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THE IMMUNOBIOLOGY OF T CELLS WITH INVARIANT $\gamma\delta$ ANTIGEN RECEPTORS

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Abstract

T cells bearing specific $\gamma\delta$ TCR are the major lymphoid population in certain epithelial tissues. There are striking differences between these and peripheral T cells. The epithelial $\gamma\delta$ T cells exhibit highly restricted V gene use, preferential pairing of TCR chains, and lack of diversity at the junctions creating populations of cells with virtually identical TCR in particular epithelia. Generation of certain epithelial $\gamma\delta$ populations appears to be restricted to a discrete stage early in development. The restricted localization and expression of invariant antigen receptors may equip the epithelial $\gamma\delta$ T cells to perform specialized functions which differ from those of circulating $\alpha\beta$ and $\gamma\delta$ TCR$^+$ cells. This review provides a summary of the characterization of $\gamma\delta$ T cells found in epithelial tissues and speculates on the in vivo role of these cells.

INTRODUCTION

In the hematolymphoid tissues, the vast majority of T cells express antigen receptors (TCR) composed of clonally variable $\alpha\beta$ heterodimers in association with the invariant components of the CD3 complex (1). The $\alpha\beta$ T cells recognize antigens as peptide fragments in the context of class-I or class-II MHC, express the coreceptors CD8 or CD4, and mediate cytotoxic reactions or perform help, respectively. Both types of $\alpha\beta$ cells draw upon

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the same pool of α and β gene segments in the production of their antigen receptors. The functional TCR repertoire of the $\alpha\beta$ T-cell population is shaped by positive and negative selective events that occur during intrathymic maturation (2).

For the past several years it has been known that a minority of T cells in the lymphoid organs express alternative receptors composed of clonally variable γ and δ chains, also in association with the CD3 complex. Since their discovery, the ontogeny, diversity, and biological function of these cells have been the subject of intense investigation (see 3 and 4 for recent reviews). Although it is not yet possible to establish a paradigm for the development and specificity of the lymphoid $\gamma\delta$ cells analogous to the MHC-directed specificity of $\alpha\beta$ cells, these cells clearly have the capacity to recognize a heterogeneous array of ligands, including classical and nonclassical MHC antigens, bacterial heat shock proteins, and various self-antigens (see 5 for a recent review). Although the $\gamma\delta$ TCR repertoire is constrained by the relatively small number of germline elements available for the construction of functional TCR genes, the existence of extensive junctional diversity in the γ and δ chains suggests that the potential repertoire is as large, or perhaps larger, than that of $\alpha\beta$ cells (6).

One fact that has emerged in the past three years is that in several species $\gamma\delta$ T cells comprise a major, if not the exclusive, T-cell component in some epithelial tissues. This compartmentalization is particularly pronounced in the mouse, where there is a tight correlation between TCR V gene segment usage and tissue localization. Recent data demonstrate that some of these epithelial $\gamma\delta$ cells arise from precursors present only early during fetal thymic development. The $\gamma\delta$ TCR of these cells have essentially no diversity, which suggests that they might play a role in a previously unrecognized form of immunological surveillance for stress-induced self-antigens rather than foreign antigens. Because of these unique features, these epithelium-associated $\gamma\delta$ T cells should perhaps be considered a separate class of cells distinct from conventional $\alpha\beta$ T cells and $\gamma\delta$ T cells of the hematolymphoid tissues. This review attempts to summarize what is known of the origin, tropism, selection, and specificity of these novel cells. The emphasis will be on the mouse, where the most data is available.

$\gamma\delta$ TCR GENE STRUCTURE

As is the case for the immunoglobulin genes, the TCR α, β, γ, and δ loci consist of V, J, or V, D, J, and C gene segments which undergo somatic rearrangement to generate functional genes. The diversity of the rearranged genes results from combinatorial diversity arising from the use of different segments present in the germline, junctional diversity arising

from differential trimming of the termini of the recombining gene segments by an exonuclease, and additional junctional diversification by insertion of template independent nucleotides (NGE) by terminal transferase (7). Additional, although limited, diversity may be generated by the inclusion of germline-derived nucleotides (P elements), perhaps by transfer of nucleotides to the blunt end of the opposite strand by a novel enzyme prior to trimming, insertion of NGE, and ligation (8).

The genomic organization of the TCR genes is shown schematically in Figure 1. (A more extensive review of γ and δ gene structures can be found in 3.) The γ locus consists of four Jγ genes, each associated with a Cγ gene, and seven Vγ genes (9–12). Cγ3 is a pseudogene and is deleted in some strains (9, 11). The Vγ genes seem to rearrange to the Jγ segments in three functional sets: Vγ5, Vγ2, Vγ4, and Vγ3 with Jγ1-Cγ1; Vγ1.2 with Jγ2-Cγ2; and Vγ1.1 with Jγ4-Cγ4 (nomenclature is that of Garman et al; 10. See 3 for concordance with other systems).

The δ locus is located within the α locus, between the Vα and the Jα segments (13). There are about 10 Vδ genes, based on the criteria of rearrangement to Dδ/Jδ-Cδ segments (13–19). Some Vα genes, or genes very similar to Vα, are used in functional δ genes. Other V genes, including those used by substantial numbers of $\gamma\delta$ cells in the thymus and spleen (Vδ1 and Vδ5), appear to rearrange only to Dδ/Jδ-Cδ segments (14–16). The δ locus has two D and two J elements upstream of a single C gene.

While the TCR γ and δ loci contain many fewer V and J segments than

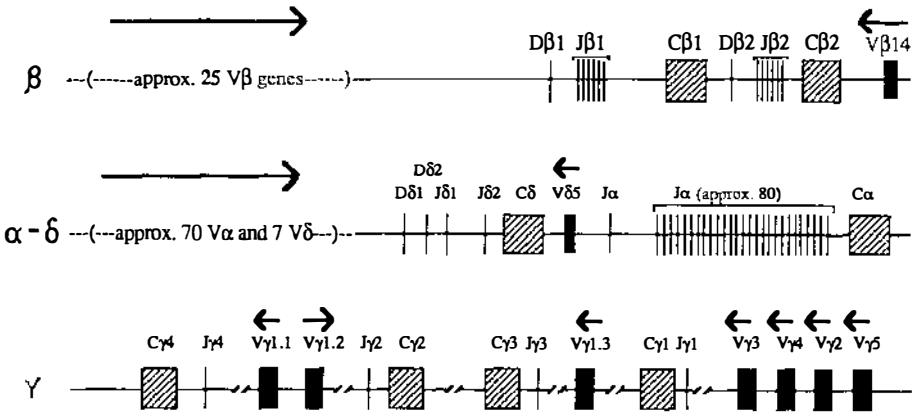

Figure 1 Genomic organization of murine T cell receptor loci. *Arrows* depict transcriptional orientation. The nomenclature used in this review is from Garman et al (10). For correspondence with other Vγ gene nomenclature, see (3).

the α and β locus, extensive junctional diversity has been observed in rearranged γ and δ genes, especially in the adult thymus (16). Since many rearranged δ genes employ both Dδ1 and Dδ2, both D segments can be read in all three frames, and N region diversity has been observed at each of the junctions, the potential diversity of δ junctions is enormous. The $\gamma\delta$ repertoire, despite the relative paucity of V segments, potentially may exceed that of the $\alpha\beta$ TCR (6). If, as proposed, the junctional regions are located in the putative third complementarity determining region and are involved in recognition of antigenic peptides, the capacity of $\gamma\delta$ cells to recognize diverse antigen might be remarkably large. However, as is later discussed, in most epithelial tissues this potential is not realized. The $\gamma\delta$ repertoire in these tissues is constrained by preferential pairing of specific Vγ and Vδ chains, and in at least three tissues by extremely limited junctional diversity.

