion that the Vγ2 gene is more accessible than the Vγ3 gene at this stage, including a report that showed a much greater degree of histone acetylation in the vicinity of the Vγ2 gene than in the vicinity of the Vγ3 gene (36). Furthermore, it was shown that an inhibitor of histone deacetylation, which is expected to increase the acetylation of the Vγ3 gene and therefore its accessibility, stimulated increased levels of Vγ3 rearrangements in the adult thymus (36).

Differences in accessibility of the different Vγ genes suggested that *cis*-acting regulatory elements associated with the Vγ genes might be responsible for controlling Vγ gene rearrangement at the adult stage. Therefore, we designed experiments to test whether the promoter segments of these genes play a role in imposing the adult pattern of rearrangement. For this purpose, we generated a new transgene based on the γB transgene discussed above. The frameshift mutations in the Vγ gene segments of this construct should prevent interference in the outcome of an experiment by cellular

selection events. The new transgene, called γB-Pr-Sw, was identical to γB except that the promoter segments of the Vγ3 and Vγ2 genes were reciprocally swapped (35) (*Fig.* 2). Compared with the γB transgene, the rearrangement patterns of Vγ2 versus Vγ3 were reversed in the adult thymus of the γB-Pr-Sw transgenic mice. Consistent with the reversed pattern of rearrangement, the pattern of germline transcription of the Vγ2 and Vγ3 genes was also reversed in adult thymocytes of γB-Pr-Sw mice, as compared with γB mice. These data showed that the promoter regions of the individual Vγ genes determine the preference for Vγ2 rearrangements in the adult thymus, probably by controlling the accessibility of the corresponding Vγ genes to the recombinase enzymes.

A distinct mechanism for establishing the rearrangement pattern in the fetal thymus

Our initial expectations were that the rearrangement patterns in the fetal and adult thymus would reflect the same basic mechanism and that the switch between these two patterns would consist of altered regulation of this basic mechanism. However, the notion that promoter activity is responsible for the fetal rearrangement pattern was rapidly refuted by analysis of γB-Pr-Sw mice. Despite the reversed rearrangement pattern in the adult thymus of these mice, the exchanged promoter segments had no significant effect on transgene rearrangements in the fetal thymus, where Vγ3 rearrangements predominated to a similar extent as in the γB transgenic mice. These results indicated that differential promoter activity cannot account for the predominance of Vγ3 (and Vγ4) rearrangements in the fetal thymus. We therefore considered other explanations.

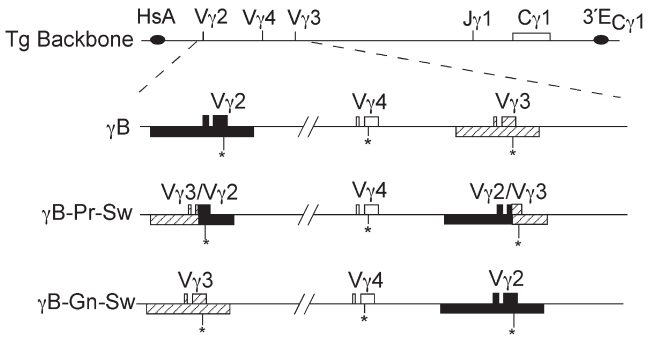

***Fig.* 2. Configuration of γB, γB-Pr-Sw, and γB-Gn-Sw transgenes.** The transgenes include all contiguous sequence as present in the endogenous locus. The configuration of γB is identical to that of the endogenous locus. In γB-Pr-Sw, the promoter regions and leader exon (which encodes the signal peptide) of the Vγ2 and Vγ3 gene segments were exchanged. In γB-Gn-Sw, 1.6- to 2.3-kb fragments containing the entire Vγ3 and Vγ2 gene segments were exchanged, including the promoters and RSS. The asterisks indicate the sites where frameshift mutations were introduced into each Vγ gene segment. [Reprinted with permission from Proceedings of the National Academy of Sciences, USA (48).]

The location of Vγ genes in the TCRγ locus is a major determinant of the rearrangement pattern in the fetal thymus

An obvious candidate as a determinant of the fetal rearrangement pattern is the location of Vγ genes within the TCRCγ1 locus. Previous studies have documented preferential rearrangement at the fetal stage of 3′- (D- or J-proximal) V genes in the IgH and TCRδ loci, in addition to the comparable findings in the TCRγ locus already discussed above (5, 29, 37–39). In the IgH locus, an IL-7-dependent shift in the adult stage to rearrangement of 5′-VH genes has been documented (40, 41). However, other studies indicate that even in the adult stage, newly generated B cells show a bias for rearrangement of 3′-VH gene segments (42, 43). Based on transfections of cell lines with plasmids containing various recombination substrates, it was proposed that the preferential rearrangement of 3′-VH genes in the IgH locus was because of the potency of the RSS associated with these genes (44). In contrast, another study showed that the rate of recombination observed *in vivo* in the natural locus did not correlate with the rate of recombination of corresponding substrates in transfected cell lines, but did correlate with the position of the gene in the locus (45). The consistency of these latter findings with ours suggested that location of a V gene within a V gene array might be a major determinant of rearrangement at the fetal stage.

To directly test the role of Vγ gene location in rearrangement, we generated a new transgene in which the entire Vγ2 and Vγ3 gene segments were swapped within the γB transgene (*Fig.* 2). The exchanged fragments were approximately 1.6–2.3 kb in length and included the promoter regions, coding regions, and some downstream sequence including the RSS. This transgene, called γB-Gn-Sw, showed a reversed pattern of rearrangement in the fetal thymus compared with γB, such that Vγ2 rearrangements were several fold more frequent than Vγ3 rearrangements (46). These data clearly showed that the relative location of Vγ genes is a dominant factor in determining their rearrangement levels in the fetal thymus. In recent unpublished experiments, we generated gene-targeted mice that have corroborated this conclusion (our unpublished data).

How does the position of Vγ genes in the locus determine rearrangement levels? One model to consider is that the Vγ gene position somehow determines whether the Vγ gene is in an open chromatin configuration and is therefore accessible to the recombinase machinery and transcription apparatus. This model is reminiscent of a study showing that proximity of

globin genes to the β globin locus-control region (LCR) determines the extent of globin gene transcription in the early yolk-sac stage of erythropoiesis (47). We have shown, however, that combined deletion of both known enhancers in the Cγ1 locus, 3′-ECγ1 and HsA did not substantially perturb the fetal or adult patterns of Vγ gene rearrangement, nor did it cause a major reduction in the extent of Vγ gene rearrangement (48). Therefore, proximity of the V genes to these elements cannot be a necessary determinant of the patterns of rearrangement. While it might be proposed that differential accessibility is controlled by proximity to a distinct unidentified regulatory element, direct tests showed that gene position did not alter the accessibility of the Vγ genes at the fetal stage, even as it did alter the tendency of the Vγ genes to rearrange. Thus, comparing fetal thymocytes from γB-Gn-Sw transgenic mice with those from γB transgenic mice, we found no significant difference in germline transcription or restriction enzyme accessibility of the Vγ2 or Vγ3 genes (46). Consistent with the transgenic studies, in the endogenous TCRγ locus, both genes showed high levels of germline transcription and histone acetylation in fetal thymocytes (36, our unpublished observations). All these data are in agreement that both Vγ2 and Vγ3 genes are 'open' and accessible in the natural locus at the fetal stage and suggest that the regulatory mechanism that dictates differential Vγ gene rearrangement at the fetal stage differs from that in the adult stage, where regulated accessibility of the Vγ genes appears to play a dominant role.

Models of regulated Vγ gene rearrangement

Two specific mechanisms are under consideration to account for preferential rearrangement of the 3′-Vγ gene at the fetal stage. One possibility, reminiscent of the one first proposed to account for preferential rearrangement of 3′-VH genes (37, 38), is that rearrangement at the Cγ1 cluster involves some form of one-dimensional 'tracking' mechanism along the DNA by the recombinase, in which downstream Vγ genes are encountered before upstream genes because of their greater proximity to the Jγ gene segment. A second possibility is that the 3′-Vγ gene RSS, being more closely tethered to Jγ1 in genomic DNA, is more likely to collide with the Jγ1 gene RSS than a 5′ Vγ gene RSS during three-dimensional diffusion, initiating rearrangement.

