

Assembly of Productive T Cell Receptor δ Variable Region Genes Exhibits Allelic Inclusion

By Barry P. Sleckman, Bernard Khor, Robert Monroe,
and Frederick W. Alt

*From the Howard Hughes Medical Institute, Children's Hospital and Department of Genetics,
Harvard Medical School and The Center for Blood Research, Boston, Massachusetts 02115*

Summary

The generation of a productive "in-frame" T cell receptor β (TCR β), immunoglobulin (Ig) heavy (H) or Ig light (L) chain variable region gene can result in the cessation of rearrangement of the alternate allele, a process referred to as allelic exclusion. This process ensures that most $\alpha\beta$ T cells express a single TCR β chain and most B cells express single IgH and IgL chains. Assembly of TCR α and TCR γ chain variable region genes exhibit allelic inclusion and $\alpha\beta$ and $\gamma\delta$ T cells can express two TCR α or TCR γ chains, respectively. However, it was not known whether assembly of TCR δ variable regions genes is regulated in the context of allelic exclusion. To address this issue, we have analyzed TCR δ rearrangements in a panel of mouse splenic $\gamma\delta$ T cell hybridomas. We find that, similar to TCR α and γ variable region genes, assembly of TCR δ variable region genes exhibits properties of allelic inclusion. These findings are discussed in the context of $\gamma\delta$ T cell development and regulation of rearrangement of TCR δ genes.

Key words: T cells • $\gamma\delta$ T cells • T cell receptor rearrangement • allelic exclusion • T cell receptor δ

Lymphocyte antigen receptor variable region genes are assembled during development from component variable (V),¹ diversity (D), and joining (J) gene segments in the case of the TCR β and δ chain genes and the Ig heavy (H) chain gene or from V and J gene segments in the case of TCR α and γ chain genes and Ig light (L) chain genes (1, 2). Productive rearrangement of TCR β or IgH chain variable region genes results in cessation of further V to DJ rearrangements on the alternate allele, a process referred to as allelic exclusion (2, 3). "Functional" rearrangement of IgL κ or λ L chain genes (i.e., rearrangements which generate an IgL chain that can pair with a pre-existing IgH chain) also lead to cessation of further IgL chain rearrangements resulting in both allelic and IgL chain isotype exclusion (4). In contrast, TCR α and TCR γ chain variable region gene assembly does not exhibit properties of allelic exclusion (3, 5–7). Consequently $\alpha\beta$ and $\gamma\delta$ T cells can express two TCR α or γ chains, respectively (6, 7).

Several models have been proposed to account for allelic exclusion. One model proposed that the probability of a productive rearrangement is low making it unlikely that an individual cell could have two productive rearrangements (8). However, it is now known that the probability of a productive rearrangement can be as high as 33% (9). Another model proposed that the probability of two complete V(D)J rearrangements in any one cell was low. However, a significant percentage of peripheral B and T cells have two IgH or TCR β V(D)J rearrangements, respectively, arguing against this model (3, 10). It has been proposed that IgH chain allelic exclusion occurs due to a toxic effect of expressing two IgH chains (11). However, the recent demonstration that B cell development proceeds normally in mice that express two IgH transgenes essentially rules out this model (12). An early model, based on analyses of rearrangement patterns in cell lines, proposed that allelic exclusion is regulated and that expression of a productively rearranged IgH or IgL chain prevents further rearrangements at the IgH and IgL chain loci, respectively (4, 13, 14). This regulated model was supported by studies demonstrating that expression of IgH or IgL transgenes resulted in a block in endogenous IgH or IgL chain gene rearrangement, respectively (15–18). Studies of TCR β transgenic mice have supported an analogous model by which the TCR β transgene feeds

¹Abbreviations used in this paper: BW, BW-1100.129.237; D, diversity; DN, double negative; DP, double positive; H, heavy; J, joining; L, light; RAG, recombinase activating gene; V, variable.

The first two authors contributed equally to this study.

back to block endogenous TCR β rearrangements (19). In addition, it has recently been demonstrated that expression of IgH or TCR β chains as pre-B or pre-T cell receptors, respectively, is required for allelic exclusion (20–23).

T cells can be divided into two distinct lineages based on expression of either $\alpha\beta$ or $\gamma\delta$ TCRs. The genes that encode the TCR β and TCR γ chains lie in distinct loci, whereas the genes that encode the TCR δ and TCR α chains lie in a single locus (TCR α/δ locus; Fig. 1; references 24, 25). In the adult thymus TCR β rearrangements are initiated at the CD4⁻/CD8⁻ (double negative, DN) stage of thymocyte development and are ordered with D β to J β rearrangement occurring on both alleles before V β to DJ β rearrangement (3, 26, 27). Once a productive V(D)J β rearrangement is made and a TCR β chain expressed, cells proceed to the CD4⁺/CD8⁺ (double positive, DP) stage of development and further V β to DJ β rearrangements cease (3, 26, 27). As a result, many $\alpha\beta$ T cells have DJ β rearrangements on a single allele (28). V α to J α rearrangements are initiated at the DP stage. However, unlike the TCR β locus, expression of a TCR α chain does not result in cessation of V α to J α rearrangements (3, 26, 27). This process continues on both alleles, and V α to J α rearrangements can result in the deletion of previously assembled productive VJ α rearrangements (29). It has been proposed that the downregulation of recombinase activating gene (RAG) gene expression may ultimately be responsible for termination of V α to J α rearrangement (30).

