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Different γδ T-cell receptors are expressed on thymocytes at different stages of development

(γδ T-cell hybridomas/transcriptional control)

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ABSTRACT **We have analyzed the structural diversity of the murine γδ T-cell receptor (TCR) heterodimer expressed on CD4⁻ CD8⁻ thymocyte populations and on TCR γδ-expressing hybridomas derived from thymocytes of fetal, newborn, and adult mice. We found that CD4⁻ CD8⁻ thymocytes derived from mice of different pre- and postnatal age preferentially express a γδ TCR encoded by different subsets of γ and δ gene segments. This age-dependent differential expression of γδ TCR on thymocytes seems to be accomplished in part by a specific control of rearranged γ genes operating at the level of transcription and/or RNA stability. We discuss the implications of these findings with respect to the recognition roles of the γδ TCR.**

The specific antigen recognition structure (T-cell receptor; TCR) of major histocompatibility complex (MHC)-restricted T-cells is composed of two polymorphic glycosylated polypeptide chains, TCR α and TCR β, noncovalently associated with an invariant protein complex, termed CD3. More recently, discovery of a third rearranging gene, γ, which is expressed specifically in T cells (1, 2), led to the identification of another CD3-associated heterodimer containing polymorphic TCR γ and TCR δ chains on subsets of immature thymocytes (3–6), peripheral T cells (7–9), dendritic epidermal cells (DEC) (10, 11), and gut intraepithelial lymphocytes (IEL) (12, 13). The role of the γδ receptor is yet to be established. Analysis of rearrangement and expression of TCR γ and δ genes during fetal ontogeny have suggested a possible coordinate control in the rearrangements and expression of these loci (14–16). In the present study, we have analyzed the structure and diversity of γ and δ polypeptide chains on CD4⁻ CD8⁻ [hereafter referred to as double negative (DN)] thymocytes and on γδ-expressing T-cell hybridomas derived from fetal and adult thymocytes. Our observations strongly suggest that different sets of γ-chain variable region (V_γ) and δ-chain variable region (V_δ) genes are expressed on fetal, newborn, and adult thymocytes and that some V_δ species preferentially pair with specific V_γ products to form a γδ TCR. In addition, we suggest the possibility of V_γ-dependent control of γ gene transcription.

MATERIALS AND METHODS

Animals. C57BL/6 (B6) and BALB/c mice were purchased from The Jackson Laboratory.

Preparation of γδ T-Cell Hybridomas. DN thymocytes were obtained after treatment of cells with anti-L3T4 monoclonal antibody (mAb) (RL172.4) and anti-Lyt-2 mAb (3.155.D14) plus rabbit complement (Cederlane Laboratories, Hornby, ON, Canada) as described (8). Hybridomas were prepared by fusing 10^7 BW5147 with 4×10^7 DN thymocytes according to standard procedures (17). The γδ hybridomas were identified by fluorocytometry with anti-CD3 mAb (2C11) (18) and immunoprecipitation by anti-γ mAb (KN365) (13).

Cell Surface Iodination, Immunoprecipitation, and Endoglycosaminidase F (endo F) Treatment. ^{125}I-Labeling, preparation of cell lysates, immunoprecipitation with anti-γ mAb (KN365), "off-diagonal" SDS/PAGE analysis, and endo F treatment were all carried out according to published methods (8).

Southern Blot Analysis. Five micrograms of digested DNA was electrophoresed into a 0.7% agarose gel, blotted on a nitrocellulose filter, and hybridized with random-primed probes in hybridization buffer [50% (vol/vol) formamide/5× SSC (1× = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7)/Denhardt's solution (0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin)/10% (wt/vol) dextran sulfate/0.02 M $NaPO_4$ buffer/single-stranded DNA at 0.25 mg/ml] at 42°C. Filters were washed for 40 min at 60°C with 2× SSC/0.1% SDS and for 30 min at 60°C with 0.2× SSC/0.1% SDS.