Whatever the specific molecular mechanism to account for the fetal pattern of rearrangement is, the available data support the general model depicted in *Fig.* 3 to account for the 'developmental switch' in Vγ gene rearrangement that occurs in ontogeny. The model proposes that in the fetal thymus, the whole TCRγ locus is open for rearrangement, but the downstream Vγ3 (and Vγ4) genes have a competitive advantage

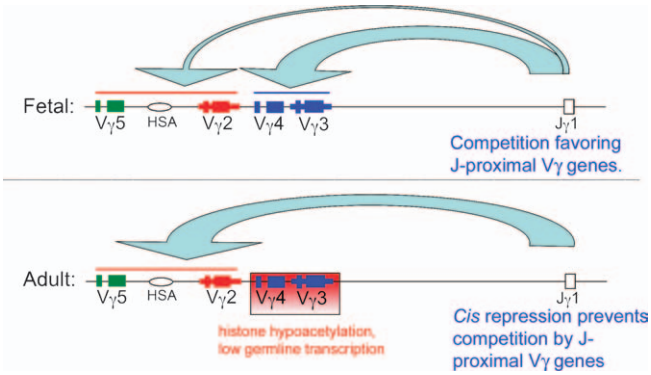

Fig. 3. A model of programmed Vγ gene rearrangement. At the fetal stage, both the 5′- and the 3′-Vγ genes are in an open 'accessible' chromatin configuration. Rearrangement of the 3′-Vγ genes (Vγ3 and Vγ4) is preferred at the fetal stage because of their location in the cluster, possibly related to their proximity to Jγ1. This location preference may reflect competition of the Vγ gene segments for the rearrangement apparatus. At the adult stage, events involving the specific Vγ promoters result in suppressed accessibility of Vγ3 and Vγ4. Rearrangement of 5′-Vγ genes is strongly increased at this stage, possibly because the inaccessible Vγ genes can no longer compete with the 5′-gene segments.

because of their proximity to the Jγ1 gene and, therefore, are preferentially rearranged over other upstream Vγ genes at the fetal stage. In the adult thymus, as a result of the activity of the associated promoter elements, the upstream Vγ2 (and Vγ5) genes are maintained in an open and accessible state, while the accessibility of the downstream Vγ3 (and 4) genes is suppressed. The altered accessibility shifts the balance in favor of rearrangement of the upstream Vγ2 (and Vγ5) genes that are therefore preferentially rearranged in the adult thymus.

The potential role of transcription factor E2A in regulating Vγ gene rearrangement

The role of specific transcription factors in regulating *cis*-acting elements in the TCRγ locus has not been comprehensively studied. However, studies have implicated the E2A transcription factor in the process, specifically in imparting the adult pattern of Vγ gene rearrangement. E2A is a member of the basic helix-loop-helix transcription factor family and has been found to play various important roles in regulating lymphocyte development (49). In mice lacking E2A, Vγ2 gene rearrangements were decreased in the adult thymus, while Vγ3 gene rearrangements were increased (50). The switch in Vγ2 versus Vγ3 rearrangement in E2A knockout mice was associated with a corresponding change in the germline transcription of these Vγ genes (50), suggesting that E2A contributes to the programmed Vγ gene rearrangement pattern at the adult stage by differentially regulating the accessibility of the Vγ2 versus Vγ3 genes. Whereas little effect of E2A deficiency on Vγ gene

rearrangement was noted in the fetal thymus at a very late stage in fetal ontogeny (day 19 of gestation) (50), we observed a substantial defect in rearrangement of both the Vγ3 and the Vγ2 genes in the early fetal thymus (day 15), at a time when Vγ3 rearrangements normally predominate (N. Xiong, C. Murre, D. H. Raulet, unpublished data). These findings suggest that E2A plays distinct roles in regulating the development of γδ T cells at different stages in ontogeny.

E2A may interact directly with *cis*-acting elements in the TCRγ locus to regulate rearrangement of Vγ genes differentially. It was reported, for example, that E2A sites are present in the RSS of most Vγ and Vδ gene segments (50), and candidate E2A sites have been identified in the promoter regions of Vγ2, Vγ3, and Vγ4 genes. The sites in the promoter regions might be more important, judging from the aforementioned evidence that the adult pattern of rearrangement is dependent on the promoter sequences and not on the Vγ gene segments or RSS. However, evidence that E2A acts directly on Vγ genes *in vivo* is lacking.

To help define *cis*-acting elements through which E2A may act in regulating Vγ gene rearrangement *in vivo*, we crossed E2A knockout mice with γB transgenic mice. In adult thymocytes from γB transgenic mice, E2A deficiency resulted in an enhancement in transgene Vγ3 rearrangements and a reduction in transgene Vγ2 rearrangements, much as it does for the endogenous Vγ genes (*Table 1*). These data are consistent with the possibility that E2A interacts with *cis*-acting elements within the span of the γB transgene to regulate Vγ gene rearrangement.

To address whether E2A regulates rearrangement through interaction with the promoter regions of Vγ genes, we crossed the E2A knockout mice with γB-Pr-Sw and γB-Gn-Sw transgenic mice. The rationale of this analysis was that if the promoter regions of Vγ genes are the dominant interaction sites for E2A to regulate rearrangement of the corresponding Vγ genes, E2A deficiency should alter rearrangement of Vγ genes in these transgenes according to the origin of the promoter regions (Vγ2 or Vγ3) that flank the Vγ gene, not the origin of the Vγ gene itself. This prediction was borne out in the case of the Vγ2 promoter, in which we observed decreased rearrangements of those Vγ genes in each construct that was flanked by the Vγ2 promoter fragment (Vγ2 in the γB-Gn-Sw transgene and Vγ3 in the γB-Pr-Sw transgene) (*Table 1*, N. Xiong, C. Murre, D. H. Raulet, unpublished data). Hence, it is plausible that E2A acts directly on the Vγ2 promoter to enhance rearrangement at the adult stage, perhaps by maintaining accessibility of this region of the locus. In contrast, the prediction was not borne out in the case of the Vγ3 promoter because in both the γB-Gn-Sw and the γB-Pr-Sw transgenes, E2A deficiency caused a decrease rather than an increase in rearrangement of those Vγ genes that were flanked by the Vγ3 promoter (*Table 1*, N. Xiong, Murre, D. H. Raulet, unpublished data). These data suggest that the Vγ3 promoter region is not a dominant interaction site for E2A regulation of Vγ gene rearrangement. In both these transgenes, the Vγ3 promoter was relocalized to the 5′ position normally occupied by the Vγ2 promoter. A possible explanation for the puzzling results with these transgenes is that E2A acts on another site near this upstream region to enhance rearrangement in wildtype cells. A deeper understanding of the role of E2A in regulating this locus will require detailed analysis of the roles of the various E2A sites in the context of endogenous genomic DNA.

Role of enhancer elements in promoting rearrangement and transcription of TCRγ genes

Likely redundancy of enhancer elements in regulating TCRγ gene rearrangement

Although the local Vγ promoters influence the accessibility of the corresponding Vγ genes, there is likely to be a higher order regulation of the chromatin condensation of the TCRγ locus by additional *cis*-acting elements, including the aforementioned enhancer elements HsA and 3′-ECγ1. Indeed, deletion of small DNA segments containing HsA and 3′-ECγ1 from a genomic transgene abrogated rearrangement of the transgene *in vivo* (51), suggesting the roles of HsA and 3′-ECγ1 in maintaining the chromatin in an open configuration for rearrangement. Surprisingly, however, simultaneous deletion of both elements in the endogenous locus by gene targeting only modestly

***Table 1*. Effect of E2A deficiency on endogenous and transgenic Vγ2 and Vγ3 gene rearrangements**

	Endogenous		γB		γB-Pr-Sw		γB-Gn-Sw	
	Vγ2	Vγ3	Vγ2	Vγ3	Vγ2	Vγ3	Vγ2	Vγ3
Change in $E2A^{-/-}$ mice*	↓ 2–10†	↑ >3	↓ 3–5	↑ 3–5	↓ 3–5	↓ 2–3	↓ 3	↓ 4–5

*Change in $E2A^{-/-}$ mice indicates increased (↑ fold change) or decreased (↓ fold change) *Vγ* gene rearrangements in $E2A^{-/-}$ mice compared with that in $E2A^{+/+}$ littermates.
†The data for endogenous Vγ2 rearrangements in $E2A^{-/-}$ mice are from Bain *et al.* (50).

inhibited Vγ gene rearrangement, and knocking out either element in isolation had no effect on rearrangement or accessibility (48). Considering that rearrangement of other Ig/TCR genes requires enhancer elements (52), it appears likely that an unidentified *cis*-acting element(s) necessary for recombination exists in the endogenous Cγ1 locus, separate from the segment that comprised our transgene. However, the putative additional regulatory element(s) cannot, by itself, support transcription of most of these rearranged Vγ genes because transcription of rearranged Vγ2, Vγ3, and Vγ4 genes was nearly abolished in the HsA/3′-ECγ1 double knockout mice. Thus, whereas HsA and 3′-ECγ1 together possess properties of an LCR and are capable of maintaining the genomic accessibility of an ectopically integrated TCRγ transgene, they probably function redundantly with other *cis*-acting regulatory elements in the endogenous locus to promote genomic accessibility for gene rearrangement.