Several notable differences exist between the developmental regulation of assembly of $\alpha\beta$ and $\gamma\delta$ TCR variable region genes. Assembly of TCR γ and TCR δ variable region genes occurs at the DN stage of thymocyte development (31). It is not known whether rearrangement of these genes is concurrent or sequential. In addition, assembly of TCR γ genes does not appear to exhibit allelic exclusion (7). Similar to the TCR β locus, assembly of TCR δ variable region genes does not proceed to completion on all alleles. However, unlike the TCR β locus, TCR δ variable region gene assembly does not appear to be ordered, since incomplete DD δ , DJ δ and VD δ rearrangements have been described (32, 33). It is unresolved whether productive TCR δ rearrangements lead to termination of further TCR δ rearrangements (allelic exclusion) or whether TCR δ rearrangements are limited by factors independent of the formation of productive rearrangements. To address this issue, we have analyzed TCR δ rearrangements in a panel of T cell hybridomas derived from splenic $\gamma\delta$ T cells. We find the percentage of cells with two in-frame V(D)J δ rearrangements is similar to that predicted in the absence of allelic exclusion. These findings are discussed in the context of $\gamma\delta$ T cell development.

Materials and Methods

Isolation of $\gamma\delta$ T Cells and Production of $\gamma\delta$ T Cell Hybridomas. Whole spleen cell suspensions from C57BL6 \times CBA mice were incubated in DMEM-15 containing 40 U recombinant human IL-2/ml (PharMingen, San Diego, CA) on plates that had been

coated with 10 μ g/ml rat anti-hamster Ig (PharMingen) followed by 10 μ g/ml of an anti-TCR δ chain mAb (GL4; PharMingen). Cultures were maintained for 6 d and the resulting cells were >90% pure $\gamma\delta$ T cells as determined by flow cytometry (data not shown). Hybridomas were produced by fusing these $\gamma\delta$ T cells to the thymoma BW-1100.129.237 using a fusion protocol that has been described elsewhere (34, 35).

Flow Cytometry. Single cell suspensions were prepared from thymus, spleen and lymph nodes as previously described (36). Hybridomas were stained with FITC-conjugated anti-TCR β chain (H57-597) and PE-conjugated anti-TCR δ chain (GL3) monoclonal antibodies from PharMingen and were analyzed by a FACScan[®] (Becton Dickinson & Co., Sparks, MD).

Genomic DNA Analysis. Genomic DNA was isolated and Southern blotting carried out as previously described using Zetaprobe membranes (Bio-Rad Laboratories, Hercules, CA) and probes generated by random hexamer priming (Boehringer Mannheim Corp., Indianapolis, IN) using α -³²P]dCTP (34, 35). Probe 1 is a 600-bp HindIII fragment (39). Probe 3 is a 550-bp MspI to NdeI fragment and probe 4 a 1-kb NdeI-XbaI fragment from pTAE-7 (40). Probe 5 is a 350-bp PCR product generated as described elsewhere (41). Probe 6 is a 1.5-kb EcoRI C δ cDNA fragment (42).

PCR and Sequence Analysis. PCR reactions were carried out using 200 ng of genomic DNA isolated from hybridomas and 2.5 U AmpliTaq polymerase (Perkin-Elmer Corp., Norwalk, CT). PCR conditions were: 92°C for 1 min 30 s, 62°C for 2 min 30 s, 72°C for 1 min 30 s cycled 30 times. PCR products were subcloned into pT7blue (Novagen Inc., Madison, WI) before sequencing on a ABI Prism 377 DNA sequencer (Perkin-Elmer Corp.). The V δ nomenclature of Arden et al. (43) is used. The V δ and J δ primers used to PCR VDJ δ joins were as follows: V δ primers: ADV7S (V δ 7/V δ 6), TCACCCTGGACTGTTCATAT; ADV11S5, ATTTTACGACCACCATGAGG; ADV17S2 (V δ 9), ATGCTGATTCTAAGCCTGCT; DV2S8 (V δ 8), AGCAGGTGAGACAAAGTCC; DV4S8, ACGATAGAGTGCAACTACTCA; DV6S2 (V δ 3), ATGGGATGTGTGAGTGGAAT; V10S7 (V δ 7), TGAAGAGGCTGCTGTGCTC; DV101S1 (V δ 1), ATGCTTTGGAGATGTCCAGT; DV102S1 (V δ 2), ATGGGGATGTTCCTTCAAGT; DV104S1 (V δ 4), CAGGTGGCAGAATCA-GCAA; DV105S1 (V δ 5), ATGATTGTTGCCGCGACCC; J δ primers: J δ 1, AGAGTCCAAGAATCATCTACG; J δ 2, CTTC-TGTGTACTACTTTTTATTTTC.

Southern blotting of PCR products was carried out with internal oligos to J δ 1 (CGACAAACTCGTCTTTGG) or J δ 2 (CTCCTGGGACACCCGACAGA).