Northern Blot Analysis. Ten micrograms of formaldehyde-treated total RNA was subjected to electrophoresis in a 1% agarose gel containing formaldehyde, transferred to nylon membranes, and hybridized to random-primed probes in the presence of 50% formamide/1% SDS/1 M NaCl/10% dextran sulfate at 42°C. Filters were then washed in 2× SSC/1% SDS at 60° for 1 hr.

RESULTS

Distinct γδ TCR on Embryonic and Adult Thymocyte Populations. It has been reported that both the DNA rearrangement and RNA expression patterns of the γ genes differ between early fetal thymocytes and adult thymocytes (14). However, earlier studies carried out at the protein level did not reveal developmental stage-dependent expression of various γ genes (6). By using the monoclonal anti-γ antibody KN365 (8, 13), which detects the protein products of a variety of γ genes, we examined the surface expression of γδ TCR on both fetal and adult thymocytes. As can be seen in Fig. 1*a*, on day 15.5 of gestation, the main γ species had a molecular mass of ≈38 kDa. On day 18, a 36-kDa γ chain appeared in addition to the 38-kDa γ chain; the 36-kDa γ chain became

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Abbreviations: TCR, T-cell receptor; mAb, monoclonal antibody; DN, double negative; MHC, major histocompatibility complex; DEC, dendritic epidermal cells; IEL, intraepithelial lymphocytes; V, variable; C, constant; D, diversity; J, joining; endo F, endoglycosaminidase F.

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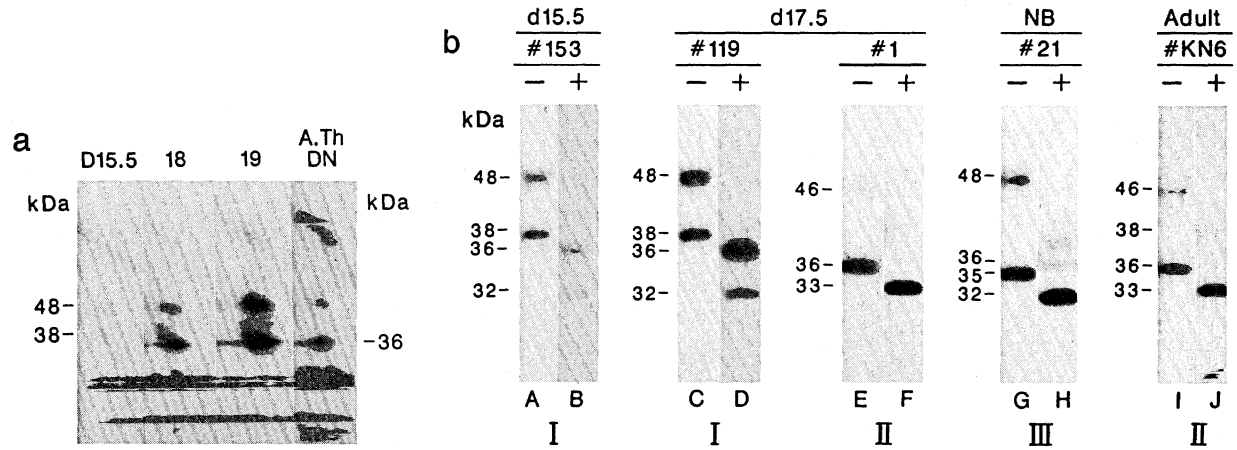

FIG. 1. $\gamma\delta$ heterodimers on the surface of murine DN thymocyte populations and thymocyte-derived hybridomas. Thymocytes or hybridomas were surface-iodinated, lysed, and immunoprecipitated with KN365. The samples were solubilized and analyzed by SDS/PAGE under reducing conditions followed by autoradiography. (*a*) Day (D) 15.5, day 18, and day 19 fetal thymocytes and DN adult (10 week old) thymocytes. A. Th, adult thymocytes. (*b*) T-cell hybridomas generated by fusing the BW5147 thymoma with C57BL/6 DN thymocytes at different stages of development. Samples were untreated (lanes A, C, E, G, and I) or treated with endo F (lanes B, D, F, H, and J). The Roman numerals at the bottom indicate the type of $\gamma\delta$ TCR.

dominant on day 19 and was the only detectable γ-chain protein on adult DN thymocytes. The observed difference in the apparent molecular mass of the γ chains may be explained by variability in the extent of N-glycosylation of the product of the same γ gene or by differential expression of various γ genes with a different number of potential N-glycosylation sites.