WAVES: PROGRAMMED APPEARANCE OF $\gamma\delta$ CELLS DURING ONTOGENY

Cells that express $\gamma\delta$ TCR are the first TCR-bearing cells to appear in ontogeny (20–22) and are detectable by day 14 of gestation. Until about day 18, $\gamma\delta$ cells comprise the major population of TCR$^+$ cells in the thymus, after which their proportional representation rapidly decreases to about 1% of total thymocytes as cells expressing $\alpha\beta$ TCR emerge (22, 23). $\gamma\delta$ cells first appear in the spleen between birth and three weeks of age (24).

The first clue of programming of $\gamma\delta$ TCR appearance during ontogeny came from comparison of V segment rearrangement and expression in the fetal and adult thymus (10, 14, 16, 25, 26). Striking differences were observed. Vγ3, Vγ4, and Vδ1 were found to be predominant in the fetal thymus but were virtually undetectable in the adult, while the Vγ1 family and Vδ5 were frequently expressed in the adult. With the development of antibodies to $\gamma\delta$ TCR, it became apparent that the regulated rearrangement and expression of V genes is reflected by an ordered appearance during ontogeny of cells bearing different $\gamma\delta$ TCR (22, 23, 27).

As shown in Figure 2, cells bearing different $\gamma\delta$ TCR appear during ontogeny as a series of overlapping waves. The first TCR$^+$ cells to appear express Vγ3 and reach a level of about 2×10^4 cells before they decline. The next cells to appear express Vγ4 and are followed by Vγ2, and finally by cells expressing Vγ5 and other $\gamma\delta$ TCR. The factors regulating this progression are at present unknown, but it is perhaps noteworthy that the order of appearance of cells correlates with the proximity of the Vγ gene segments to Jγ1.

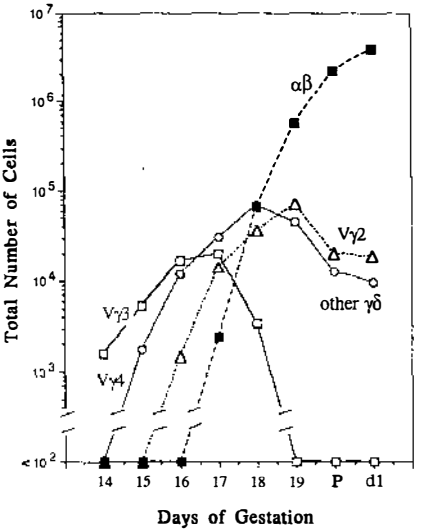

Figure 2 Expression of TCR chains during fetal thymic development. The values for Vγ3 (mAb 536) (22), Vγ2 (mAb UC3-10A6) (S. Widacki, J. Bluestone, submitted), and pan αβ (mAb H57-597) (122) represent actual data points. The values for the Vγ4 and other γδ curves represent the number of cells remaining after subtraction of the sum of cells expressing Vγ3 and Vγ2 from the total number of γδ cells obtained with pan γδ (mAb GL3) (54). The other γδ cells are primarily Vγ4 expressing from day 14 until day 17, based on PCR analysis of cDNA obtained from timed fetal tissue (8) (J. Noble, J. P. Allison, personal observation). The Vγ4 expressing cells decline after birth and Vγ5+ cells become the other major γδ TCR bearing population in the adult thymus.

In the adult thymus Vγ2-Jγ1/Cγ1 and Vδ5 predominate (18, 23), although Vγ1.1-Jγ4/Cγ4 is also present (3, 27). The composition of the adult spleen is very similar, with Vγ2-Jγ1/Cγ1 and Vγ1.1-Jγ4/Cγ4 expressed by a large fraction of γδ cells, along with Vδ5 and lower levels of Vδ2, Vδ4, and Vδ6 (24, 28, 29).

In addition to differences in V gene usage, another striking difference appears in the TCR of fetal and adult γδ cells. The TCR of the adult γδ cells in both the thymus and spleen have extensive junctional diversity (16, 18, 29). Productively rearranged genes exhibit considerable trimming, extensive NGE addition, and in the δ genes both D elements are regularly used. This junctional diversification ensures that the repertoire, although constrained by limited use of V regions, has the potential for recognition of a very large array of antigens. However, in startling contrast, essentially no junctional diversity appears in productively rearranged Vδ1, Vγ3, or

Vγ4 genes in the fetal thymus (8, 16). NGE are virtually absent, there is little exonucleolytic trimming, and in the vast majority of δ rearrangements a single D element is used. The canonical fetal sequences are shown in Figure 3. The selective and instructional mechanisms that might be involved in generating these nondiverse junctions are discussed below.

The absence of cells bearing the predominant fetal TCR from the adult raises questions as to their role and fate. One possibility is that these early cells might perform some role essential for the subsequent development of cells bearing diverse receptors (30, 31) and then perish. Alternatively, the fetal γδ cells might migrate to nonlymphoid sites and carry out whatever immunological role their puny repertoire allows.