Theoretical Determination of In-Frame Rearrangement Percentages. All mature $\gamma\delta$ T cells must have at least one productive VDJ δ rearrangement. If the probability that a VDJ δ rearrangement will be in-frame equals P then the probability that a VDJ δ rearrangement will be out of frame will be (1 – P). If there is an equal chance of a rearrangement in each of the three reading frames then P = 1/3. In the absence of allelic exclusion and in cells with two VDJ δ rearrangements, the percentage of cells with two in-frame TCR δ rearrangements will be equal to the probability that the cell will have two in-frame rearrangements divided by the probability that the cell will have at least one in-frame rearrangement.

$$\frac{P^2}{1 - (1 - P)^2} = \frac{\left(\frac{1}{3}\right)^2}{1 - \left(1 - \frac{1}{3}\right)^2} = \frac{\frac{1}{9}}{1 - \left(\frac{2}{3}\right)^2} = \frac{1}{5}$$

Results

Generation of $\gamma\delta$ T Cell Hybridomas. Splenic $\gamma\delta$ T cell hybridomas were generated from C57BL6 \times CBA F1 mice by stimulating unfractionated spleen cells with plate-bound anti-TCR δ antibody (GL3) as described in Materials and Methods section. The resulting cell population was $>90\%$ pure $\gamma\delta$ T cells as determined by flow cytometry (data not shown). These cells were fused to the BW-1100.129.237 (BW) thymoma which is incapable of producing TCR δ , β , or α chains (35). T cell hybridomas generated by fusion of a $\gamma\delta$ T cell to BW were identified by flow cytometric analysis of cell surface TCR δ expression (data not shown). Only those hybridomas that expressed TCR δ were chosen for further analysis.

To ensure that both TCR δ alleles were present in the resulting panel of $\gamma\delta$ T cell hybridomas genomic DNA isolated from these hybridomas was assayed by Southern blot analyses using TCR δ restriction fragment length polymorphisms that exist between C57BL6 and CBA mice (44). Genomic DNA isolated from $\gamma\delta$ T cell hybridomas was digested with HindIII and subjected to Southern blot analysis using probe 6 which is directed against the TCR δ constant region gene ($C\delta$; Figs. 1 and 2). Probe 6 hybridizing bands are not found in BW as $C\delta$ has been deleted on both alleles due to $V\alpha$ to $J\alpha$ rearrangements (Fig. 2). Using probe 6, distinct size bands are generated by the C57BL6 and CBA TCR δ alleles, and hybridomas that had lost either allele (for example F1D.11) were excluded from further analysis (Fig. 2).

To determine whether the $\gamma\delta$ T cell hybridomas chosen for analysis were clonal, genomic DNA was subjected to Southern blot analysis using probe 4 to detect rearrangements to $J\delta 1$ (Fig. 3 a), probe 5 to detect rearrangements to $J\delta 2$ (Fig. 3 b) or a probe that detects rearrangements to $J\beta 2$ (data not shown). Hybridomas that were oligoclonal on the basis of having three or more TCR δ or TCR β rearrangements were excluded from further analysis. The resulting 27 $\gamma\delta$ T cell hybridomas that satisfied the above criteria were characterized further.

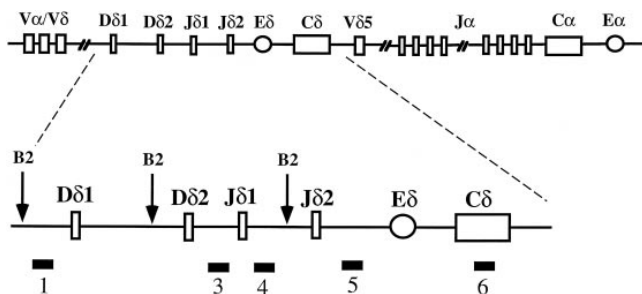


Figure 1. Schematic of the mouse TCR α/δ locus. Shown are the $V\alpha/V\delta$ gene segments, the $D\delta$ and $J\delta$ gene segments, the TCR δ enhancer ($E\delta$), the TCR δ constant region gene ($C\delta$), and the $V\delta 5$ gene segment. This is followed by the $J\alpha$ gene segments, the TCR α constant region gene ($C\alpha$), and the TCR α enhancer ($E\alpha$). Also shown are probes 1 and 3 through 6 and the approximate position of the BglII ($B2$) sites. The schematic is not drawn to scale.

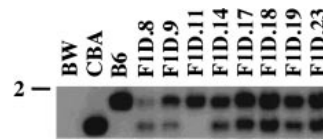


Figure 2. Analysis for the presence of the CBA and C57BL6 TCR δ alleles. Genomic DNA from the hybridoma fusion partner BW 1100.129.237 (BW), CBA kidney (CBA), C57BL6 kidney (B6), or $\gamma\delta$ T cell hybridomas (F1D) was digested with HindIII and subjected to Southern blot analysis using probe 6 (Fig. 1). The 2-kb marker is shown.