To determine the gene–protein relationship of the γ and δ chains expressed on thymocytes, we generated $\gamma\delta$-bearing T-cell hybridomas by fusing DN thymocytes from day 15.5 and day 17.5 embryos as well as newborn and adult mice with the AKR thymoma BW5147, which does not express a TCR–CD3 complex on its surface. The hybridomas were first screened for CD3 expression with the anti-CD3 mAb 2C11 (18). $CD3^+$ hybridomas were surface-iodinated and subjected to immunoprecipitation with KN365 (8, 13). As shown in Fig. 1*b*, lanes A, C, E, G, and I, two bands of 35–38 kDa and 46–48 kDa were observed. In each case only the lower molecular mass band appeared when the lysate was reduced and alkylated before immunoprecipitation (data not shown), indicating that it is the γ chain. Also shown in Fig. 1*b* are migration patterns of the γ and δ chains from which N-linked carbohydrates have been removed by treatment with endo F (lanes B, D, F, H, and J). On the basis of these results, three types of $\gamma\delta$ heterodimers could be identified.

$\gamma\delta$ TCR Preferentially Expressed on Early Fetal Thymocytes Are Encoded by $V_{\gamma5}$ and $V_{\delta1}$. Type I $\gamma\delta$ TCR was composed of a high molecular mass γ chain (about 38 kDa) and a high molecular mass δ chain (about 48 kDa) and was expressed on all three day 15.5 hybridomas (represented by hybridoma 153 in Fig. 1*b*) and one of the three day 17.5 hybridomas, hybridoma 119. The apparent molecular mass contributed by N-linked carbohydrates was 6 kDa (see Fig. 1*b*, lanes A and B or lanes C and D), a value too high to be attributed to one moiety. Since $V_5J_1C_1$ γ is the only γ chain identified to date that carries multiple (i.e., two) potential N-glycosylation sites (14), we suspected that the type I γ chain is encoded by this γ gene.

This supposition was tested by the analysis of nucleic acids. At least one copy of $V_{\gamma4}$ and $V_{\gamma5}$, but no $V_{\gamma6}$ and $V_{\gamma7}$ DNA segments, is rearranged to the $J_{\gamma1}$ DNA segment (Fig. 2*a*), and the transcripts of only the rearranged $V_{\gamma5}$ gene are detected in type I hybridomas (Fig. 3*a*). Rearranged $V_2J_2C_2$ γ gene bands are also detected in both the hybridomas and BW5147, but this γ gene, which carries no potential N-glycosylation site (1), is unlikely to code for the endo F-sensitive type I γ chains. Thus these results confirm that it is the $V_5J_1C_1$ γ chain that is preferentially expressed as the component of the type I $\gamma\delta$ TCR during the early phase of fetal development. We cloned a γ-chain cDNA from hybridoma 129 and determined the sequence. As expected, $V_{\gamma5}$ and $J_{\gamma1}$ gene segments were joined "in-frame" in this hybridoma (data not shown).