DIFFERENTIAL EXPRESSION OF γδ TCR BY CELLS IN EPITHELIAL TISSUES

Skin Epithelium (dEC, s-IEL)

Several years ago it was demonstrated that mouse skin contained a unique population of intraepithelial lymphocytes (IEL). These cells express Thy-1

	Vγ3	N	Jγ1
Germline	TGT GCC TGC TGG GAT CT		AT AGC TCA GGT TTT
Fetal[a]	TGT GCC TGC TGG GA T		AGC TCA GGT TTT
DEC[b]	TGT GCC TGC TGG GAT		AGC TCA GGT TTT

	Vγ4	N	Jγ1
Germline	TGT GCA TGC TGG GAT		AT AGC TCA GGT TTT
Fetal[a]	TGT GCA TGC TGG GAT		AGC TCA GGT TTT
r-IEL[c]	TGT GCA TGC TGG GAT		AGC TCA GGT TTT

	Vδ1	N	Dδ2	N?	Jδ2
Germline	TCA GAT AT		ATC GGA GGG ATA CGA G		C TCC TGG
Fetal[a]	TCA GAT AT		C GGA GGG A	G	C TCC TGG
DEC[b]	TCA GAT AT		C GGA GGG A	G	C TCC TGG
r-IEL[c]	TCA GAT AT		C GGA GGG A	G	C TCC TGG

Figure 3 Canonical sequences of skin and reproductive epithelial T cells. Junctional sequences were obtained by pcr amplification of DNA obtained from freshly isolated cells, clones, or hybridomas generated from C57BL/6, Balb/C, or AKR mice. Due to shared sequences at the ends of some germline gene segments, assignment of nucleotides to a particular segment was arbitrary. (*a*) Fetal thymocytes and fetal hybridomas were prepared from C57BL/6 and Balb/c mice (8). (*b*) Freshly isolated dEC obtained from Balb/c mice and AKR derived dEC clones were analyzed (44, 49). (*c*) Vaginal and uterine cells were isolated from Balb/c and C57BL/6 mice (50) and Balb/c mice (D. Nandi, unpublished observation).

and have a pronounced dendritic morphology (32, 33). Designated Thy-1$^+$ dendritic epidermal cells (dEC), these cells are evenly distributed within the epidermis at a density of between 200 and 500 cells/mm^2 surface area in abdominal skin (32–34); they are found in intimate contact with keratinocytes. The dEC differ from Langerhans cells in that they lack expression of class-II MHC and are ultrastructurally and phenotypically distinct from the other epidermal cell populations (32, 33). The dEC uniformly express Ly-5 and do not express either CD4 or CD8. Thy-1$^+$ dEC can be found in low numbers in the skin of athymic nu/nu mice but are absent from the skin of SCID mice (32, 35, 36). While it was suggested that these cells might represent a unique population of epithelium-associated T cells (33), at the time of discovery reagents were not yet available to detect CD3 or cell surface TCR. Subsequently, immunoprecipitation analysis with antibodies to CD3 and TCR γ and δ chains allowed demonstration that dEC express $\gamma\delta$ TCR (37–40).

In vitro studies have demonstrated that dEC have functional properties similar to $\alpha\beta$ T cells. Activation with concanavalin A or anti-TCR/CD3 antibodies leads to expression of IL-2 receptor, secretion of IL-2, and proliferation (41, 42). Proximal signalling events are also analogous to those in $\alpha\beta$ T cells, in that cytoplasmic free calcium is elevated and components of the CD3 complex are phosphorylated, presumably by protein kinase C (42). At least some dEC clones also secrete IL-3 and IFN-γ (R. Tigelaar, W. Havran, and T. Mossman, unpublished results). In addition to secretion of lymphokines, dEC can mediate cytolysis of cells coated with anti-CD3 or anti-TCR antibodies in retargeting assays (42), but dEC do not appear to lyse tumor or NK target cells (42, 43). These results indicate that dEC are capable of mediating functional responses typical of mature T cells and have the potential to perform protective immunological roles in the skin.

In striking contrast to the TCR diversity of $\alpha\beta$ and $\gamma\delta$ cells in lymphoid tissues, the dEC TCR is essentially monomorphic and is uniquely expressed in the skin in adult mice. The first hint of this was obtained in the initial molecular analysis of TCR gene expression by a series of independent dEC clones. Each clone was found to express Vγ3, Vδ1, and Jδ2 mRNA (38, 44). In addition, the vast majority of CD3$^+$ cells freshly isolated from adult skin of several mouse strains (BALB/c, AKR, C3H/He, C57BL/6) were found to be reactive with a monoclonal antibody to Vγ3, while Vγ3$^+$ cells were undetectable in lymphoid tissues (22, 45) or gut or vaginal epithelia (W. L. Havran, D. Nandi, unpublished results). In situ immunofluorescent staining of skin with CD3, Vγ3, and Thy-1 antibodies also indicates that most if not all dEC express Vγ3 (E. Payer, personal communication). While there have been reports of skin-derived hybridomas

that express other TCR, including both $\alpha\beta$ and $\gamma\delta$ (19, 46–48), the preponderance of evidence indicates that the skin is virtually exclusively populated by $V\gamma3^+$ T cells.

While these results suggested that the dEC TCR repertoire might be severely restricted in combinatorial diversity, there remained the possibility of diversity contributed by junctional events. However, sequence analysis of the clones as well as freshly isolated polyclonal dEC revealed essentially invariant $V\gamma3$-$J\gamma1$ and $V\delta1$-$D\delta2$-$J\delta2$ sequences (44, 49). The canonical sequences of the dEC TCR junctions are shown in Figure 3. It is noteworthy that the canonical sequence of the dEC γ and δ genes are the same as those of the canonical $V\gamma3$ and $V\delta1$ sequences found in the fetal thymus (8), and that the same canonical sequences are found in at least three MHC disparate mouse strains (AKR, BALB/c, and C57BL/6). These observations have important implications as to the origin, selection, and biological role of these cells, issues discussed below.

Female Reproductive Epithelium (r-IEL)

The female reproductive tract contains both $\alpha\beta$ and $\gamma\delta$ T cells (50). Immunohistostaining analysis indicates that in the vagina most $\gamma\delta$ cells are found in the basal layer of stratified squamous epithelium, while in the uterus the $\gamma\delta$ cells are broadly distributed through the endometrium and myometrium or are associated with simple columnar luminal epithelium. The $\alpha\beta$ cells are mainly found in subepithelial areas (50). The r-IEL $\gamma\delta$ cells do not express either CD4 or CD8, and approximately half express Thy-1. T cells are absent from the vaginal epithelium of athymic nu/nu mice (D. Nandi, J. Allison, unpublished observations). The capacity of the r-IEL to secrete lymphokines or mediate cytotoxic reactions is at present unknown.

Like the dEC, the r-IEL have a severely restricted TCR repertoire. The TCR are composed of a unique γ chain, $V\gamma4$-$J\gamma1$, and a δ chain composed of $V\delta1$-$J\delta2$-$D\delta2$. Sequence analysis has demonstrated that, as in the dEC, there is essentially no diversity in the junctions of the γ or δ chains of the r-IEL, and the same canonical sequences are found in at least two MHC disparate mouse strains (50) (D. Nandi, J. Allison, unpublished observations). As shown in Figure 3, the canonical sequences of the r-IEL γ and δ junctions are identical to those of fetal $V\gamma4$ and $V\delta1$. It is intriguing that the r-IEL δ chain is identical to that of the dEC TCR. It is also striking that at the amino acid level, the junctions of the dEC $V\gamma3$ chain and the r-IEL $V\gamma4$ chain are also identical. As shown in Figure 4, the sequence identity extends nine residues upstream from the V-J joint. It is possible, though unlikely, that conservation of the γ chain junctional sequences is necessary for pairing with the δ chain. In any event, the similarities of

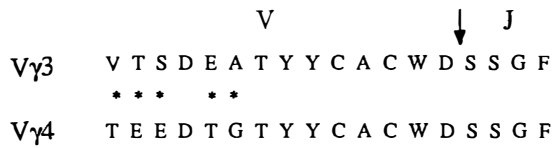

Figure 4 Amino acid sequence of canonical Vγ3 and Vγ4 junctions. Sequences were derived from junctional nucleotide sequences shown in Figure 3 and additional 5′ V germline sequence. *Arrow* indicates V-J boundary. *Asterisks* indicate positions at which the residues differ.

these canonical receptors may have important implications as to specificity and selection.