TCR δ Allele Configurations in $\gamma\delta$ T Cell Hybridomas. To determine the extent of TCR δ rearrangement in the panel of $\gamma\delta$ T cell hybridomas, hybridoma genomic DNA was digested with BglII and subjected to Southern blot analysis using probe 4 (Figs. 1 and 3 a, data not shown). None of the hybridomas exhibited germline size bands, demonstrating that most TCR δ alleles are rearranged in splenic $\gamma\delta$ T cells (Fig. 3 a, data not shown). In addition, most hybridomas gave two bands with probe 4, showing that most TCR δ rearrangements in splenic $\gamma\delta$ T cells use the $J\delta 1$ gene segment. F1D.58 exhibited single nongerm-line bands with probes 4 and 5, demonstrating that it had undergone rearrangements to $J\delta 1$ and $J\delta 2$ (Fig. 3 b). All

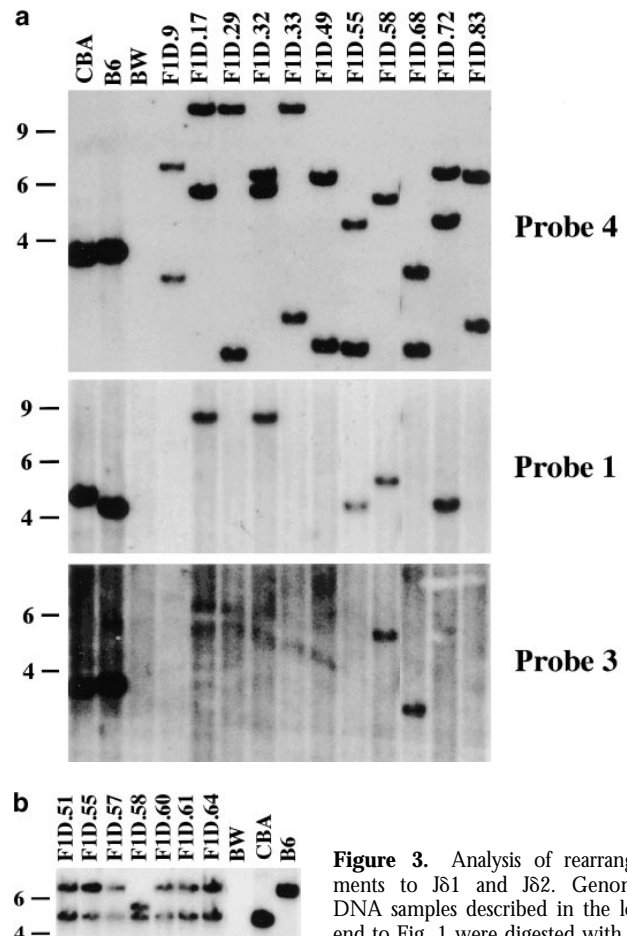


Figure 3. Analysis of rearrangements to $J\delta 1$ and $J\delta 2$. Genomic DNA samples described in the legend to Fig. 1 were digested with either BglII (a) or HindIII (b) and subjected to Southern blot analysis using probes 1, 3, and 4 (a) or probe 5 (b). Shown are the 9-, 6- and 4-kb molecular mass markers.

other hybridomas exhibited germline bands from the C57BL6 and CBA TCR δ alleles using probe 5, showing that there is minimal rearrangement to the J δ 2 gene segment in splenic $\gamma\delta$ T cell hybridomas analyzed here (Fig. 3 *b*, data not shown).

To assay for incomplete TCR δ rearrangements, BglII-digested hybridoma genomic DNA was probed with probes 1 and 3 (Figs. 1 and 3 *a*, data not shown). Probe 1 hybridizing bands of similar size to probe 4 hybridizing bands would be generated by alleles that have undergone D δ 1 to D δ 2 or D δ 1 to J δ 1 rearrangements. Hybridomas F1D.19, 45, 51, 55, 71, and 72 all exhibit a 4.5-kb BglII band with probes 1 and 4 (Fig. 3 *a*, Table 1, data not shown), whereas hybridoma F1D.58 yields a 5.5-kb BglII band with probes 1 and 4 (Fig. 3 *a* and Table 1). To determine which hybridomas had undergone a D δ 1 to D δ 2 rearrangement, BglII-digested DNA was assayed with a probe (probe 3) to the region between D δ 1 and D δ 2 which will be deleted upon D δ 1 to D δ 2 rearrangement (Fig. 1). F1D.58 has a 5.5-kb BglII band that hybridizes to probe 3, demonstrating that one of the alleles in this hybridoma has undergone a D δ 1 to D δ 2 rearrangement (Fig. 3 *a*, Table 1). The hybridomas that yielded a 4.5-kb BglII band with probes 1 and 4 do not have probe 3 hybridizing bands, demonstrating that they have undergone D δ 1 to J δ 1 rearrangements (Fig. 3 *a*).