In all three type I hybridomas studied, one of the previously reported V_δ gene segments, $V_{\delta1}$ (15, 16), was rearranged to $J_{\delta2}$, generating a 4.2-kilobase (kb) band (Fig. 2*b*). In addition, an incomplete rearrangement of $V_{\delta1}$ to $D_{\delta2}$ (15) was found in hybridomas 153 and 129, whereas a second complete rearrangement, V_1DJ_1C δ, was found in hybridoma 119. Transcripts of apparently full length were detected with the $V_{\delta1}$ (Fig. 3*b*) and $J_{\delta2}$ (data not shown) probes in all three hybridomas. In addition, transcripts were detected in hybridoma 119 with the $J_{\delta1}$ probe at a low level (data not shown). These results indicate that the δ chains expressed in hybridomas 153 and 129 are encoded by the V_1DJ_2C δ gene. With hybridoma 129, cDNA sequencing analysis supported this conclusion (data not shown). In hybridoma 119, the V_1DJ_2C δ gene may also encode the surface-expressed δ chain, but an ambiguity remains because of the coexistence of the $V_{\delta1}DJ_{\delta1}$ rearrangement and its transcripts.

"Adult-Type" $\gamma\delta$ TCR Encoded by $V_{\gamma4}$ and Several V_δs Are Infrequent on Fetal Thymocytes. The γ chain from type II $\gamma\delta$ TCR migrated faster (36 kDa) than the type I γ chain, and after endo F treatment, its molecular mass was reduced only by 3 kDa, suggesting that type I and type II γ chains are different (Fig. 2*b*, lanes E and F and also lanes I and J). In addition, the type II δ chain had a molecular mass (46 kDa) significantly lower than that of the type I δ chain (48 kDa) and was conspicuously difficult to label with ^{125}I (Fig. 2*b*, lanes E and I). Type II $\gamma\delta$ TCR was found on 2 out of 3 day 17.5 hybridomas, 1 (Fig. 1*b*) and 159 (data not shown), 2 out of 12 newborn hybridomas (data not shown, see Table 2), as well as on most adult hybridomas, which are represented by KN6 (Fig. 1*b*).

Other than the rearrangement detected by the $V_{\gamma2}$ probe (not relevant for the reason given above), only $V_4J_1C_1$ γ rearrangement was observed for γ genes in hybridomas 159 and KN6, and $V_4J_1C_1$ γ and $V_5J_1C_1$ γ rearrangements were observed in hybridoma 1 (Fig. 2*a*). These hybridomas contain full-length RNA detected by the $V_{\gamma4}$ probe but not by the $V_{\gamma5}$, $V_{\gamma6}$, or $V_{\gamma7}$ probes (Fig. 3*a*). The fusion partner BW5147 also carries one copy of the rearranged $V_4J_1C_1$ γ gene (Fig. 2*a*),