There is an indication that the putative additional regulatory element(s) that promotes recombination in the Cγ1 cluster may reside upstream of HsA. In gene-targeted mice in which HsA was replaced by a *PGK-neo* gene cassette, development of $V\gamma2^+$ cells was severely reduced (data not shown), whereas deletion of PGK-*neo* resulted in restored numbers of $V\gamma2^+$ cells. In contrast, replacement of 3′-ECγ1 with *PGK-neo* did not significantly affect γδ T-cell development (data not shown). Because insertion of a *PGK-neo* gene between a *cis*-acting element and the gene it regulates often inhibits the function of the element (53), these data may imply the existence of an unidentified regulatory element(s) upstream of HsA, which plays a role in Vγ gene recombination and/or transcription. These results together with the results described earlier in this review suggest that V–J rearrangement in the Cγ1 cluster is regulated by multiple *cis*-acting regulatory elements that function in a hierarchical complex fashion, in which some elements regulate larger segments of the cluster while others play a fine tuning role by regulating accessibility of individual Vγ genes.

Redundant roles of HsA and 3′-ECγ1 in regulating TCRγ gene transcription and γδ T-cell development in the thymus

As expected, in addition to their roles in regulating Vγ gene rearrangement, *cis*-acting elements in the Cγ1 cluster are also important in controlling expression of the rearranged TCRγ genes and development of corresponding γδ T cells. The various elements contribute differentially to rearrangement versus transcription. In particular, although the HsA and 3′-ECγ1 enhancer elements are not required for rearrangement in the endogenous locus, they play critical roles in transcription.

The roles of HsA and 3′-ECγ1 in regulating TCRγ gene expression and γδ T-cell development were probed with gene-targeted mice lacking one or both of these elements (48). Although germline deletion of HsA and 3′-ECγ1 only impaired Vγ gene rearrangement modestly (by twofold), the numbers of $V\gamma2^+$ and $V\gamma3^+$ (and presumably $V\gamma4^+$) γδ T cells in the thymus were dramatically decreased by more than 20-fold. Impaired development of these subsets was correlated with a dramatic reduction in the transcription of the TCRγ genes located between HsA and 3′-ECγ1, including Vγ2, Vγ4, and Vγ3 genes, whereas the upstream Vγ5 and Vγ genes in other clusters were not substantially affected. Interestingly, separate deletions of HsA or 3′-ECγ1 had little or no effect on transcription of TCRγ genes and development of γδ T cells in the thymus. This study, along with a study of enhancer elements in the Igκ locus, provided the first direct evidence of redundantly functioning enhancer elements in regulating transcription of an antigen-receptor gene (48). It is noteworthy that although expression of rearranged Vγ2–4 genes was highly impaired in the double knockout mice, a few T cells expressing Vγ2 and Vγ3 at normal levels were detectable in these mice, suggesting that while initiation of gene expression is greatly reduced in the absence of these elements, a few cells that succeed in initiating gene expression can express adequate levels of the corresponding mRNAs.

A unique function of HsA in upregulating TCRγ gene expression in peripheral $V\gamma2^+$ γδ T cells

Although separate deletions of HsA or 3′-ECγ1 did not impair expression of TCRγ genes in the thymus or thymic development of γδ T cells, deleting HsA resulted in a significant defect in the periphery. The number of $V\gamma2^+$ γδ T cells in the spleen or lymph nodes of HsA knockout mice was reduced by a factor of 4 when compared with wildtype mice (48). No such defect was evident in 3′-ECγ1 knockout mice, suggesting a unique role for HsA at the post-thymic stage of γδ T-cell development.

Analysis of the cell surface phenotype of γδ T cells provided a possible clue as to the cause of the depressed $V\gamma2^+$ γδ T-cell compartment in the periphery of HsA knockout mice. Most peripheral $V\gamma2^+$ γδ T cells in wildtype mice have probably been previously exposed to antigen, as they exhibit a memory phenotype characterized by expression of CD44 but not CD62L (54) (*Fig.* 4). In contrast, the majority of residual $V\gamma2^+$ T cells in HsA knockout mice were $CD44^{low}$ cells, suggesting that memory $V\gamma2^+$ T cells either fail to form or fail to survive in the absence of HsA. As a possible explanation for this defect, it may be significant that in wildtype mice, the TCR levels were elevated by approximately threefold on $CD44^+V\gamma2^+$ T cells as

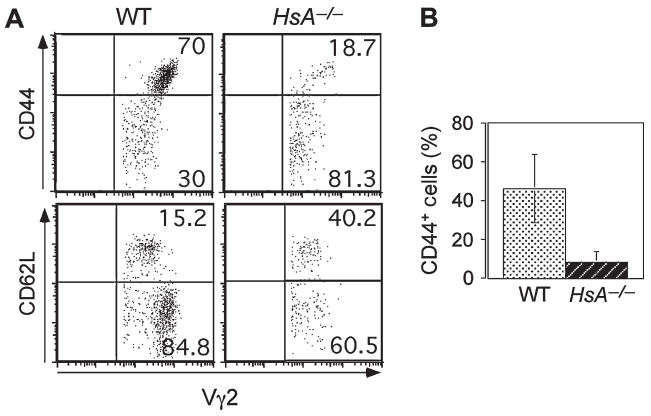

***Fig. 4.* Role of HsA in peripheral development of Vγ2$^+$ cells.** (A) Reduced percentage of CD44$^+$ cells or CD62L$^-$ cells among Vγ2$^+$ lymph node cells in *HsA*$^{-/-}$ mice. Gated Vγ2$^+$ cells are shown. (B) Mean percentages (± standard deviation) of CD44$^+$ cells among Vγ2$^+$ cells in lymph nodes of wildtype versus *HsA*$^{-/-}$ mice ($n > 5$). The total number of lymph node Vγ2$^+$ cells was also reduced (by 4- to 5-fold) in the *HsA*$^{-/-}$ mice.

compared with CD44$^-$Vγ2$^+$ T cells, suggesting that formation of Vγ2$^+$ memory cells is associated with increased levels of Vγ2$^+$ TCRs. Memory αβ T cells usually show depressed TCR levels. In HsA knockout mice, γδ TCR levels (and transcripts) were relatively low on residual Vγ2$^+$ T cells, consistent with the possibility that upregulation of the Vγ2$^+$ TCR on memory cells requires the action of HsA (48). We propose that TCR upregulation and consequent increased TCR signaling may be necessary for differentiation and/or maintenance of Vγ2$^+$ memory cells.

Thymic selection and development of tissue-specific γδ T cells

The findings discussed above have documented the role of programmed Vγ gene rearrangement in the sequential development of γδ subtypes during ontogeny. In principle, programmed Vγ (and Vδ) rearrangement might be a sufficient explanation for the sequential appearance of γδ T-cell subsets, but it has long been proposed that cellular selection also plays a role in the process. Findings have been somewhat contradictory on this point. A related issue concerns how the appearance of γδ T-cell subsets in the thymus is coordinated with the localization of these cells to distinct peripheral sites and, potentially, to the acquisition of different functional activities. Recent studies provide compelling evidence that thymic selection plays a key role in the development of at least some γδ T-cell subsets in the periphery, especially the invariant γδ T-cell subsets that arise in the fetal thymus and in localization of these γδ T cells to their preferred home in the periphery. Studies examining selection in the adult versus fetal thymus are reviewed separately.

Selection of γδ T cells in the adult thymus

The necessity for class I and class II major histocompatibility complex (MHC) molecules in the positive selection of αβ T cells in the thymus was clearly shown by the absence of these cells in mice lacking MHC class I and/or class II molecules (55, 56). In contrast, the absence of MHC class I and class II molecules had no observable effect on the development of γδ T cells in the thymus (56–58). These data suggested that most γδ T cells are not selected by classical MHC molecules or by non-classical class I molecules that depend on β2-microglobulin (β2m) for expression on the cell surface. However, it remained possible that MHC does play a role in selection of a subset of γδ T cells. Furthermore, the natural ligands for γδ T cells are in most cases not known and it remains possible that many or all the cells are positively selected by interactions with unidentified ligands in the thymus.

Because of limited knowledge of the natural ligands for γδ TCRs, many studies designed to examine whether developing γδ T cells undergo selection have used the G8 and KN6 transgenic mouse strains. The two transgenic strains harbor different γδ TCRs, although both include a Vγ2 chain. The two TCRs are specific for the same ligands, the T10 and T22 non-classical MHC class I molecules (59–62). T10 and T22 are encoded in the TL region of the MHC and are expressed highly in B6 mice (H-2^b haplotype) but only weakly in BALB/c mice (H-2^d haplotype) because of defective T22 gene expression in the latter strain (59). Studies with T22 tetramers show that a small subset of naturally arising mature peripheral γδ T cells in normal mice is specific for these same ligands (63).