The DV105S1 (V δ 5) gene segment rearranges by inversion and, therefore, a nongermline probe 1 hybridizing band should be generated by the reciprocal product of a DV105S1 to D δ 1 rearrangement. Furthermore, this band would likely be of a different size than the probe 4 hybridizing band generated by the same rearrangement. Hybridomas F1D.17, 23, 32, and 61 all have 9-kb BglII probe 1 hybridizing bands (Fig. 3 *a*, data not shown). None of these hybridomas has a 9-kb probe 4 hybridizing BglII band, and each was found to have a DV105S1 to J δ 1 rearrangement

Table 1. $\gamma\delta$ T Cell Hybridomas with One Complete VDJ δ Rearrangement

Hybridoma	V(D)J δ rearrangement	Incomplete rearrangement
F1D.19	DV104S1-J δ 1	D(D) J δ 1
F1D.45	DV7S-J δ 1	D(D) J δ 1
F1D.51	DV104S1-J δ 1	D(D) J δ 1
F1D.55	DV104S1-J δ 1	D(D) J δ 1
F1D.58	DV101S1-J δ 2	DD
F1D.68	DV104S1-J δ 1	DV6S2-(D)D
F1D.71	DV104S1-J δ 1	D(D) J δ 1
F1D.72	DV7S-J δ 1	D(D) J δ 1

The V δ gene segments utilized were determined by PCR as described in Materials and Methods section. The D δ 2 gene segment is indicated in brackets in some rearrangements as it can not be determined from this analysis whether it is utilized. The V δ gene segment nomenclature of Arden et al. (43) is used.

by PCR analysis (Table 2). Finally, V δ to D δ rearrangements by V δ gene segments other than DV105S1 will result in loss of probe 1 hybridizing bands and generation of a non-germline probe 3 hybridizing band that should be similar in size to the band generated by probe 4 when probing BglII-digested DNA. In this regard F1D.68 has a 3.5-kb BglII band that hybridizes to probes 3 and 4 and was found to have a V δ to D δ 2 rearrangement by PCR analysis (Fig. 3 *a*, Table 1).

These Southern blot analyses revealed that, of the 27 $\gamma\delta$ T cell hybridomas analyzed, all had complete V(D)J δ rearrangements on one allele (Fig. 3, *a* and *b*, Tables 1 and 2). On the other allele, 19 hybridomas also had complete V(D)J δ rearrangements, one had a D δ 1D δ 2 rearrangement, one had a VD δ 2 rearrangement and 6 had D δ 1J δ 1 rearrangements (Fig. 3 *a*, Table 1). In addition, the J δ 2 gene segment was used in only one rearrangement (Fig. 3 *b*, Table 1).

Analysis of V(D)J δ Rearrangements in $\gamma\delta$ T Cell Hybridomas. Using primers that should recognize the members of the 11 known mouse V δ gene families in conjunction with primers that were just downstream of J δ 1 or J δ 2, PCR

Table 2. $\gamma\delta$ T Cell Hybridomas with Two Complete VDJ δ Rearrangements

Hybridoma	Rearrangement	In-frame	Rearrangement	In-frame
F1D.9	DV107S7-J δ 1	Y	ADV7S2-J δ 1	N
F1D.17	ADV7S1-J δ 1	Y	DV105S1-J δ 1	N
F1D.23	ADV7S1-J δ 1	Y	DV105S1-J δ 1	N
F1D.29	ADV7S1-J δ 1	Y	DV104S1-J δ 1	N
F1D.32	DV7S-J δ 1	Y	DV105S1-J δ 1	Y
F1D.33	ADV7S1-J δ 1	Y	ADV17S2-J δ 1	N
F1D.36	DV104S1-J δ 1	Y	ND	
F1D.42	DV10S7-J δ 1	Y	ADV17S2-J δ 1	N
F1D.49	DV7S5-J δ 1	Y	DV104S1-J δ 1	Y
F1D.57	DV104S1-J δ 1	Y	DV7S4-J δ 1	N
F1D.60	ADV7S2-J δ 1	Y	DV104S1-J δ 1	Y
F1D.61	DV105S1-J δ 1	Y	DV104S1-J δ 1	Y
F1D.64	ND	Y	DV7S6-J δ 1	N
F1D.67	DV104S1-J δ 1	Y	DV7S5-J δ 1	N
F1D.73	DV104S1-J δ 1	Y	DV104S1-J δ 1	Y
F1D.75	ADV7S1-J δ 1	Y	DV104S1-J δ 1	Y
F1D.83	DV102S1-J δ 1	Y	DV7S5-J δ 1	N
F1D.89	DV104S1-J δ 1	Y	DV7S5-J δ 1	N
F1D.91	DV7S6-J δ 1	Y	ND	

F1D.32 has a rearrangement utilizing a DV7S V δ gene segment that differs from other known family members by at least five nucleotides (data not shown). This V δ gene segment may represent a novel DV7S family member or is due to strain differences. The two DV104S1 rearrangements in F1D.73 are distinct as determined by differences in junctional diversity (data not shown). The in-frame TCR δ rearrangement in F1D.64 is presumed as the cell expresses a TCR δ chain.

analysis was carried out on all hybridomas to determine V δ gene segment usage (Tables 1 and 2; reference 43). By this analysis none of the hybridomas analyzed gave more than two distinct PCR products (data not shown). PCR products from the 19 hybridomas that had two complete V(D)J δ rearrangements were cloned and sequenced. None of the V(D)J δ rearrangements isolated used known V δ pseudogenes (43). Two distinct rearrangements were isolated from 16 of the 19 hybridomas determined to have two V(D)J δ rearrangements. All hybridomas had at least one in-frame V(D)J δ rearrangement except for F1D.64 in which only a single out of frame V(D)J δ rearrangement was isolated (Table 2). The other allele of this hybridoma must have an in-frame V(D)J δ rearrangement, that was undetected by this analysis, as it expresses a TCR δ chain (data not shown). Only a single in-frame rearrangement was isolated from F1D.36 and F1D.91. The inability to detect more than a single rearrangement in these hybridomas could be due to the use of novel V δ gene segments unable to be detected by the primer set used in this analysis. Alternatively, these hybridomas may have two rearrangements involving members of the same V δ gene family that were not both detected upon nucleotide sequence analysis. Significantly, analyses of the 17 hybridomas with two defined V(D)J δ joins revealed that 6 had two in-frame rearrangements, demonstrating that assembly of TCR δ variable region genes does not exhibit allelic exclusion (Table 2).