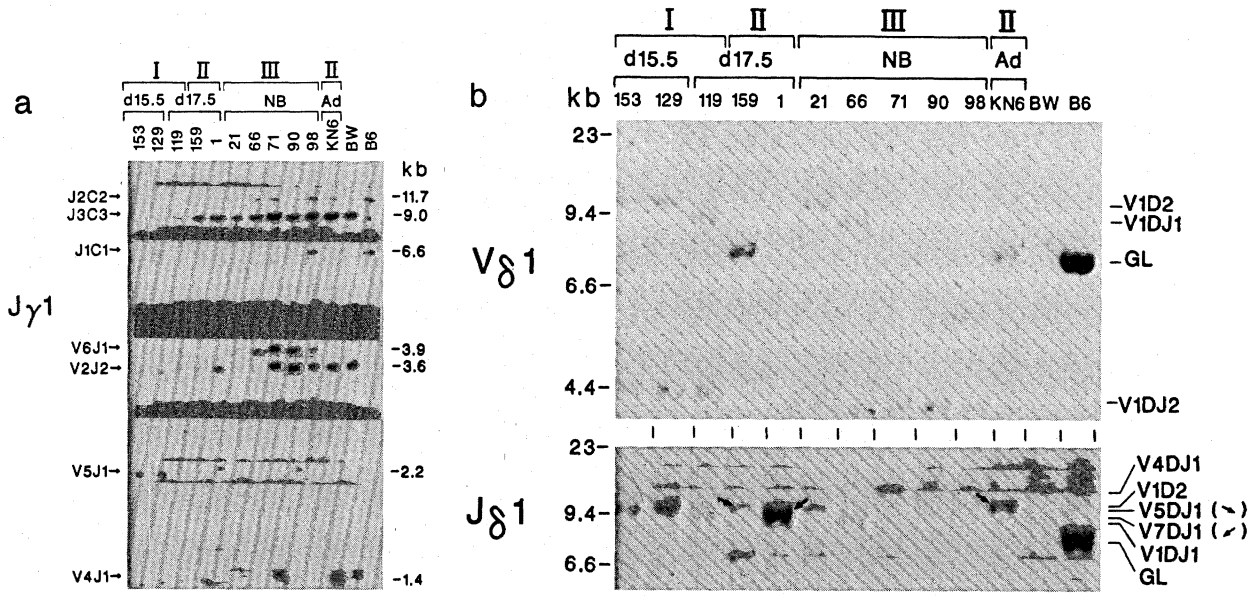

FIG. 2. Southern blot analysis of DNA from γδ-bearing hybridomas. Indicated on top of each lane are the hybridoma number, the stage of development of the thymocyte fusion partner [day 15.5, day 17.5, newborn (NB) or adult (Ad)] and the type of γδ receptor as defined in Fig. 1*b* (I, II, or III). (*a*) *Hin*dIII-digested DNA was analyzed with a $J_{\gamma 1}$ probe, which cross-hybridizes with $J_{\gamma 2}$ and $J_{\gamma 3}$ gene segments. Assignment of bands to germ-line or rearranged genes (indicated at left) was done according to a previous report (19) and confirmed by hybridization with corresponding V_γ probes. BW5147 carries one copy of out-of-frame, untranscribed $V_4J_1C_1$ γ gene, one copy of $V_7J_4C_4$ (an unusual *V–J* joining), and one copy of out-of-frame $V_2J_2C_2$ γ gene. (*b*) *Eco*RI-digested DNA was analyzed either with a $V_{\delta 1}$ probe or with a $J_{\delta 1}$ probe. The 9.7-, 8.8-, and 7.3-kb $V_{\delta 1}$ bands were assigned to V_1D_2, V_1DJ_1, and germ line (GL), respectively, by a previous report (15). Note that V_1D_2 and V_1DJ_1 bands hybridized to both $V_{\delta 1}$ and $J_{\delta 1}$ probes as described previously (15). The 4.2-kb $V_{\delta 1}$ band was assigned to V_1DJ_2 rearrangement according to restriction map analysis of a cosmid clone from hybridoma 129, which contained this rearranged gene (data not shown). The assignments of 13.0-, 9.6-, 9.3- and 7.8-kb $J_{\delta 1}$ bands to V_4DJ_1 δ, V_5DJ_1 δ, V_7DJ_1 δ, and germ line, respectively, were done according to previous reports (15, 16) and after probing with the respective V_δ probes. J_δ and C_δ gene segments are entirely deleted from the genome of the thymoma BW5147 (15). Thus all observed δ gene rearrangements in the hybridomas are derived from the thymocyte fusion partners.

but this *V–J* joining is out-of-frame (19). Thus the simplest interpretation of the Southern and Northern blot analyses is that type II hybridomas carry at least two copies of the $V_4J_1C_1$ γ genes, one being in-frame joined and coding for the surface-expressed γ chain and the other being out-of-frame joined and derived from BW5147. That this is indeed the case has recently been confirmed by cDNA cloning and sequencing (Y.T., unpublished data).