Early studies with G8 and KN6 transgenic mice reported that the transgenic γδ T cells underwent negative selection in mice that express strong ligands for the receptors, such as the B6 strain, but not in mice that express a weak ligand, such as the BALB/c strain (64, 65). It has been much more controversial whether interactions of these transgenic γδ T cells with 'weak' ligands are necessary for positive selection of the cells. Several studies suggested that positive selection was necessary, based on the finding that transgenic γδ T cells developed much less efficiently in β2m-deficient mice, which lack surface expression of T10 and T22, than in BALB/c mice, which express a weak ligand (14, 66, 67). Another study attributed much of this difference to negative selection, because of variations in the genetic backgrounds of the mice under study (68). On top of this contradiction, a study that used T22 tetramers to directly assay the development of natural T10/T22-specific γδ T cells in non-transgenic mice concluded that development of these cells is not impaired in β2m-deficient mice (K. Jensen & Y. Chien, personal communication). In lieu of positive selection, these

authors attribute the relatively high frequency of T10/T22-reactive γδ T cells in normal mice to programmed rearrangement mechanisms that assemble a reactive TCR in a small but significant population of developing γδ T cells. Given the variability in experimental outcomes and the possibility that these TCRs may be positively selected by an unknown β2m-independent ligand, it remains uncertain whether positive selection is required for the development of T10/T22-reactive γδ T cells in the adult thymus.

Selection of γδ T cells in the fetal thymus

As an approach to investigate the requirement for thymic selection of γδ T cells in the early fetal thymus, especially the Vγ3$^+$ DETC population, several studies have investigated mice equipped with various γδ TCR transgenes non-native to the skin. The underlying assumption of most of these studies was that the transgenic TCR would exclude expression of TCRs normally found in the fetal thymus, especially the canonical Vγ3Vδ1 TCR found on most DETCs. Positive selection of DETCs, if necessary, should be aborted if the transgenic TCR had the inappropriate specificity. It was shown that γδ T cells expressing 'inappropriate' transgenic TCRs were able to develop normally into DETCs in the skin, suggesting that DETC development is not dependent on expression of Vγ3 or Vδ1 and therefore is not dependent on positive thymic selection by ligands that are specifically recognized only by the Vγ3Vδ1 DETC TCR (69, 70). The assumption that the transgenic TCR necessarily excludes expression of endogenous TCRs was later proven incorrect, however, raising doubts about the earlier conclusions. For example, whereas plentiful transgene-expressing skin-resident γδ T cells developed in Vδ6.3 transgenic mice, none developed when the transgene was crossed onto a TCRδ$^{-/-}$ background in which endogenous TCRδ chains cannot be expressed (71). These data suggested that development of the skin-resident γδ T cells depends on endogenously encoded TCRδ chains in the transgenic mice, such as the Vδ1 chain. This finding resurrected the possibility that DETC development requires positive selection on specific ligands in the thymus.

As a related approach to this question, the requirement for the Vγ3Vδ1 TCR in development of DETCs was assessed by knocking out the Vγ3 or Vδ1 genes. Disabling either of these genes did not prevent development of DETCs, proving that TCRs other than Vγ3Vδ1 are compatible with DETC development (72, 73). Analysis of γδ TCR$^+$ DETCs in the *Vγ3*$^{-/-}$ mice detected Vγ1.1, Vγ2, and Vγ5 paired Vδ1 and other Vδ chains. The possibility that the DETCs in Vγ3 knockout mice had undergone specific intrathymic positive selection was inferred from the finding that the TCRs on these DETCs had a specific 'idiotypic' marker detected with the 17D1 monoclonal antibody, a marker that is also found on Vγ3Vδ1 TCRs (72). However, this marker was absent on the DETCs that arose in Vδ1 knockout mice, suggesting that 17D1 reactivity might be associated with Vδ1-containing TCRs rather than with specificity of the TCR (73). A more compelling suggestion that the DETCs in Vγ3 knockout mice had undergone selection was the finding that these cells produced IL-2 when stimulated with keratinocyte cell lines, a specificity previously shown to be associated with DETCs, but not other subsets of γδ T cells (72).

Evidence for thymic positive selection of DETC precursors

The previous studies were consistent with the selection of DETC precursors, but did not distinguish whether the selection occurred in the skin itself or was the result of selection in the thymus. We recently used gene targeting to reinvestigate the requirement for specific Vγ chains in the development of DETCs. The results provided compelling evidence for selection of DETCs and clear evidence that it occurs in the thymus, and at the same time these studies showed a possible mechanism to couple thymic selection with emigration of the T cells to the epidermis (74).

We generated mice with a large deletion of the Cγ1 cluster that encompassed HsA, Vγ2, Vγ4, Vγ3, Jγ1, Cγ1, and 3′-ECγ1 (*234JCγ1*$^{-/-}$ mice). The remaining Cγ clusters remained intact, and Vγ1.1$^+$ and probably Vγ1.2$^+$ T cells developed normally in the thymus of the knockout mice, where they were first detected at days 15 and 16 of gestation. Normal numbers of γδ T cells, most of them Vγ1.1$^+$ cells, were detected in the spleen and intestines of these mice, but Vγ2$^+$, Vγ3$^+$, and Vγ5$^+$ T cells were absent, as expected. Remarkably, despite the abundance of γδ T cells in other peripheral sites and in the fetal thymus, there were virtually no TCRγδ$^+$ DETCs in the epidermis of these mice (74). Therefore, DETC development was dependent on expression of specific γ chains, consistent with a requirement for positive selection of the cells. These data were in contrast to the findings using Vγ3 knockout mice reported above, where Vγ1.1 and other γδ T cells were detected in the epidermis in lieu of Vγ3$^+$ T cells. The basis for this discrepancy in results is currently under investigation.

Additional evidence that the DETC precursors undergo specific positive selection and that selection occurs in the fetal thymus came from analysis of marker expression on fetal thymocytes. Consistent with earlier studies (75, 76), we found that approximately 70% of Vγ3$^+$ γδ T cells in the fetal thymus express various activation markers, including CD122, which is the β chain of the receptors for IL-2 and IL-5. We showed that CD122 expression on Vγ3$^+$ fetal thymocytes was correlated

with expression of Vδ1, the chain characteristic of DETCs (74). $V\gamma3^+$ fetal thymates lacking CD122 showed a much more diverse expression of TCR Vδ chains, indicating that CD122 expression occurred specifically on $V\gamma3^+V\delta1^+$ fetal thymocytes and not on thymocytes with Vγ3 paired to other TCRδ chains. Remarkably, CD122 was completely absent on the γδ T cells in the fetal thymus of $234JC\gamma1^{-/-}$ mice, which lack DETCs, showing a correlation between the expression of activation markers in the thymus and development of DETCs. Thus, expression of DETC-specific Vδ1 was associated with CD122 expression in the thymus, which was in turn correlated with DETC development. These data suggested that among fetal thymic precursors expressing various γδ TCRs, those expressing Vγ3 and Vδ1 engaged a thymic ligand, leading to expression of activation markers and ultimately development of DETCs.

While highly suggestive of selection, the correlations described above could theoretically arise by other mechanisms. However, as a result of an unexpected observation, we were able to provide strong additional evidence that these events reflected positive selection. As one approach to test whether the absence of DETC development in $234JC\gamma1^{-/-}$ mice was specifically because of the absence of Vγ3, we attempted to complement the $234JC\gamma1^{-/-}$ mice with a transgene encoding a rearranged functional Vγ2–Jγ1Cγ1 chain. Surprisingly, the transgene restored DETC development and all the resulting DETCs expressed the transgenic Vγ2 chain (74). While this finding could have been seen to resurrect the notion that DETC development does not depend on specific TCR expression, analysis of the DETCs showed instead a very specific TCR configuration: all the cells expressed Vγ2, as expected, and showed great enrichment for Vδ7 expression and little or no expression of other TCRδ chains, including Vδ1. Furthermore, the $V\gamma2^+V\delta7^+$ DETCs, but not peripheral γδ T cells, produced IL-2 when stimulated with keratinocyte cell lines, suggesting that the Vγ2Vδ7 receptor combination, like the Vγ3Vδ1 combination, confers specificity for a keratinocyte ligand. The story was brought full circle when the analysis of the Vγ2 transgenic/knockout mice showed that the Vγ2 transgene resulted in restored expression of CD122 on a subset of $V\gamma2^+$ fetal thymocytes. Furthermore, the $V\gamma2^+$ fetal thymocytes that expressed CD122 predominantly expressed Vδ7, whereas $V\gamma2^+$ fetal thymocytes that did not express CD122 expressed a diversity of Vδ genes. These data strongly supported the conclusion that only specific subsets of developing fetal thymic γδ T cells, including cells expressing the Vγ3Vδ1 pair and the Vγ2Vδ7 pair, exhibit reactivity with an unknown ligand that is expressed by fetal thymic stromal cells and probably shared with keratinocytes. Moreover, such reactivity leads to expression of CD122 on developing fetal thymocytes and is necessary for development of DETCs (74).