Discussion

To determine if assembly of TCR δ variable region genes is regulated in the context of allelic exclusion, we have analyzed a panel of 27 clonal hybridomas derived from mouse splenic $\gamma\delta$ T cells. Of the 17 hybridomas with defined V(D)J δ rearrangements on both alleles, 6 (35%) have two in-frame rearrangements. This demonstrates that TCR δ variable region gene assembly does not exhibit allelic exclusion. Although this percentage is higher than the 20% (see Materials and Methods for calculations), which would be expected in the absence of allelic exclusion, this difference is not statistically significant ($P > 0.10$). Two human $\gamma\delta$ T cell clones with in-frame TCR δ rearrangements on both alleles have been described previously (33, 45). However, given the number of cells analyzed in these studies, it was not possible to determine whether these clones represented rare events or a general lack of TCR δ allelic exclusion. As TCR γ rearrangements do not exhibit allelic exclusion, failure of TCR δ allelic exclusion further increases the possibility that a single $\gamma\delta$ T cell will express two or more distinct $\gamma\delta$ TCRs (7).

It is possible that one of the TCR δ rearrangements in each of the six cells with two in-frame rearrangements encodes for a TCR δ chain that cannot be expressed on the surface of the cell and therefore would not signal a block of further TCR δ rearrangements. This may occur, for example, if the TCR δ chain were not able to pair with a TCR γ chain or a component of a $\gamma\delta$ pre-TCR, if such a receptor exists. In this regard, it has recently been shown that 2–4% of peripheral B cells have two in-frame IgH rearrangements but that only one encodes for an IgH chain that is capable of forming a pre-B cell receptor (22). Our data is more consistent with the notion that assembly of TCR δ variable region genes exhibits properties of allelic inclusion as the percentage of $\gamma\delta$ T cell hybridomas with two in-frame TCR δ rearrangements is in agreement with the percentage expected in the absence of allelic exclusion. Furthermore, this percentage is similar to that of $\alpha\beta$ T cells with two in-frame rearrangements at the TCR α locus, which also exhibits allelic inclusion (3).

It has been proposed for the IgH locus (and by analogy for the TCR β locus) that the precise ordering of variable gene segment rearrangement during lymphocyte development may be important for effecting allelic exclusion (14). In both of these loci, D to J rearrangement occurs on both alleles before V to DJ rearrangement. Presumably V to DJ rearrangement proceeds initially on one allele, at which point the rearrangement is "tested." If it encodes a protein that can be expressed, signals are generated that prevent further V to DJ rearrangements on the other allele. In accordance with this model, the expected number of B and T cells have V(D)J/DJ configured rearrangements of their IgH and TCR β alleles, respectively (3, 10).

Unlike the IgH and TCR β loci, assembly of TCR δ variable gene segments is not ordered during development, and we now show that the TCR δ locus is not regulated in the context of allelic exclusion. However, the finding that many $\gamma\delta$ T cells have incomplete TCR δ rearrangements demonstrates that rearrangement is frequently terminated before completion. The events that lead to termination of TCR δ rearrangement are not known. Thymic $\gamma\delta$ T cells do not express RAG-1 or RAG-2, and it is possible, as proposed for TCR α rearrangement, that down regulation of RAG expression leads to termination of TCR δ rearrangement (30, 46). Termination of TCR δ rearrangement, by whatever mechanism, may be part of a developmental program that is independent of TCR δ expression. Alternatively, rearrangement may cease upon TCR δ expression, and failure of allelic exclusion may be due to the unordered simultaneous rearrangement of TCR δ alleles.

We thank F. Livak and D. Schatz for providing us with probes and C.H. Bassing for critical review of the manuscript.

This work is supported by the Howard Hughes Medical Institute and by National Institutes of Health grants

AI20047 (F.W. Alt) and AI01297-01 (B.P. Sleckman). B.P. Sleckman is a recipient of a Career Development Award in the Biomedical Sciences from the Burroughs Wellcome Fund.

Address correspondence to Frederick W. Alt, Howard Hughes Medical Institute, Children's Hospital, Longwood Ave., Boston, MA 02115. Phone: 617-355-7290; Fax: 617-730-0432; E-mail: alt@rascal.med.harvard.edu

B.P. Sleckman's present address is Department of Pathology, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110.

Received for publication 28 July 1998.