As to the δ locus, both day 17.5 type II hybridomas showed only $J_{\delta 1}$ rearrangements (Fig. 2*b* and Table 1): $V_{\delta 5}$ to $J_{\delta 1}$ in hybridoma 159 and a previously unreported $V_{\delta 7}$ to $J_{\delta 1}$ in hybridoma 1. Consistent with the Southern blot studies, these hybridomas contained full-length transcripts detected by the

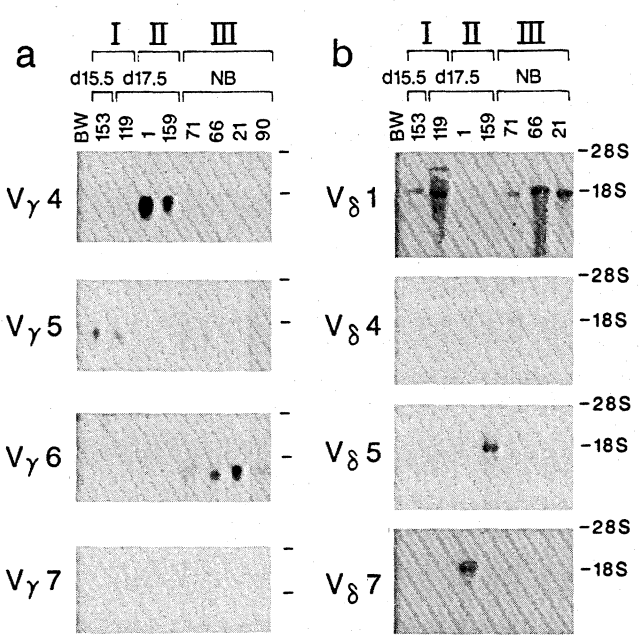

FIG. 3. Northern blot analysis of RNA from γδ hybridomas. Total RNA was analyzed with the $V_{\gamma 4}$, $V_{\gamma 5}$, $V_{\gamma 6}$, or $V_{\gamma 7}$ probe (*a*) or with the $V_{\delta 1}$, $V_{\delta 4}$, $V_{\delta 5}$, or $V_{\delta 7}$ probe (*b*). No transcripts hybridizing to $C_{\gamma 4}$ or $V_{\delta 2}$ probe were detected (data not shown). d, Day; NB, newborn.

Table 1. Summary of the genomic arrangement and transcription state of γ and δ genes in γδ-bearing hybridomas

Age	Hybridoma number	J₁ γ	δ		
			J_1	J_2	Partial
Day 15.5	153	[V_5]/*	d/G	[V_1]/G	V_1D_2
	129	[V_5]/G	d/G	[V_1]/G	V_1D_2
Day 17.5	119	[V_5]/*	d/[V_1]	[V_1]/G	
	159	[V_4]/G	[V_5]/‡	G/G	
	1	[V_4]/V_5	[V_7]/§	G/G	
Newborn	21	[V_6]/†	d/G	[V_1]/G	V_1D_2
	66	[V_6]/†	d/[V_1]	[V_1]/G	
	71	[V_6]/†	d/[V_4]	[V_1]/G	
	90	[V_6]/V_5	d/d	[V_1]/¶	
	98	[V_6]/G	d/d	[V_1]/G	
Adult	KN6	[V_4]/[V_4]	[V_5]/[V_5]	G/G	
	BW	V_4/d	d/d	d/d	

Rearranged and transcribed *VJC* γ or *VDJC* δ genes are boxed. G, germ line; d, deletion.
*V_5J_1 or V_4J_1 rearrangement.
†V_6J_1 or V_4J_1 rearrangement.
‡Unidentified rearrangement.
§V_7J_1 or unidentified rearrangement.
¶V_1J_1 or unidentified rearrangement.

$V_{\delta 5}$ and $V_{\delta 7}$ probe, respectively (Fig. 3*b*), indicating that the δ chains expressed on the surface of hybridomas 159 and 1 are encoded by V_5DJ_1C and V_7DJ_1C δ genes, respectively. Further analysis of adult type II hybridomas showed that the γ chains are frequently encoded by the $V_4J_1C_1$ γ gene, whereas the δ chain is encoded by the $V_{\delta 4}$, $V_{\delta 5}$, or $V_{\delta 7}$ gene segments all rearranged to the $DJ_{\delta 1}C_\delta$ sequence (Y.T., unpublished data).