A recent report provides independent support for positive selection of DETC precursors in the thymus. A comparison of mouse strains showed normal development of $V\gamma3^+$ DETCs in FVB-Jax strain mice but none in the related FVB/N-Taconic strain, and chimera experiments showed that the defect in FVB/N-Taconic was consistent with the absence of a selecting ligand for DETCs in the fetal thymus (77). The identity of the putative selecting ligand has not yet been reported.

Preference for the Vγ3Vδ1 pair among DETCs in wildtype mice

If different VγVδ pairs are compatible with DETC development, why does the Vγ3Vδ1 pair predominate in wildtype mice? A likely possibility, already discussed above, is that the Vγ3Vδ1 pair is more likely to arise in the fetal thymus than other VγVδ pairs, as a result of favored rearrangement of these genes in the fetal period. However, this preference cannot fully account for the dominance of the Vγ3Vδ1 pair. In wildtype mice, a small but significant percentage of DETCs in the epidermis are $V\gamma3^-$ in the neonatal period, whereas such cells are nearly undetectable in the adult stage (73, 78). Thymic production of $V\gamma3^+$ cells ceases by the time of birth, so the change cannot reflect greater thymic production of $V\gamma3^+$ cells at the adult stage.

Other evidence suggests that the Vγ3Vδ1 TCR is favored among DETCs, even when it is not the most commonly expressed γδ TCR in the early fetal thymus. In the previously discussed gene-targeted mice in which the 3′-ECγ1 and HsA enhancer elements were simultaneously deleted in the Cγ1 cluster, the number of $V\gamma3^+$ T cells in the fetal thymus was reduced by fivefold to 20-fold at all time points, but $V\gamma1.1^+$ and $V\gamma5^+$ γδ T cells developed normally. Therefore, $V\gamma3^+$ T cells represented only a small percentage of γδ T cells in the fetal thymus of these mice (47) (Fig. 5). Nevertheless, approximately 75% of DETCs in the skin of adult knockout mice expressed Vγ3, suggesting selection for the canonical Vγ3Vδ1 TCR among DETCs (47) (Fig. 5).

Between the neonatal and adult period, DETCs are believed to expand greatly in the epidermis, and it is likely that the local environment in the skin is important for selective expansion of the $V\gamma3^+$ γδ T cells. The expansion could result from local engagement of the DETC TCR with epidermal ligands, although it is believed that the putative ligand is only expressed well in damaged or stressed epidermis (79). Alternatively, one could speculate that the $V\gamma3V\delta1^+$ DETCs may receive more potent positive selection signals in the fetal thymus, perhaps because of a higher affinity of the Vγ3Vδ1 TCR for the selecting ligand, which may enhance the ability of the cells to survive and expand later in the epidermis.

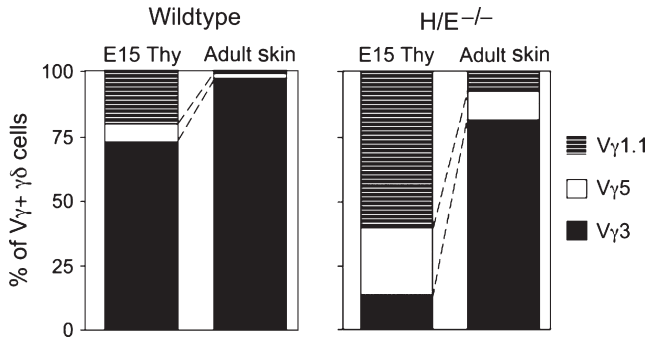

***Fig. 5.* The composition of different γδ subsets in the fetal thymus (day 15 of gestation) and adult skin (DETC), comparing wildtype littermates with $H/E^{-/-}$ mice lacking both known Cγ1 enhancer elements (HsA and 3′-ECγ1).** Most DETCs express Vγ3 in mice of both genotypes, but $V\gamma3^+$ cells are quite rare in the fetal thymus of mice. These data suggest a strong preference for $V\gamma3^+$ DETCs, even when these cells are produced in limiting numbers. Data are based on analysis of cells from three or more mice of each type on a mixed (C57BL/6/129Sv) genetic background.

Positive versus negative selection in the thymus may vary at different developmental stages

Differences have been noted in comparing negative selection in the thymus of adult and fetal (or neonatal) mice, which may be instructive in understanding the different roles of the γδ T-cell subsets that arise at these stages. In the adult thymus, T cells expressing the G8 γδ TCR underwent negative selection in mice that express the strong TL^b ligand, but little or no negative selection occurred in neonatal mice of the same genotype, despite indications that the G8 TCR was engaged with its ligand at that stage (80). Another report indicated that transgenic $V\gamma3V\delta1^+$ γδ T cells underwent negative selection in adult but not fetal thymi in certain genetic backgrounds (81). Finally, treatment of fetal thymic organ cultures with anti-γδ-TCR antibodies did not delete γδ T cells, whereas the same treatment causes deletion of adult thymic γδ T cells (82). These indications suggest that developing fetal thymic γδ T cells are highly resistant to negative selection. Perhaps this resistance reflects the specialization of the fetal thymus to select cells with highly defined, invariant specificities. The highly constrained repertoire of rearrangements and junctional diversification that occur in the fetal thymus is predicted to produce a large number of cells with receptors of the optimal affinity for the correct ligand. If the selecting ligands in the thymus are identical to or have the same affinity for the receptor as the ligands recognized by the cells in the periphery, negative selection, if it occurred, would presumably result in deletion of the cells. Because γδ T cells that arise at the adult stage are highly diverse, effective negative selection is probably necessary to reduce the risk of generating pathogenic autoreactive γδ T cells. These considerations may aid in understanding why and how the thymus specializes in producing different types of T cells at different stages of development.

How does positive selection in the fetal thymus enable development of γδ T cells in the epidermis?

Having provided evidence for positive selection of DETCs, we sought to understand how selection in the thymus is coordinated with T-cell development in the skin. In theory, thymic selection could influence migration from the thymus, migration to the epidermis, and survival and/or expansion in the epidermal environment.

Consistent with a role in lymphocyte migration, we found that positive selection in the fetal thymus resulted in a coordinated switch in expression of several chemokine and homing receptors in the selected cells (74). Chemokine receptor CCR10 and sphingosine 1-phosphate receptor-1 (S1P1) were upregulated, while CCR6 was downregulated in the positively selected fetal thymic γδ T cells. Together, these changes are expected to enhance emigration from the thymus and may direct cells specifically to the epidermis. Recent studies show, for example, that S1P1 is essential for the exiting of mature αβ T cells from the thymus in adult mice (83). Also, the ligand for CCR6, CCL20, is highly expressed in the thymus (84–87). Upregulation of SIP1 and loss of CCR6 are therefore predicted to promote thymic export. Furthermore, the ligand for CCR10, the chemokine CCL27, is constitutively expressed in the skin (88–90), including the fetal skin (74). Hence, CCR10 upregulation may promote skin homing of positively selected γδ T cells after they exit the fetal thymus. These predictions remain to be tested experimentally.

Positive selection in the thymus may also influence propagation of DETCs in the epidermis in other ways. A likely possibility is that the survival and expansion of DETCs in the skin are enabled by the upregulation of CD122 that occurs as a result of positive selection of DETC precursors in the fetal thymus. CD122 is the β chain of the IL-2/IL-15 receptor, and IL-15 is known to be essential for DETC development (91), whereas IL-2 is not essential (92). In both CD122 and IL-15 knockout mice, $V\gamma3^+$ γδ T cells were generated in the fetal thymus nearly as efficiently as in wildtype mice, but DETCs did not develop (91, 93). It has been proposed that IL-15 expressed by keratinocytes maintains survival/expansion of DETCs in the skin, apparently through interaction with IL-15 receptors expressed on skin-specific γδ T cells (91). Therefore, the survival and expansion of DETCs in the skin are probably promoted by the upregulation of cytokine receptors, which occurs during positive selection in the thymus. It remains

possible that additional stimulation of the cells in the epidermis is also important in maintaining expression of these cytokine receptors.

A proposed model for development of tissue-localized γδ T-cell subsets

Programmed Vγ gene rearrangement and cellular selection cooperate to regulate development of different tissue-specific γδ T cells (*Fig.* 6). In the early fetal thymus, a programmed rearrangement process based on proximity of the gene to Jγ1 favors the generation of Vγ3Vδ1$^+$ γδ T cells, the natural skin-specific γδ T-cell precursors, and Vγ4Vδ1$^+$ T cells, destined for other epithelial locations. Subsequently, the Vγ3Vδ1$^+$ γδ T cells are positively selected by engagement of the Vγ3Vδ1 TCR with a ligand expressed in the fetal thymus, resulting in a coordinate switch in expression of chemokine receptors and cytokine receptors, which in turn directs the specific migration of these cells to the skin and enhances their subsequent expansion and survival there.