Intestinal Epithelium (i-IEL, IEL)

Intestinal intraepithelial T lymphocytes are found primarily in the small intestine interspersed between the villous epithelial cells (51). The i-IEL predominately express γδ TCR (52, 53), although many preparations also contain significant numbers of αβ TCR$^+$ cells (51, 54). As in the chicken (55), αβ$^+$ cells in the mouse gut are found primarily in the lamina propria (R. P. Bucy, personal communication) and may be present in i-IEL preparations as contaminants. Unlike γδ cells in the skin or reproductive tract, nearly all of the i-IEL express CD8 (52). Like many other CD8$^+$ γδ T cells, the i-IEL do not express the CD8β (Lyt-3) chain (51). The functional significance of the lack of CD8β is unknown, but CD8α alone is sufficient for signal transduction (56). The γδ i-IEL are thus far the only peripheral T cells that express CT1, a carbohydrate antigen associated with CD45 (52). Expression of Thy-1 by i-IEL is variable. The i-IEL of normal mice maintained in a germ-free environment were reported to lack Thy-1 expression, but Thy-1 expression was observed on about half of the cells after the mice were returned to conventional housing (57). Freshly isolated i-IEL are capable of effectively lysing target cells coated with anti-CD3 antibodies, indicating that they are functionally active cells (52). Little cytolytic activity was detected in i-IEL from germfree mice, and cytolysis was exclusively found in the Thy-1$^+$ population in conventionally housed mice (57). Environmental stimuli, possibly bacterial, may induce expression of Thy-1 and cytolytic activity. However, other studies have failed to demonstrate a correlation between Thy-1 expression and germ-free versus conventional housing (58), and it has been suggested that Thy-1 is a marker of intrathymic differentiation (58).

As is the case in other epithelia, the TCR of γδ cells in the gut use predominately a single Vγ segment, in this case Vγ5 (59, 53, 59, 60). However, unlike the other epithelial γδ cells, the IEL can employ a variety

of Vδ segments, with Vδ4 being the most prevalent, and Vδ5, 6, and 7 being used less frequently (49, 51, 59). Also unlike other epithelial $\gamma\delta$ cells; the junctions of both the γ and δ chains exhibit considerable junctional diversity, including extensive N-regions and the use of both D elements in many of the δ chains (49, 59). Thus, unlike the other epithelia, i-IEL have a very large potential antigenic repertoire.

Lung (RPL)

A subset of resident pulmonary lymphocytes (RPL) expresses the $\gamma\delta$ TCR (61). The localization of these cells within the tissue and their association with epithelial cells have not been established. Major subsets of lung-derived $\gamma\delta$ cells proliferate in response to exposure in vivo to aerosols containing *Myobacterium tuberculosis* or in vitro to heat shock (61, 62). TCR gene utilization is more complex in the lung than in other tissues. Vγ2 appears to predominate, but Vγ4 can also be detected in DNA amplified from cDNA by the polymerase chain reaction (PCR) (62). In the in-frame δ transcripts obtained from BALB/c mice, Vδ6 predominates and Vδ5 is expressed at a high level, while Vδ4 and Vδ7 are also detectable (63). Junctional sequences are highly diverse, with extensive NGE addition and the use of both D-elements in the majority of rearrangements (63). Thus, like the i-IEL, $\gamma\delta$ cells in the lung have a potentially large antigenic repertoire. Of interest, a large fraction of the Vδ5 genes expressed in the RPL of BALB/c, but not C57BL/6, mice are composed of an invariant delta sequence designated BID (63). This invariant sequence is also dominant in RPL of (C57BL/6 $\times$ BALB/c)F1, BALB/b, and athymic BALB/c nu/nu mice, and it is not found in the thymus of adult BALB/c mice. These observations suggest that RPL with this invariant sequence might be positively selected or expanded extrathymically by a structure not encoded for in the classical BALB/c H-2^d haplotype. The invariant sequence contains the complete germline sequences of Vδ5, Dδ2, and Jδ1 joined with no NGE additions. In contrast to the canonical sequences of the skin and reproductive $\gamma\delta$ TCR, the lung invariant sequence does not appear to be fetal derived because Vδ5 is rarely rearranged and expressed in the fetal thymus (64).

Other Epithelia

A variety of mouse epithelial tissues have been surveyed by immunohistochemistry and PCR amplification of transcripts for the presence of cells expressing $\gamma\delta$ TCR. These cells have been detected in contact with epithelial cells in the tongue, and in small numbers in the esophagus, stomach, and bladder (50). Essentially nothing is known of the phenotypic or functional

properties of γδ cells in these tissues. A single Vγ4/Vδ1 TCR with junctional sequence identical to that in the r-IEL predominates in the tongue (50).

Other Species

Analysis of human epithelial sites indicated the presence of γδ T cells, but in significantly lower numbers than in the mouse. In human epithelial tissues, αβ cells seem to predominate, at least in the adult. γδ T cells are present in adult human skin, but their fractional representation in the total T-cell population is not significantly different than what is seen in peripheral blood (65, 66). Human intestinal epithelium contains higher numbers of γδ T cells than are found in the peripheral blood or lamina propria (51, 66). The highest cell numbers are seen during gestation, followed by a decrease after birth (51) possibly due to an increase in absolute numbers of αβ cells as a result of exposure to antigenic stimulation.

In the chicken, a major population of T cells in the thymus and blood express γδ TCR (67, 68). As in the mouse, chicken γδ cells precede αβ cells during development and do not appear to go through a TCR^{lo} stage (69). Distribution of T cells in the intestinal epithelium is similar to the mouse, with γδ cells localized to intraepithelial sites in the villi and αβ cells located primarily in the lamina propria (55). However, there is no evidence of γδ cells in the skin. In the rat, about half of the $CD3^+$ cells in preparations of intestinal lymphocytes were $\alpha\beta^+$, suggesting that γδ cells are a major population in this epithelium (70). No information is available on the presence of T cells in rat skin. In cattle and sheep, as in the chicken, γδ cells comprise a major fraction of T cells in the thymus and blood. In both species, αβ and γδ cells are present in the intestine, and γδ cells are abundant in the skin (71). Probes are not yet available to assess V gene usage or junctional diversity in these species.