$\gamma\delta$ TCR Preferentially Expressed on Thymocytes of Newborn Mice Are Encoded by $V_{\gamma 6}$ and $V_{\delta 1}$. The molecular mass of the type III γ chain (35 kDa) was lower than that of either the type I γ (38 kDa) or type II γ (36 kDa) chain and was decreased by 3 kDa after endo F treatment (Fig. 2*b*). The type III δ chain was similar to the type I δ chain before (48 kDa) and after (36 kDa) endo F treatment. This type of $\gamma\delta$ heterodimer was identified on 10 out of 12 newborn and 3 out of 9 adult hybridomas. In these hybridomas, $V_6J_1C_1$ γ is the only γ gene that is both rearranged (Fig. 2*a*) and expressed at the RNA level (Fig. 3*a*). The $V_{\gamma 4}$ gene segments are also rearranged, but their transcripts are undetected (Fig. 3*a*). $V_6J_1C_1$ γ-chain cDNA clones isolated from one of the newborn hybridomas (no. 21) confirmed that the *V–J* joining was in-frame (data not shown). Thus these results suggest that among the $\gamma\delta$ receptor-bearing DN thymocytes of newborn mice the $V_6J_1C_1$ γ chain is expressed preferentially on the surface. This is in stark contrast with the equally favored expression of the $V_5J_1C_1$ γ chain on the surface of day 15.5 thymocytes (see above).

As in type I hybridomas, V_1DJ_2C δ genes (Fig. 2*b*) and their full-length transcripts (Fig. 3*b* and Table 1) were detected in all type III hybridomas. In addition, hybridomas 21, 66, 71, and 98 carry an incomplete V_1D_2 δ, an apparently complete V_1DJ_1C δ, V_4DJ_1C δ, and an unidentified rearrangement involving $V_{\delta 1}$ (5.7 kb), respectively (Fig. 2*b*). Hybridomas 66 and 71 seem to carry full-length transcripts of the rearranged V_1DJ_1C δ and V_4DJ_1C δ genes (Fig. 3*b*), respectively. Thus, in the majority (three out of five) of the type III hybridomas, a $V_1DJ_2C_\delta$ gene seems to be used to code for the surface-expressed δ chains. In the rest of the cases (two out of five), the same *V* genes may also be used for δ expression, although coexistence of other δ gene transcripts in these hybridomas makes this conclusion less certain.

DISCUSSION

Developmental Age-Dependent Occurrence of Intrathymic $\gamma\delta$ TCR Encoded by Different γ and δ Gene Segments. The results of the analysis of hybridomas are summarized in Table 2. It is clear that during development specific γ and δ genes are preferentially utilized to code for the $\gamma\delta$ receptors on thymocytes in an age-dependent fashion. This conclusion is supported by the results obtained with thymocyte populations (Fig. 1*a*). Rearrangement and RNA expression of different V_γ and V_δ gene segments in fetal vs. adult thymocytes has been reported (15, 16). However, these studies were carried out using a population of thymocytes or hybridomas, the majority of which do not bear $\gamma\delta$ receptors. Our present study focused on the cells expressing these receptors and demonstrated the occurrence of developmental age-dependent rearrangement and surface-expression of specific *V* and *J* gene segments for both γ and δ chains.

Table 2. Distribution of various types of $\gamma\delta$ hybridomas

$\gamma\delta$ protein complex type	Rearranged and expressed TCR gene		Age			
	γ	δ	Day 15.5	Day 17.5	NB	Adult
I	V_5J_1	V_1DJ_2 V_4DJ_1	3	1	—	—
II	V_4J_1	V_5DJ_1 V_7DJ_1	—	2	2	6
III	V_6J_1	V_1DJ_2	—	—	10	3