In the adult thymus, Vγ3 and Vγ4 (and Vδ1) rearrangements are suppressed by the programmed rearrangement process, favoring production of Vγ2$^+$ and other adult-type γδ T cells. Although it remains somewhat murky whether positive selection acts on adult-type γδ T cells, it is likely that a maturation process, whether selection or another process, confers these cells with their specific homing properties, enabling them to exit the thymus and home to secondary lymphoid organs.

Presumably, the directed differential homing of specific subsets of γδ T cells is ultimately important for positioning these different T cells appropriately for their specialized roles. In the case of invariant γδ subsets, such as the Vγ3Vδ1$^+$ DETCs, the cells are localized to the site where they can recognize their specialized ligands, which are induced in the skin by the pathophysiological changes (79). The much more variable adult γδ T-cell subsets, such as Vγ2$^+$ T cells, are believed to be involved in surveillance for more diverse sets of ligands, appropriate to their localization to the secondary lymphoid organs.

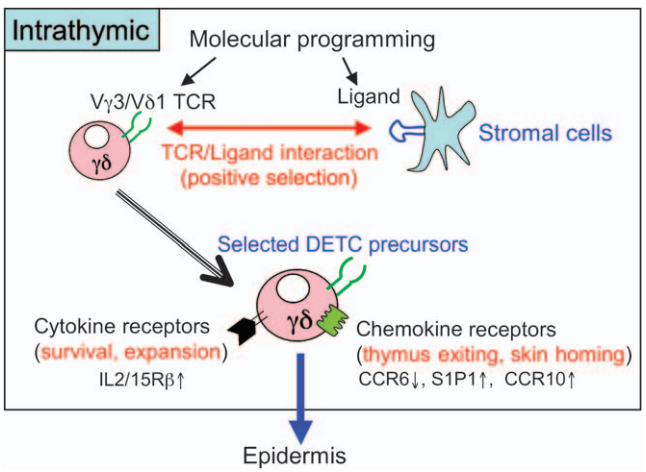

***Fig.* 6. Proposed overall scheme of developmental events leading to development of γδ T cells in the skin (DETCs).** In the first phase, programmed rearrangement events lead to a striking bias in TCR gene rearrangements, favoring production of the Vγ3Vδ1 canonical TCR, and may direct expression on thymic stromal cells of ligands necessary for positive selection of DETCs. Positive selection ensures that only cells with the correct specificity differentiate further and bestow the selected cells with chemokine receptors, which enable the cells to exit the thymus and migrate to the epidermis, and with cytokine receptors, which aid in subsequent survival and expansion of the cells.

Concluding remarks

Our research has addressed all stages of the development of γδ T cells, including the decision to differentiate along the αβ versus γδ lineage, ordered production of Vγ-defined subsets of γδ T cells over the course of ontogeny and the acquisition during development of guidance information that directs the cell to its appropriate destination. Our findings together with those of others show that programmed events, such as Vγ gene rearrangement and junctional diversification mechanisms, cooperate with subsequent cellular selection processes, ultimately generating distinct sets of specific tissue-localized γδ T cells. For example, in the absence of selection, most of the productive Vγ rearrangements in the early fetal period are canonical Vγ3 rearrangements (9). Subsequent development of DETCs nevertheless requires a cellular selection process. Hence, with respect to γδ T-cell specificity, the molecular program greatly restricts the possible repertoire at the fetal stage, and selection reinforces this restriction. In the normal course of events, many of the cells subjected to selection will already express the canonical Vγ3Vδ1 receptor, so it might be surmised that shaping the repertoire is not the sole or even main role of positive selection. But under exceptional circumstances, the selection process is capable of selecting alternative TCRs, including the Vγ2Vδ7 receptor, which apparently provides specificity similar to that of the canonical receptor.

The role of selection extends beyond shaping the repertoire, however, by inducing expression of specific chemokine receptors and cytokine receptors that are likely to determine the subsequent localization, survival, and proliferation of the cells. This outcome of positive selection at the fetal stage may be 'hardwired' in the sense that all γδ lineage cells at this stage will make this response to TCR ligation. We found, for example, that

fetal thymocytes stimulated with anti-γδ-TCR antibodies upregulated the same chemokine and cytokine receptors as DETC precursors, even if the thymocytes lacked a known DETC-compatible receptor (74). These considerations suggest that the specialization of the fetal thymus to produce DETCs is imposed at numerous levels of development and prompt the question of why such stage-specific specialization evolved. We suggest that it is necessary because the repertoires, diversity, and functions of the cells produced at the fetal versus adult stages differ so greatly. Specialization of the thymus for production of different subsets at different stages is one way to vary developmental processes at different stages to impart different repertoires and functionality on the corresponding cells. As examples, the absence of TdT at the fetal stage is critical to producing a high frequency of canonical Vγ3 rearrangements appropriate for DETC production (24, 25), and we have proposed in this review that the reduced susceptibility of fetal thymocytes to negative selection may be important for producing cells like DETCs, with a fixed specificity for a self-ligand.

Despite its role in the production of DETCs and Vγ4Vδ1 invariant T cells at the fetal stage, ontogenic specialization is clearly not required to produce other specialized T-cell subsets. Aside from the obvious CD4/CD8 subsets, some of the T-cell subsets that arise in the adult thymus have properties similar to those of DETCs. Examples are natural killer (NK) T cells, CD8αα$^+$ T cells, mucosal-associated invariant T cells (MALT), which exhibit restricted TCR repertoires, are localized to specific peripheral tissues, and exhibit distinctive immune functions. The development of these cells is not separated in ontogeny from the production of conventional αβ T cells and adult-type γδ T cells, but the differentiation process they undergo may share one or more of the features of DETC development outlined in this review. Developmentally, many of these cells undergo selection processes that are unlike those of conventional αβ T cells but similar in some respects to those of γδ T cells. For example, NK T cells are positively selected by a self-glycolipid antigen associated with CD1 (94, 95), while MALT cells are positively selected by the self-antigen MR1 (96, 97). Strong TCR/antigen interactions, which usually result in negative selection of conventional T cells, were found to promote development of CD8αα$^+$ T cells (98). It remains to be seen whether positive selection of these and other T-cell subsets also induces chemokine and cytokine receptors that aid in peripheral localization and survival of the cells.

γδ T cells in the human also display unique tissue distribution patterns. For example, Vδ1$^+$ γδ T cells are predominant in intestines and skin (99, 100), where they are believed to play an important role in tumor surveillance and maintenance of tissue integrity, while Vδ2$^+$ γδ T cells are the dominant population of secondary lymphoid organs and blood (101). The findings in the mouse system will ultimately provide a guide for understanding the development and localization of γδ T cells in humans.

Detailed molecular events that dictate programmed Vγ gene rearrangement and generation of corresponding γδ T cells remain to be defined. In understanding the adult pattern, identification of transcription factors that interact with the *cis*-acting elements in the TCRγ locus, especially the promoters, is one of the most prominent issues to be resolved. Understanding the role of E2A in this process will be an important step. However, E2A cannot be the sole determinant of the adult developmental pattern because E2A-deficient mice still have plentiful Vγ2 γδ T cells, but no Vγ3$^+$ γδ T cells in the adult thymus (N. Xiong, C. Murre, D. H. Raulet, unpublished data). In understanding the fetal pattern, it will be necessary to dissect how the rearrangement pattern is controlled by gene location in the cluster, independent of differences in Vγ gene accessibility. With respect to γδ T-cell selection, it is critical to identify the ligands that mediate positive selection of DETCs and potentially other γδ T-cell subsets and to determine if and how their expression is restricted to specific developmental stages or cell types in the thymus. To understand tissue localization, it will be essential to test directly the roles of specific chemokine receptors and other molecules that influence cell localization. Finally, an essential issue to explore is whether and how positively selected γδ T cells at different stages of ontogeny (and therefore selecting environments) are bestowed with different homing properties.

References

1. Raulet DH. The structure, function, and molecular genetics of the γ/δ T cell receptor. Annu Rev Immunol 1989;**7**:175–207.
2. Haas W, Pereira P, Tonegawa S. Gamma/delta cells. Annu Rev Immunol 1993;**11**:637–685.
3. Allison JP, Havran WL. The immunobiology of T cells with invariant γδ antigen receptors. Annu Rev Immunol 1991;**9**:679–705.
4. Hayday AC. [gamma][delta] cells: a right time and a right place for a conserved third way of protection. Annu Rev Immunol 2000;**18**:975–1026.
5. Garman RD, Doherty PJ, Raulet DH. Diversity, rearrangement and expression of murine T cell gamma genes. Cell 1986;**45**:733–742.
6. Capone M, Hockett RD, Zlotnik A. Kinetics of T cell receptor β, γ, and δ rearrangements during adult thymic development: T cell receptor rearrangemnts are present in CD44+CD25+ Pro-T thymocytes. Proc Natl Acad Sci U S A 1998;**95**:12522–12527.