It is apparent from this rather limited data that, with the possible exception of human and chicken skin and the human intestine, the distribution of γδ cells in epithelia is similar to that of mice. The differences in representation of γδ cells in mouse and human epithelial tissues could be the result of several differences between the species. The human lifespan and exposure to antigens is significantly greater than that of inbred mice housed in relatively clean environments. The observation that higher numbers of γδ cells are present in at least some tissues early in human life (51) raises the possibility that increased exposure to antigen leads to expansion of cells expressing αβ TCR, thereby decreasing the percentage of γδ cells. Comparison of epithelial tissues taken from wild mice with tissues from conventional inbred populations could address this possibility. Additionally, there are obvious differences between human and mouse skin. The

difference in hair distribution and density may play a role in localization of $\gamma\delta$ cells in the skin or affect the presence or density of the antigen recognized by these cells.

TISSUE TROPISM: EPITHELIAL HOMING AND THE T-CELL RECEPTOR

The nature and properties of the receptors expressed by $\gamma\delta$ T cells in different epithelia are summarized in Table 1. It is evident that, with the exception of the lung, a single Vγ gene segment is used in each tissue. The close correlation of Vγ gene usage and tissue localization raises the possibility that the TCR itself might be responsible for tissue-specific homing, perhaps by encountering specific ligands and arresting migration of the cells. However, analysis of the relevant cells in a number of mice bearing TCR transgenes indicates that this is not the case. For example, the i-IEL and the dEC of mice transgenic for a Vγ2/Vδ5 TCR of the type expressed by most adult $\gamma\delta$ thymocytes were found to bear TCR encoded by the transgenes, rather than Vγ5 or Vγ3 (58). Similarly, the i-IEL of mice transgenic for the dEC Vγ3/Vδ1 TCR uniformly and exclusively express the Vγ3 transgenes (58) (M. Iwashima, A. Green, M. Bonyhadi, M. Davis, J. Allison, Y.-H. Chien, submitted). The fact that epithelium-associated $\gamma\delta$ cells in these mice express inappropriate TCR indicates that the TCR itself is not responsible for tissue-specific homing, despite the close correlation between Vγ usage and tissue localization in normal mice. Regulation of TCR gene rearrangement may be closely correlated with expression of specific homing receptors during development.

THYMIC ORIGIN OF EPITHELIUM-ASSOCIATED $\gamma\delta$ T CELLS

As shown in Figure 5, individual V segments associated with the Jγ1-Cγ1 cluster are ultimately found in T cells associated with specific epithelial

Table 1 Epithelial $\gamma\delta$ T lymphocytes

Tissue	γ	δ	Diversity	$\alpha\beta$ Cells
Skin	V_3/J_1	$V_1/D_2/J_2$	minimal	absent
Vagina, uterus	V_4/J_1	V_1/D_2J_2	minimal	subepithelial
Tongue	V_4/J_1	$V_1/D_2/J_2$	minimal	?
Intestine	V_5/J_1	V_5, V_4, V_6, V_7	+++	lamina propria
Lung	V_2, V_4	V_6, V_5, V_4, V_7	++	?

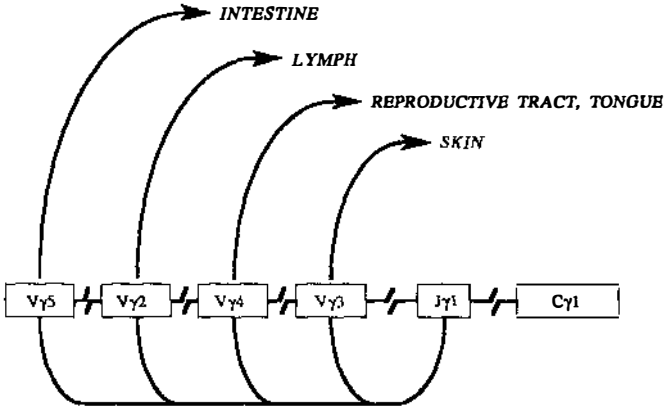

Figure 5 Order of Vγ gene segments in the Cγ1 Cγ1 cluster relative to Cγ1 and tissue localization in the adult mouse.

tissues in the adult mouse. As previously noted, analysis of Vγ3, Vγ4, and Vδ1-Dδ2-Jδ2 rearrangements in the fetal thymus revealed canonical sequences for each which were the same as those found in γδ cells associated with the skin, vaginal epithelium, and tongue. These observations are consistent with the possibility that these tissues are colonized by early thymic emigrants. In contrast, the intestinal γδ cells use V segments not present early in development, raising the possibility that the intestine is seeded in another manner.

Skin

The observation that the dEC of adult mouse skin and the early fetal thymocytes have identical TCR structure is suggestive but does not prove that dEC arise from precursors in the thymus. Indeed, the role of the thymus in the generation of the dEC is controversial. Thymus and skin epithelium arise from common embryologic tissues and possess keratinizing, stratified squamous epithelium which can support lymphocyte differentiation (34, 72). Bone marrow stem cells may migrate directly into the skin and undergo extrathymic differentiation and maturation (34). Thy-1$^+$ cells can be detected in the skin of athymic nu/nu mice, an observation consistent with an extrathymic origin of these cells (32, 35). However, once reagents to detect the CD3 complex and γδ TCR became available, it was established that the Thy-1$^+$ epidermal cells in nude mice do not express detectable cell-surface CD3 or associated γδ TCR, and the TCR genes in these cells were demonstrated to be unrearranged (35). These results suggested that either the thymus was required for generation of

dEC, or that the skin of nude mice was unable to support the homing or differentiation of dEC precursors. Engraftment of adult nude or newborn euthymic mice with fetal thymic lobes or isolated $V\gamma3^+$ fetal thymocytes results in the appearance of donor type $V\gamma3^+$ cells in the skin (73). These results demonstrate that the skin of nude mice supports the homing of $V\gamma3^+$ thymocytes and suggest that the absence of mature dEC in these mice is due to the lack of a functional thymus. If dEC arise from fetal precursors, elimination of $V\gamma3^+$ cells during fetal development might be expected to result in the absence of dEC in the adult. Indeed, the skin of 4-month-old mice which had been exposed to anti-$V\gamma3$ antibodies in utero contained no $V\gamma3^+$ cells (73). Together, these data strongly support the hypothesis that dEC arise from fetal thymic precursors and cannot be generated in the adult mouse.