References

1. Sleckman, B.P., J.R. Gorman, and F.W. Alt. 1996. Accessibility control of antigen-receptor variable-region gene assembly: role of *cis*-acting elements. *Annu. Rev. Immunol.* 14:459-481.
2. Okada, A., and F.W. Alt. 1994. Mechanisms that control antigen receptor variable region gene assembly. *Sem. Immunol.* 6:185-196.
3. Malissen, M., J. Trucy, E. Jouvin-Marche, P.-A. Cazenave, R. Scollay, and B. Malissen. 1992. Regulation of TCR α and β gene allelic exclusion during T cell development. *Immunol. Today.* 13:315-322.
4. Alt, F.W., V. Enea, A.L. Bothwell, and D. Baltimore. 1980. Activity of multiple light chain genes in murine multiple myeloma cells producing a single, functional light chain. *Cell.* 21:1-12.
5. Casanova, J.-L., P. Romero, C. Widmann, P. Kourilsky, and J.L. Maryanski. 1991. T cell receptor genes in a series of class I major histocompatibility complex-restricted cytotoxic T lymphocyte clones specific for *Plasmodium berghei* nonapeptide: Implications for T cell allelic exclusion and antigen-specific repertoire. *J. Exp. Med.* 174:1371-1383.
6. Padovan, E., G. Casorati, P. Dellabona, S. Meyer, M. Brockhaus, and A. Lanzavecchia. 1993. Expression of two T cell receptor α chains: dual receptor T cells. *Science.* 262:422-424.
7. Davodeau, F., M.-A. Peyrat, I. Houde, M.-M. Hallet, G. De Libero, H. Vie, and M. Bonneville. 1993. Surface expression of two distinct functional antigen receptors on human $\gamma\delta$ T cells. *Science.* 260:1800-1802.
8. Coleclough, C., R.P. Perry, K. Karjalainen, and M. Weigert. 1981. Aberrant rearrangements contribute significantly to allelic exclusion of immunoglobulin gene expression. *Nature.* 290:372-378.
9. Lansford, R., A. Okada, J. Chen, E.M. Oltz, T.K. Blackwell, F.W. Alt, and G. Rathbun. Mechanism and control of immunoglobulin gene rearrangement. In *Molecular Immunology-Frontiers in Molecular Biology*. 2nd edition. D.B. Hames, and D.M. Glover, editors. IRL Press, New York. 1-100.
10. ten Boekel, E., F. Melchers, and A. Rolink. 1995. The status of Ig rearrangements in single cells from different stages of B cell development. *Int. Immunol.* 7:1013-1019.
11. Wabl, M., and C. Steinberg. 1982. A theory of allelic exclusion for the immunoglobulin genes. *Proc. Natl. Acad. Sci. USA.* 79:6976-6978.
12. Sonoda, E., Y. Pewzner-Jung, S. Schwerts, S. Taki, S. Jung, D. Eilat, and K. Rajewsky. 1997. B cell development under the condition of allelic inclusion. *Immunity.* 6:225-234.
13. Alt, F.A., N. Rosenberg, S. Lewis, E. Thomas, and D. Baltimore. 1981. Organization and reorganization of immunoglobulin genes in A-MuLV-transformed cells: Rearrangement of heavy but not light chain genes. *Cell.* 27:381-390.
14. Alt, F.W., G.D. Yancopoulos, T.K. Blackwell, C. Wood, E. Thomas, M. Boss, R. Coffman, N. Rosenberg, S. Tonegawa, and D. Baltimore. 1984. Ordered rearrangement of immunoglobulin heavy chain variable region genes. *EMBO (Eur. Mol. Biol. Organ.)* 3:1209-1219.
15. Rusconi, S., and G. Kohler. 1985. Transmission and expression of a specific pair of rearranged immunoglobulin μ and κ genes in a transgenic mouse line. *Nature.* 314:330-334.
16. Weaver, D., F. Costantini, T. Imanishi-Kari, and D. Baltimore. 1985. A transgenic immunoglobulin mu gene prevents rearrangement of endogenous genes. *Cell.* 42:117-127.
17. Nussenzweig, M.C., A.C. Shaw, E. Sinn, D.B. Danner, K.L. Holmes, H.C. Morse III, and P. Leder. 1987. Allelic exclusion in mice that express the membrane form of immunoglobulin μ . *Science.* 236:816-819.
18. Storb, U., C. Pinkert, B. Arp, P. Engler, K. Gollahon, J. Manz, W. Brady, and R. Brinster. 1986. Transgenic mice with μ and κ encoding anti-phosphorylcholine antibodies. *J. Exp. Med.* 164:627-638.
19. Uematsu, Y., S. Ryser, Z. Dembic, P. Borgulya, P. Krimpenfort, A. Berns, H. von Boehmer, and M. Steinmetz. 1988. In transgenic mice the introduced functional T cell receptor β gene prevents expression of endogenous β genes. *Cell.* 52:831-841.
20. Loffert, D., A. Ehlich, W. Muller, and K. Rajewsky. 1996. Surrogate light chain expression is required to establish heavy chain allelic exclusion during B cell development. *Immunity.* 4:133-144.
21. Kitamura, D., and K. Rajewsky. 1992. Targeted disruption of μ chain membrane exon causes loss of heavy-chain allelic exclusion. *Nature.* 356:154-156.
22. ten Boekel, E., F. Melchers, and A.G. Rolink. 1998. Precursor B cells showing H chain allelic inclusion display allelic exclusion at the level of pre-B cell receptor surface expression. *Immunity.* 8:199-208.
23. Aifantis, I., J. Buer, H. von Boehmer, and O. Azogui. 1997. Essential role of the pre-T cell receptor in allelic exclusion. *Immunity.* 7:601-608.
24. Chien, Y.-H., M. Iwashima, K.B. Kaplan, J.F. Elliot, and M.M. Davis. 1987. A new T-cell receptor gene located within the α locus and expressed early in T cell differentiation. *Nature.* 327:677-682.
25. Satyanarayana, K., S. Hata, P. Devlin, M.C. Roncarolo, J.E. De Vries, H. Spits, J.L. Strominger, and M.S. Krangel. 1988. Genomic organization of the human T-cell antigen-receptor α/δ locus. *Proc. Natl. Acad. Sci. USA.* 85:8166-8170.
26. Fehling, H.J., and H. von Boehmer. 1997. Early $\alpha\beta$ T cell development in the thymus of normal and genetically altered