7. Livák F, Tourigny M, Schatz DG, Petrie HT. Characterization of TCR gene rearrangements during adult murine T cell development. J Immunol 1999;**162**:2575–2580.
8. Livak F, Petrie HT, Crispe IN, Schatz DG. In-frame TCR δ rearrangements play a critical role in the αβ/γδ lineage decision. Immunity 1995;**2**:617–627.
9. Dudley EC, Girardi M, Owen MJ, Hayday AC. αβ and γδ T cells can share a late common precursor. Curr Biol 1995;**5**:659–669.
10. Kang J, Baker J, Raulet D. Evidence that productive rearrangements of TCRγ genes influence the fate of developing T cells. Eur J Immunol 1995;**25**:2706–2709.
11. Kang J, Volkmann A, Raulet DH. Evidence that gammadelta versus alphabeta T cell fate determination is initiated independently of T cell receptor signaling. J Exp Med 2001;**193**:689–698.
12. Ciofani M, Knowles GC, Wiest DL, von Boehmer H, Zuniga-Pflucker JC. Stage-specific and differential notch dependency at the alphabeta and gammadelta T lineage bifurcation. Immunity 2006;**25**:105–116.
13. Saint-Ruf C, Panigada M, Azogui O, Debey P, von Boehmer H, Grassi F. Different initiation of pre-TCR and gammadeltaTCR signalling. Nature 2000;**406**:524–527.
14. Haks MC, et al. Attenuation of gammadeltaTCR signaling efficiently diverts thymocytes to the alphabeta lineage. Immunity 2005;**22**:595–606.
15. Hayes SM, Li L, Love PE. TCR signal strength influences alphabeta/gammadelta lineage fate. Immunity 2005;**22**:583–593.
16. Raulet D, et al. Control of γδ T cell development. Immunol Rev 1991;**120**:185–204.
17. Allison JP. Gamma delta T-cell development. Curr Opin Immunol 1993;**5**:241–246.
18. Havran WL, Allison JP. Thy-1^+ dendritic epidermal cells of adult mice arise from fetal thymic precursors. Nature 1990;**344**:68–70.
19. Itohara S, et al. Homing of a γδ thymocyte subset with homogenous T-cell receptors to mucosal epithelia. Nature 1990;**343**: 754–757.
20. Viney JL, MacDonald TT, Kilshaw PJ. T-cell receptor expression in intestinal intra-epithelial lymphocyte subpopulations of normal and athymic mice. Immunology 1989;**66**:583–587.
21. Rocha B, Vassalli P, Guy-Grand D. Thymic and extrathymic origins of gut intraepithelial lymphocyte populations in mice. J Exp Med 1994;**180**:681–686.
22. Pereira P, Hermitte V, Lembezat MP, Boucontet L, Azuara V, Grigoriadou K. Developmentally regulated and lineage-specific rearrangement of T cell receptor Valpha/delta gene segments. Eur J Immunol 2000;**30**:1988–1997.
23. Davis MM, Bjorkman PJ. T cell antigen genes and T cell recognition. Nature 1988;**334**:395–402.
24. Tigelaar RE, Lewis JM. Immunobiology of mouse dendritic epidermal T cells: a decade later, some answers, but still more questions. J Invest Dermatol 1995;**105**:43S–49S.
25. Asarnow DM, Cado D, Raulet DH. Selection is not required to produce invariant T cell receptor γ-gene junctional sequences. Nature 1993;**362**:158–160.
26. Zhang Y, et al. The role of short homology repeats and TdT in generation of the invariant γδ antigen receptor repertoire in the fetal thymus. Immunity 1995;**3**:439–447.
27. Itohara S, et al. T cell receptor δ gene mutant mice: independent generation of αβ T cells and programmed rearrangements of γδ TCR genes. Cell 1993;**72**:337–348.
28. Korman A, Marusic-Galesic S, Spencer D, Kruisbeek A, Raulet DH. Predominant variable region gene usage by γ/δ T cell receptor-bearing cells in the adult thymus. J Exp Med 1988;**168**:1021–1040.
29. Heilig JS, Tonegawa S. Diversity of murine gamma genes and expression in fetal and adult T lymphocytes. Nature 1986;**322**:836–840.
30. 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.
31. Guy-Grand D, Cerf-Bensussan N, Malissen B, Malassis-Seris M, Briottet C, Vassalli P. Two gut intraepithelial CD8+ lymphocyte populations with different T cell receptors: a role for gut epithelium in T cell differentiation. J Exp Med 1991;**173**:471–481.
32. Lefrancois L, Puddington L. Extrathymic intestinal T-cell development: virtual reality? Immunol Today 1995;**16**:16–21.
33. Ikuta K, et al. A developmental switch in thymic lymphocyte maturation potential occurs at the level of hematopoietic stem cells. Cell 1990;**62**:863–874.
34. Goldman J, Spencer D, Raulet D. Ordered rearrangement of variable region genes of the T cell receptor gamma locus correlates with transcription of the unrearranged genes. J Exp Med 1993;**177**: 729–739.
35. Baker JE, Cado D, Raulet DH. Developmentally programmed rearrangement of T cell receptor Vγ genes is controlled by sequences immediately upstream of the Vγ genes. Immunity 1998;**9**:159–168.
36. Agata Y, et al. Histone acetylation determines the developmentally regulated accessibility for T cell receptor gamma gene recombination. J Exp Med 2001;**193**:873–879.
37. Yancopoulos GD, Desiderio SV, Paskind M, Kearney JF, Baltimore D, Alt FW. Preferential utilization of the most J_H-proximal V_H gene segements in pre-B-cell lines. Nature 1984;**311**:727–733.
38. Yancopoulos GD, Malynn BA, Alt FW. Developmentally regulated and strain-specific expression of murine V_H gene families. J Exp Med 1988;**168**:417–435.
39. Chien Y-H, et al. T cell receptor δ gene rearrangements in early thymocytes. Nature 1987;**330**:722–727.
40. Peschon JJ, et al. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J Exp Med 1994;**180**:1955–1960.
41. Corcoran AE, Riddell A, Krooshoop D, Venkitaraman AR. Impaired immunoglobulin gene rearrangement in mice lacking the IL-7 receptor. Nature 1998;**391**:904–907.
42. Malynn BA, Yancopoulos GD, Barth JE, Bona CA, Alt FW. Biased expression of J_H-proximal V_H genes occurs in the newly generated repertoire of neonatal and adult mice. J Exp Med 1990;**171**:843–859.
43. Jung D, Giallourakis C, Mostoslavsky R, Alt FW. Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus. Annu Rev Immunol 2006;**24**:541–570.
44. Connor AM, Fanning LJ, Celler JW, Hicks LK, Ramsden DA, Wu GE. Mouse VH7183 recombination signal sequences mediate recombination more frequently than those of VHJ558. J Immunol 1995;**155**: 5268–5272.
45. Williams GS, et al. Unequal VH gene rearrangement frequency within the large VH7183 gene family is not due to recombination signal sequence variation, and mapping of the genes shows a bias of rearrangement based on chromosomal location. J Immunol 2001;**167**:257–263.
46. Xiong N, Baker JE, Kang C, Raulet DH. The genomic arrangement of T cell receptor variable genes is a determinant of the developmental rearrangement pattern. Proc Natl Acad Sci U S A 2004;**101**:260–265.
47. Tanimoto K, Liu Q, Bungert J, Engel JD. Effects of altered gene order or orientation of the locus control region on human beta-globin gene expression in mice. Nature 1999;**398**:344–348.
48. Xiong N, Kang CH, Raulet DH. Redundant and unique roles of two enhancer elements in the TCR gamma locus in gene regulation and gamma delta T cell development. Immunity 2002;**16**:453–463.
49. Greenbaum S, Zhuang Y. Regulation of early lymphocyte development by E2A family proteins. Semin Immunol 2002;**14**: 405–414.