Thy-1^+, TCR^- cells can be detected in the late fetal epidermis. These are replaced by Thy-1^+, TCR^+ cells within a few days of birth, and the number of cells continues to increase, reaching maximum levels in the skin by two weeks post partum (74). These observations have led to the hypothesis that the early TCR^- cells differentiate into the TCR^+ cells in the epidermis (74). On the basis of the kinetics of expression of $V\gamma3$ by fetal thymocytes and grafting studies, it appears more likely that the epidermis is seeded by low numbers of $V\gamma3^+$ thymic emigrants prenatally and that these cells proliferate and gradually replace the early TCR^- population.

If dEC precursors can be generated only in the fetal thymus, it is possible that fetal and adult stem cells differ in their developmental potential. Alternatively, the thymic microenvironment might change so that in the adult it is no longer capable of supporting the development of $V\gamma3^+$ cells from pluripotential stem cells. In fact, several observations suggest that both the developmental potential of the stem cell and the thymic microenvironment are important in the generation of dEC. First, fetal liver cells, but not adult bone marrow, gave rise to $V\gamma3^+$ cells after repopulation of deoxyguanosine-treated fetal thymic lobes (A. Carbone, unpublished results). Similarly, $V\gamma3^+$ cells arise in fetal thymic organ cultures seeded with highly enriched hematopoietic stem cells from fetal liver, but not with enriched stem cells from adult bone marrow (75). Second, in vivo experiments using grafted chimeras showed that donor type $V\gamma3^+$ dEC could be detected in mice with fetal thymic grafts reconstituted with fetal liver cells, but no donor type dEC were detected in similarly grafted mice reconstituted with adult bone marrow (W. L. Havran, unpublished observations). These data clearly indicate that the stem cells undergo a developmental switch, and those in the adult do not have the capacity to generate $V\gamma3^+$ cells. However, no donor type $V\gamma3^+$ dEC could be detected

in the skin of irradiated adult mice which had received either fetal liver cells or adult bone marrow, suggesting that in the adult the thymus has also changed and is no longer capable of supporting dEC development from fetal stem cells. Together, these results strongly suggest that both the stem cells and the microenvironment in which they develop may be important for the generation of dEC. It is possible that only the fetal stem cell carries the program for proper recombination of Vγ3 and Vδ1 gene segments, and the cells which successfully rearrange require selection on elements present only in the fetal thymus for rescue and emigration. The issue of selection is further discussed below.

Reproductive Tract

Definitive information regarding the origin of reproductive epithelium–associated γδ cells is not yet available. However, the fact that the cells express TCR with the fetal canonical Vγ4 and Vδ1 junctional sequences and the absence of r-IEL in athymic nude mice suggest that this population, like the dEC, arises from fetal thymic precursors.

Intestine

Several lines of evidence suggest that the i-IEL arise in a manner distinct from that of γδ cells in the other epithelia. First, the V gene segments used by i-IEL are not abundant in the fetal thymus, and the junctions exhibit the extensive diversity characteristic of rearrangements occurring in the adult. In addition, i-IEL are found in athymic nude mice (58, 76, 77). Finally, IEL of donor type arise following bone marrow reconstitution of irradiated thymectomized mice (51). These results indicate that, unlike dEC, i-IEL can arise in the adult in the absence of thymic influence.

THE ROLE OF SELECTION IN GENERATION OF CANONICAL Vγ3, Vγ4, AND Vδ1 TCR SEQUENCES

It is now well established that the functional αβ T-cell repertoire in an individual animal is selected from a population of cells containing randomly rearranged α and β genes by a combination of positive and negative events that occur during differentiation in the thymus (2). The earliest cells to enter the thymus do not express TCR, CD4, or CD8. TCR gene rearrangement proceeds, and upon successful rearrangement of both α and β, immature $CD4^+8^+$ "double positive" thymocytes that express low (about 1/10) levels of αβ TCR emerge. Subsequently, some of these cells mature into $CD4^+8^-$ or $CD4^-8^+$ "single positive" cells that express high levels of αβ TCR with affinity for MHC class II or class I, respectively. In the past few years intrathymic selection has been assessed by following

development of individual TCR Vβ genes with known reactivity to certain MHC gene products or other self antigens, and by analysis of mice bearing $\alpha\beta$ TCR transgenes conferring single specificity for antigen and MHC (78–88). This work has established that cells bearing TCR with high affinity for self-antigens are clonally deleted at the double positive stage (negative selection), and that an interaction of the TCR with the appropriate MHC molecule is required for rescue of the cells from programmed cell death and emergence into the appropriate mature single positive population (positive selection). In addition to negative selection, induction of functional anergy in the absence of physical deletion of self-reactive $\alpha\beta$ clones can play a role in tolerance (89, 90).

The role of selection in the generation of the $\gamma\delta$ TCR repertoire is much less clear. Unlike $\alpha\beta$ cells, there are no phenotypic markers that allow the clear identification of an immature stage of $\gamma\delta$ thymocyte development. Neither do thymic $\gamma\delta$ cells pass through any obvious stage of low TCR expression, and even the earliest $\gamma\delta$ cells to appear in the fetal thymus uniformly express levels of TCR that are as high as those of peripheral $\gamma\delta$ cells (23) (W. Havran, unpublished observation). In addition, the relative paucity of $\gamma\delta$ clones with known specificity and/or MHC restriction have hampered assessment of selection.

The issue of selection has been recently addressed in two studies of mice transgenic for TCR γ and δ genes from clones reactive with non-classical MHC gene products (91, 92). In one study, which employed Vγ2-J1 and Vδ5-Jδ1 isolated from a TL^b specific hybridoma (93, 94), similar numbers of cells expressing the transgenic receptor were observed in the TL^b and TL^d mice; however, cells from the TL^b mice were unreactive with TL^b stimulator cells, while cells from the TL^d mice were highly reactive (91). It was concluded that cells expressing the transgenes were rendered anergic in the reactive TL^b background. The other study employed TCR genes isolated from a clone with reactivity for an as yet unidentified MHC gene mapping in the TL region of H-2^b: Vγ2/Jγ1 and a δ gene composed of a member of the Vα11 family rearranged to Jδ1 (17). Cells expressing the $\gamma\delta$ transgene were found in the spleen of H-$2^{d/d}$ mice but were greatly reduced in the spleens of H-$2^{b/d}$ mice, facts consistent with negative selection (92).

These transgenic mouse studies suggest that induction of self-tolerance in $\gamma\delta$ cells might be obtained by the same mechanisms operative on cells of the $\alpha\beta$ lineage. However, it should be emphasized that both transgenic models utilized $\gamma\delta$ TCR genes typical of major populations found in the adult lymphoid tissues, and that different mechanisms might be operative in those cells arising in the early stages of fetal development. It would be an enormous task to pare the entire potential repertoire down to the canonical sequences observed in the fetal Vγ3 and Vγ4 populations, and

the dEC and r-IEL of the adult, by negative selection alone, and it is probable that multiple mechanisms are involved.