TCR γ Gene Transcription May Be Controlled by *V* Gene Segment-Specific Mechanisms. Control at the level of DNA rearrangement does not, however, seem to be the sole mechanism for the differential expression of various γ genes. The thymoma BW5147 carries a transcriptionally silent $V_4J_1C_1$ γ gene (Figs. 2*a* and 3*a*). When a thymocyte expressing its own $V_4J_1C_1$ γ gene is fused with the thymoma, the transcription of the latter's $V_4J_1C_1$ γ gene is induced (Fig. 3*a*; Y.T., unpublished data). By contrast, transcripts of the BW5147-derived $V_4J_1C_1$ γ gene are not detectable in $V_5J_1C_1$ γ (type I)- or $V_6J_1C_1$ γ (type III)-expressing hybridomas (Fig. 3*a*). Likewise, in two hybridomas each carrying two different thymocyte-derived rearranged γ genes, namely hybridoma 1, which carries $V_5J_1C_1$ and $V_4J_1C_1$, and hybridoma 98, which carries $V_5J_1C_1$ and $V_6J_1C_1$ (Table 1), only the transcripts of one of the two γ genes ($V_4J_1C_1$ for hybridoma 1 and $V_6J_1C_1$ for hybridoma 98) are detectable (Fig. 3*a*). These findings are striking because in immunoglobulin or in the other TCR gene loci studied to date no such *V* segment-specific control of transcription (or RNA stability) has been observed. Indeed this mutual exclusion of *V*-dependent transcriptions does not seem to apply to the δ gene loci (see Fig. 3*b*, hybridoma 71). The above finding may mean that in the γ gene loci "allelic exclusion" at the level of DNA rearrangement is restricted to true alleles and does not operate between different V_γ gene segments.

Independent Recognition Roles May Be Borne by the Distinct $\gamma\delta$ TCR Subsets. The separate transcriptional control combined with the relatively low level of sequence homology among the V_γ gene segments suggest independent roles of the $\gamma\delta$ receptors encoded by different V_γ gene segments. This view is supported by the preferential tissue distribution of various types of $\gamma\delta$-bearing cells: whereas type II cells are confined to somatic lymphoid tissues such as spleen, lymph nodes, and peripheral blood, type I cells are mostly found in epidermis (20). More recently we found that the gut IEL preferentially utilize yet another V_γ gene segment (13).

Another feature of $\gamma\delta$ TCR that suggests distinct recognition roles by the various subsets is their difference in the extent and distribution of diversity within the *V* regions. Thus $\gamma\delta$ TCR of early fetal thymocytes and DEC as well as a majority of newborn thymocytes have very limited complementarity determining region 1 (CDR1) and CDR2 diversity, whereas $\gamma\delta$ TCR of adult thymocytes exhibit more diversity in these regions. This fetal/newborn/DEC vs. adult difference in the relative extent of *V* region diversity seems to be even more pronounced in the CDR3 regions (15, 16). This suggests distinct recognition repertoires for the fetal vs. adult $\gamma\delta$ thymocytes and epithelium-associated vs. somatic lymphoid tissue-associated $\gamma\delta$ cells. Type II $\gamma\delta$ cells have been shown to recognize class I, class II, and class IB (21) MHC gene products (refs. 22 and 23; M.B. and K.I., unpublished results) and, consequently, could have a repertoire quite similar to that of TCR $\alpha\beta$-bearing cells. On the other hand, the ligand of type I/DEC, type III, or IEL $\gamma\delta$ TCR, each utilizing a very limited and distinct V_γ-V_δ combination, has not yet been identified. However, since IEL expresses CD8 molecules (12, 13), which have affinity for MHC class I products, we favor the hypothesis that the ligand includes autologous MHC class I or class I-like (such as TLa) molecules. It may be that each of these $\gamma\delta$ TCR subsets is designed to recognize peptides that are derived from pathogens prone to infect the epithelial cells of the respective anatomical sites and are presented by a MHC product that is specifically expressed on epithelial cells under "patholog-

ical" conditions. MHC class IB molecules that are distinct from MHC class I, K, D, and L gene products (21) could be a candidate for the ligand of such cells since their expression has been found to be tissue specific and dependent on cell differentiation and activation state.

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