50. Bain G, Romanow WJ, Albers K, Havran WL, Murre C. Positive and negative regulation of V(D)J recombination by the E2A proteins. J Exp Med 1999;**189**:289–300.
51. Baker JE, Kang J, Xiong N, Chen T, Cado D, Raulet DH. A novel element upstream of the Vγ2 gene in the murine T cell receptor γ locus cooperates with the 3′ enhancer to act as a locus control region. J Exp Med 1999;**190**:669–679.
52. Sleckman BP, et al. Accessibility control of variable region gene assembly during T-cell development. Immunol Rev 1998;**165**: 121–130.
53. Seidl KJ, et al. Position-dependent inhibition of class-switch recombination by PGK-neor cassettes inserted into the immunoglobulin heavy chain constant region locus. Proc Natl Acad Sci U S A 1999;**96**:3000–3005.
54. Tough DF, Sprent J. Lifespan of gamma/ delta T cells. J Exp Med 1998;**187**:357–365.
55. 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.
56. Grusby M, et al. Mice lacking major histocompatibility complex class I and class II molecules. Proc Natl Acad Sci U S A 1993;**90**:3913–3917.
57. Correa I, Bix M, Liao N-S, Zijlstra M, Jaenisch R, Raulet D. Most γδ T cells develop normally in β2-microglobulin-deficient mice. Proc Natl Acad Sci U S A 1992;**89**:653–657.
58. Bigby M, et al. Most γδ T cells develop normally in the absence of MHC class II molecules. J Immunol 1993;**151**: 4465–4475.
59. Ito K, Van KL, Bonneville M, Hsu S, Murphy DB, Tonegawa S. Recognition of the product of a novel MHC TL region gene (27b) by a mouse gamma delta T cell receptor. Cell 1990;**62**:549–561.
60. Schild H, et al. The nature of MHC recognition by γδ T cells. Cell 1994;**76**:29–37.
61. Bonneville M, et al. Recognition of a self major histocompatibility complex TL region product by γδ T-cell receptors. Proc Natl Acad Sci U S A 1989;**86**:5928–5932.
62. Bluestone JA, Cron RQ, Cotterman M, Houlden BA, Matis LA. Structure and specificity of T cell receptor γ/δ on major histocompatibility complex antigen-specific $CD3^{+}$,$CD4^{-}$,$CD8^{-}$ T lymphocytes. J Exp Med 1988;**168**:1899–1916.
63. Crowley MP, et al. A population of murine gammadelta T cells that recognize an inducible MHC class Ib molecule. Science 2000;**287**:314–316.
64. 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.
65. Bonneville M, et al. Blockage of αβ T-cell development by TCR γδ transgenes. Nature 1989;**342**:931–934.
66. Pereira P, Zijlstra M, McMmaster J, Loring JM, Jaenisch R, Tonegawa S. Blockade of transgenic gamma delta T cell development in beta-2-microglobulin deficient mice. EMBO J 1992;**11**:25–31.
67. Wells F, Tatsumi Y, Bluestone J, Hedrick S, Allison J, Matis L. Phenotypic and functional analysis of positive selection in the γ/δ T cell lineage. J Exp Med 1993;**177**:1061–1070.
68. Schweighoffer E, Fowlkes BJ. Positive selection is not required for thymic maturation of transgenic gd T cells. J Exp Med 1996;**183**:2033–2041.
69. Ferrick DA, et al. T cell function and expression are dramatically altered in T cell receptor Vγ1.1Jγ4Cγ4 transgenic mice. Cell 1989;**57**:483–492.
70. Bonneville M, et al. Transgenic mice demonstrate that epithelial homing of γ/δ T cells is determined by cell lineages independent of T cell receptor specificity. J Exp Med 1990;**171**:1015–1026.
71. Ferrero I, Wilson A, Beermann F, Held W, MacDonald HR. T cell receptor specificity is critical for the development of epidermal gammadelta T cells. J Exp Med 2001;**194**:1473–1483.
72. Mallick-Wood C, Lewis JM, Richie LI, Owen MJ, Tigelaar RE, Hayday AC. Conservation of T cell receptor conformation in epidermal γδ cells with disrupted primary V_γ gene usage. Science 1998;**279**:1729–1733.
73. Hara H, et al. Development of dendritic epidermal T cells with a skewed diversity of gamma delta TCRs in V delta 1-deficient mice. J Immunol 2000;**165**:3695–3705.
74. Xiong N, Kang C, Raulet DH. Positive selection of dendritic epidermal γδ T cell precursors in the fetal thymus determines expression of skin-homing receptors. Immunity 2004;**21**:121–131.
75. Leclercq G, Plum J, Nandi D, De Smedt M, Allison J. Intrathymic differentiation of Vγ3 T cells. J Exp Med 1993;**178**:309–315.
76. Van Beneden K, De Creus A, Stevenaert F, Debacker V, Plum J, Leclercq G. Expression of inhibitory receptors Ly49E and CD94/ NKG2 on fetal thymic and adult epidermal TCR V gamma 3 lymphocytes. J Immunol 2002;**168**:3295–3302.
77. Lewis JM, Girardi M, Roberts SJ, Barbee SD, Hayday AC, Tigelaar RE. Selection of the cutaneous intraepithelial gammadelta(+) T cell repertoire by a thymic stromal determinant. Nat Immunol 2006;**7**:843–850.
78. Minagawa M, Ito A, Shimura H, Tomiyama K, Ito M, Kawai K. Homogeneous epithelial gamma delta T cell repertoire of the skin is shaped through peripheral selection. J Dermatol Sci 2001;**25**:150–155.
79. Havran WL, Chien Y, Allison JP. Recognition of self antigens by skin-derived T cells with invariant gamma delta antigen receptors. Science 1991;**252**:1430–1432.
80. Lin T, et al. Autospecific gammadelta thymocytes that escape negative selection find sanctuary in the intestine. J Clin Invest 1999;**104**:1297–1305.
81. Iwashima M, Green A, Bonyhadi M, Davis MM, Allison JP, Chien YH. Expression of a fetal gamma delta T-cell receptor in adult mice triggers a non-MHC-linked form of selective depletion. Int Immunol 1991;**3**:385–393.
82. Itohara S, Tonegawa S. Selection of γδ T cells with canonical T-cell antigen receptors in fetal thymus. Proc Natl Acad Sci U S A 1990;**87**:7935–7938.
83. Matloubian M, et al. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Nature 2004;**427**:355–360.
84. Greaves DR, et al. CCR6, a CC chemokine receptor that interacts with macrophage inflammatory protein 3alpha and is highly expressed in human dendritic cells. J Exp Med 1997;**186**:837–844.
85. Baba M, et al. Identification of CCR6, the specific receptor for a novel lymphocyte-directed CC chemokine LARC. J Biol Chem 1997;**272**:14893–14898.
86. Liao F, Alderson R, Su J, Ullrich SJ, Kreider BL, Farber JM. STRL22 is a receptor for the CC chemokine MIP-3alpha. Biochem Biophys Res Commun 1997;**236**:212–217.
87. Varona R, et al. Molecular cloning, functional characterization and mRNA expression analysis of the murine chemokine receptor CCR6 and its specific ligand MIP-3alpha. FEBS Lett 1998;**440**:188–194.
88. Morales J, et al. CTACK, a skin-associated chemokine that preferentially attracts skin-homing memory T cells. Proc Natl Acad Sci U S A 1999;**96**:14470–14475.
89. Homey B, et al. Cutting edge: the orphan chemokine receptor G protein-coupled receptor-2 (GPR-2, CCR10) binds the skin-associated chemokine CCL27 (CTACK/ ALP/ILC). J Immunol 2000;**164**: 3465–3470.
90. Jarmin DI, et al. Cutting edge: identification of the orphan receptor G-protein-coupled receptor 2 as CCR10, a specific receptor for the chemokine ESkine. J Immunol 2000;**164**:3460–3464.
91. De Creus A, Van Beneden K, Stevenaert F, Debacker V, Plum J, Leclercq G. Developmental and functional defects of thymic and epidermal V gamma 3 cells in IL-15-deficient and IFN regulatory factor-1-deficient mice. J Immunol 2002;**168**:6486–6493.

92. Schimpl A, Hünig T, Elbe A. Development and Function of the Immune System in Mice with Targeted Disruption of the Interleukin 2 Gene. San Diego: Academic Press, 1994.
93. Kawai K, Suzuki H, Tomiyama K, Minagawa M, Mak TW, Ohashi PS. Requirement of the IL-2 receptor beta chain for the development of Vgamma3 dendritic epidermal T cells. J Invest Dermatol 1998;**110**:961–965.
94. Zhou D, et al. Lysosomal glycosphingolipid recognition by NKT cells. Science 2004;**306**:1786–1789.
95. Kronenberg M. Toward an understanding of NKT cell biology: progress and paradoxes. Annu Rev Immunol 2005;**23**:877–900.
96. Tilloy F, et al. An invariant T cell receptor alpha chain defines a novel TAP-independent major histocompatibility complex class Ib-restricted alpha/beta T cell subpopulation in mammals. J Exp Med 1999;**189**:1907–1921.
97. Treiner E, et al. Selection of evolutionarily conserved mucosal-associated invariant T ecells by MR1. Nature 2003;**422**:164–169.
98. Yamagata T, Mathis D, Benoist C. Self-reactivity in thymic double-positive cells commits cells to a CD8 alpha alpha lineage with characteristics of innate immune cells. Nat Immunol 2004;**5**:597–605.
99. Groh V, Steinle A, Bauer S, Spies T. Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells. Science 1998;**279**:1737–1740.
100. Ebert LM, Meuter S, Moser B. Homing and function of human skin gammadelta T cells and NK cells: relevance for tumor surveillance. J Immunol 2006;**176**:4331–4336.
101. Parker CM, et al. Evidence for extrathymic changes in the T cell receptor gamma/delta repertoire. J Exp Med 1990;**171**: 1597–1612.