Multiple developmental factors are likely to constrain the diversity of the $\gamma\delta$ TCR repertoire of the cells that rearrange in the early fetal thymus. First is the ordered rearrangement of V gene segments. In the majority of a series of dEC clones the alternative alleles carried rearrangements, albeit nonproductive, involving the same Vγ and Vδ, and in the δ locus the same Dδ, as those found on the productively rearranged alleles (44). Thus, there is probably a developmental program directing rearrangement of both γ and δ gene segments operative in the precursor cells, rather than a selective rescue of cells bearing the proper TCR γ and δ combination from a population that is independently rearranging γ and δ gene segments. This regulation may be related to the accessibility of the TCR genes. Second, it is also likely that the state of the recombinase at the time rearrangements are occurring limits the potential diversity. Levels of terminal transferase, the enzyme thought to be responsible for the incorporation of nongermline nucleotides, are low in the early fetal thymus (95, 96). This may account for the virtual absence of NGE in the canonical sequences. The absence of these elements would constrain diversity by limiting productive rearrangements to those combinations of exonuclease-trimmed germline sequences that can yield in-frame joints. Additional developmental or genetic factors may also play a role in the generation of the canonical sequences, since the Vδ1/Dδ2 junction shows an unexpectedly high degree of precision, even in nonproductive rearrangements which cannot be subjected to selection (8, 44, 49, 97).

Nonetheless, given the fact that nonproductive rearrangements of Vγ3, Vγ4, and Vδ1 are detected in both fetal thymus and in dEC from adult, any instructional sequence specific rearrangement that may occur does not inevitably lead to the canonical rearrangements. This raises the issue of whether selective pressures might also play a role in generation of the canonical junctional sequences. The sequences of the nonproductive rearrangements have somewhat limited diversity, although they are more variable than the essentially monomorphic productive rearrangements. However, since a cell must successfully rearrange the TCR genes in order to survive and the productive rearrangements that do occur are essentially invariant, the question of instruction versus selection cannot be answered on the basis of this sequence information alone.

The role of selection in the generation of the canonical $\gamma\delta$ TCR cannot be directly addressed in the absence of knowledge of the reactivity of the dEC and r-IEL. Nonetheless, several observations suggest that selection of these cells, if it occurs, probably involves different mechanisms than those operative in generation of the $\alpha\beta$ TCR repertoire. First, the early $\gamma\delta$ cells

may not be susceptible to negative selection. Treatment of fetal thymic organ cultures with antibody to CD3 results in the rapid loss of the $CD3^{lo}$, $CD4^+8^+$ $\alpha\beta$ T cells by induction of apoptosis in a manner analogous to that which occurs during negative selection (98, 99). However, similar exposure of organ cultures to anti-CD3, anti-$\gamma\delta$, or anti-Vγ3 antibodies has no effect on the subsequent emergence of cells that bear Vγ3 TCR (A. Carbone, J. Allison, unpublished observations). These results suggest that $V\gamma3^+$ cells do not pass through a developmental stage in which they are susceptible to TCR-mediated deletion, or by analogy, negative selection.

Second, a difference in the maturational mechanisms of $\gamma\delta$ and $\alpha\beta$ cells has been shown in experiments using the immunosuppressive drug cyclosporin A (CsA). CsA has profound effects on the development of $\alpha\beta$ T cells. CsA treatment of adult mice blocks the differentiation of $CD4^+8^+$ thymocytes into mature single positive cells expressing high levels of $\alpha\beta$ TCR (100, 101). The level of $\gamma\delta$ cells, as assessed by immunoprecipitation, was unaffected (100). Treatment of fetal thymic organ cultures with CsA also results in greatly diminished yields of mature $\alpha\beta$ cells; however, yields of total $\gamma\delta^+$ cells (102. 103) and $V\gamma3^+$ cells (A. Carbone, J. Allison, unpublished observations) are not affected. CsA has been demonstrated to interfere with lymphokine production and proliferation of mature T cells, presumably by interfering with the activity of nuclear proteins involved in T-cell activation (104, 105). The inhibitory effect of CsA on the maturation of $\alpha\beta$ cells may thus be due to interference with receptor-mediated signals required for positive selection (100, 101, 103). Since CsA blocks activation of mature dEC cells (W. Havran, unpublished observations), its failure to block development of $V\gamma3^+$ cells in fetal thymic organ culture may reflect a difference in the requirement for, or the nature of, TCR-mediated signals necessary for development. However, CsA has pleiotropic effects in the thymus, including induction of atropy of the medulla (101), so the differential effects of the drug on the development of $\alpha\beta$ and $\gamma\delta$ cells could reflect a difference in requirement for interaction with stromal elements or other factors. Nonetheless, these differential effects suggest a major difference in the maturational requirements of $\alpha\beta$ and $\gamma\delta$ cells.

The role of positive selection in generation of the canonical $\gamma\delta$ TCR has been difficult to address because at present it is not possible to compare the unselected and selected repertoires. However, the frequency of productive noncanonical rearrangements of Vγ3, Vγ4, and Vδ1 is increased in fetal thymic cultures treated with an antibody directed to constant region determinants of the TCR δ chain (106). The accumulation of these sequences, not normally observed, suggests TCR-mediated rescue of cells from a pool bearing diverse rearrangements in a manner mimicking positive selection.

On the basis of these findings it has been proposed that the canonical sequences may be positively selected from a diverse pool by interaction with self-determinants in the thymus (106). Proof of this possibility must await identification of the selecting element, or genetic and cellular analysis of mice exhibiting different canonical sequences.

Together, these data suggest that the canonical sequences ultimately detected in the $\gamma\delta$ TCR of cells in the skin, vaginal, and tongue epithelium are the result of targeted gene rearrangement, lack of NGE diversification, low exonucleolytic activity during recombination, and possibly positive selection. It is tempting to speculate that these factors may also be responsible for the apparent limitation of the capacity to generate the dEC to the fetal period. The program directing gene rearrangement may be limited to fetal stem cells, while expression of the selecting element might be limited to the fetal thymus.

Peptides have been implicated in the positive selection of $\alpha\beta$ T cells (107, 108). Since the putative selection of the γ and δ chains is directed toward the junctional sequences, which have been postulated to participate in binding of antigenic peptides (6), sclf-peptides might be involved in the process. Products of the TL region may be the selecting structures for the canonical $\gamma\delta$ sequences (97); however, several observations suggest that this is not the case. First, the canonical sequences are the same in several MHC-disparate strains (8, 44, 49). It is of course possible that these strains share currently unidentified invariant class-I genes. However, expression of such nonclassical MHC gene products should, like other class-I MHC, require the presence of β2-microglobulin. In mice in which the β2-microglobulin gene has been eliminated by gene targeting (109), the skin contains normal numbers of $V\gamma3^+$ cells. Moreover, the junctional γ and δ sequences in the dEC TCR of these mice are identical to the canonical sequences of normal mice (I. Correa, D. Raulet, unpublished observations). It is therefore unlikely that class-I MHC plays a role in selection of the dEC.

SPECIFICITY AND ROLE OF EPITHELIAL $\gamma\delta$ CELLS

The contrast of the high degree of diversity apparent in the TCR of $\gamma\delta$ cells in the intestinal epithelium with the extremely restricted repertoire of $\gamma\delta$ cells in the skin, vaginal, and tongue epithelium suggests that the cells might have fundamentally different roles in immunological surveillance. The essential unitary repertoire of $\gamma\delta$ cells in the latter epithelia poses a conundrum: What role in surveillance could be played by a T-cell population with identical antigen recognition structures? The mobility of the various T cells might provide some insight. Both $\alpha\beta$ and $\gamma\delta$ cells in the

lymphoid tissues recirculate and thus have the opportunity to encounter a large variety of antigens. In the gut the $\gamma\delta$ cells are localized in the villi, but these are constantly bathed in a wide variety of biological compounds and microorganisms, and pathogenic invasion may be quite frequent. The repertoire of the i-IEL might thus be required to be quite large to provide protection against the spectrum of microbial and viral antigens likely to be encountered. The situation in the other epithelia is probably very different. In the skin, the stratum corneum serves as a physical barrier which isolates the epidermis from the environment. Within the epidermis, the dEC are quite sessile, with little lateral mobility (110). The utility of a highly diverse clonally elaborated repertoire in this tissue would be quite limited, since contact of any individual cell with its appropriate antigen would be expected to be quite rare. It is likely that each individual dEC encounters only those few keratinocytes that surround it. Thus, the function of dEC may be to recognize damage-induced self-antigens induced in keratinocytes, rather than the agent that induces the cellular damage (44, 49). This type of immune protection, "trauma signal surveillance," might also be the role of $\gamma\delta$ cells with similarly restricted repertoire in other epithelia (111).

Expression of heat shock proteins, or peptides derived therefrom, may be a possible signal for this mode of surveillance (44, 49). Heat shock or stress proteins are phylogenetically highly conserved proteins that are induced or become more abundant in cells stressed by a variety of insults (112). Bacterial heat shock proteins are targets for a variety of immune responses (see 113 for recent review). Many human (114–117) and murine (118–120) $\gamma\delta$ cells exhibit reactivity for purified protein derivative (PPD) of *Mycobacterium tuberculosis*. In some instances, this reactivity has been attributed to mycobacterial HSP-65 (116, 121). Interestingly, a panel of murine thymus-derived $\gamma\delta$ hybridomas that spontaneously produced IL-2 were subsequently found to produce high levels of IL-2 when stimulated with an HSP-65 peptide (119, 121). Some of these cells were also weakly stimulated by the murine homologue of the mycobacterial peptide (121), suggesting a capacity to recognize self-HSP. Similarly, the resident lung $\gamma\delta$ cells proliferate in response to PPD and to heat shock, suggesting that these cells may also have self-HSP reactivity (61, 62). These findings suggest that recognition of heat shock proteins, and those related to mycobacterial HSP-65 in particular, may be a common property of $\gamma\delta$ cells. There is no indication that HSP-65 reactivity is a property of the epithelial $\gamma\delta$ cells with canonical TCR sequences. Neither dEC clones nor r-IEL lines are stimulated by PPD (W. Havran, D. Nandi, unpublished observations).

However, evidence does exist for recognition of self-antigens by dEC. Freshly isolated dEC, as well as dEC clones, respond to cultured ker-

atinocytes by IL-2 secretion and proliferation (W. L. Havran, J. P. Allison, in preparation). The stimulatory capacity of the keratinocytes is increased by heat shock or by exposure to sodium arsenite, suggesting that stress-related antigens might be involved in the response. The stimulation is specific, in that cells of non-epidermal origin are not recognized by the dEC, and γδ T cells that express TCR other than the dEC Vγ3/Vδ1 combination do not respond to keratinocytes. The response is blocked by Fab fragments of anti-γδ TCR antibodies, and transfection of the dEC TCR genes into a nonresponsive human T lymphoma confers reactivity, confirming that recognition is mediated through the dEC TCR. The stimulatory antigen appears to be a peptide, since live or fixed fibroblasts incubated with tryptic digests of cultured keratinocytes, but not other cells, can stimulate IL-2 release by dEC. This stimulatory activity is destroyed by further digestion of the preparations with proteinase K. Essentially no reactivity can be demonstrated in digests of freshly isolated keratinocytes that are not subjected to at least brief periods of in vitro culture before lysis (W. L. Havran, J. P. Allison, in preparation). These observations strongly suggest that dEC have the capacity to recognize stress-induced, keratinocyte-specific self-peptides. The stimulatory peptide may be derived from a protein that is specifically expressed or processed in stressed skin keratinocytes, but its identity remains to be established.

No obvious role for products of the MHC was observed in these studies. Combinations of dEC and keratinocytes or antigen-presenting cells from MHC disparate strains were equally effective, nor was the stimulation blocked by antibodies to classical MHC gene products. Thus, recognition of keratinocytes by the dEC clearly is not MHC-restricted in the conventional sense. The identity of molecules that may serve as antigen-presenting structures remains to be established.

It is not known whether r-IEL have specificity for self-peptides. However, the similarity of the r-IEL and dEC TCR suggests that this might be the case. If the junctions confer peptide reactivity, tissue specificity might be conferred by recognition of distinct antigen-presenting structures by the different Vγ chains. Alternatively, if V regions as well as the junctions play a role in peptide recognition, tissue-specific antigens could be the target.

CONCLUSIONS

The existence of cells expressing γδ TCR that are essentially invariant is paradoxical. Why is a system capable of creating an array of receptors with potentially astronomical diversity reduced to generating populations of cells with essentially identical structure? It is reasonable to take an evolutionary perspective and propose that the original γδ receptor was

entirely constrained to a limited panel of antigens; with selective pressure for diversity the receptor gave rise to the more complicated machinery which generates the $\gamma\delta$ and $\alpha\beta$ repertoires operative in recirculating cells (3). The phylogenetic separation of the two systems may be reflected in their ontogenic partition. The early cells with their invariant receptors may be important only early in life, providing a general means for dealing with invasion in the period before the host is competent for the more specific and elaborate protective responses offered by the later waves of T cells. Later in life, these cells may serve as sentinels in sites where cells with diverse receptors are not generally found, and their response to stress-induced self-antigens may help initiate more specific attack of invasive agents. Identification of the target antigen and presenting structures recognized by cells with invariant receptors will help to clarify their role. An understanding of this response might also provide insight into the cellular immune responses of primitive organisms.

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