- mice. *Curr. Opin. Immunol.* 9:263–275.
27. Willerford, D.M., W. Swat, and F.W. Alt. 1996. Developmental regulation of V(D)J recombination and lymphocyte differentiation. *Curr. Opin. Gen. Dev.* 6:603–609.
 28. Seboun, E., N. Joshi, and S. Hauser. 1992. Haplotypic origin of beta-chain genes expressed by human T-cell clones. *Immunogenetics.* 36:363–368.
 29. Marolleau, J.-P., J.D. Fondell, M. Malissen, J. Trucy, E. Barbier, K.B. Marcu, P.-A. Cazenave, and D. Primi. 1988. The joining of germ-line V α to J α genes replaces the preexisting V α -J α complexes in a T cell receptor α , β positive T cell line. *Cell.* 55:291–300.
 30. Borgulya, P., H. Kishi, Y. Uematsu, and H. von Boehmer. 1992. Exclusion and inclusion of α and β T cell receptor alleles. *Cell.* 69:529–537.
 31. Kang, J., and D.H. Raulet. 1997. Events that regulate differentiation of $\alpha\beta$ TCR+ and $\gamma\delta$ TCR+ T cells from a common precursor. *Semin. Immunol.* 9:171–179.
 32. Chien, Y.-H., M. Iwashima, D.A. Wettstein, K.B. Kaplan, J.F. Elliot, W. Born, and M.M. Davis. 1987. T-cell receptor δ gene rearrangements in early thymocytes. *Nature.* 330:722–727.
 33. Migone, N., S. Padovan, C. Zappador, C. Glachino, M. Bot-taro, G. Matullo, C. Carbonara, G. De Libero, and G. Casorati. 1995. Restriction of the T-cell receptor V delta gene repertoire is due to preferential rearrangement and is independent of antigen selection. *Immunogenetics.* 42:323.
 34. Sleckman, B.P., C.G. Bardon, R. Ferrini, L. Davidson, and F.W. Alt. 1997. Function of the TCR α enhancer in $\alpha\beta$ and $\gamma\delta$ T cells. *Immunity.* 7:505–515.
 35. White, J., M. Blackman, J. Bill, J. Kappler, P. Marrack, D. Gold, and W. Born. 1989. Two better cell lines for making hybridomas expressing specific T cell receptors. *J. Immunol.* 143:1822–1825.
 36. Chen, J., R. Lansford, V. Stewart, F. Young, and F.W. Alt. 1993. RAG-2-deficient blastocyst complementation: an assay of gene function in lymphocyte development. *Proc. Natl. Acad. Sci. USA.* 90:4528–4532.
 37. Laird, P.W., A. Zijderveld, K. Linders, M.A. Rudnicki, R. Jaenisch, and A. Berns. 1991. Simplified mammalian DNA isolation procedure. *Nucleic Acids Res.* 19:4293.
 38. Yancopoulos, G.D., T.K. Blackwell, H. Suh, L. Hood, and F.W. Alt. 1986. Introduced T cell receptor variable region gene segments recombine in pre-B cells: evidence that B and T cells use a common recombinase. *Cell.* 44:251–259.
 39. Korman, A.J., J. Maruyama, and D. Raulet. 1989. Rearrangement by inversion of a T-cell receptor δ variable region gene located 3' of the δ constant region gene. *Proc. Natl. Acad. Sci. USA.* 86:267–271.
 40. Winoto, A., and D. Baltimore. 1989. Separate lineages of T cells expressing the $\alpha\beta$ and $\gamma\delta$ receptors. *Nature.* 338:430–432.
 41. Livak, F., H.T. Petrie, I.N. Crispe, and D.G. Schatz. 1995. In-frame TCR δ gene rearrangements play a critical role in the $\alpha\beta/\gamma\delta$ T cell lineage decision. *Immunity.* 2:617–627.
 42. Elliott, J.F., E.P. Rock, P.A. Patten, M.M. Davis, and Y.-H. Chen. 1988. The adult T-cell receptor δ -chain is diverse and distinct from that of fetal thymocytes. *Nature.* 331:627–631.
 43. Arden, B., S.P. Clark, D. Kabelitz, and T.W. Mak. 1995. Mouse T-cell receptor variable gene segment families. *Immunogenetics.* 42:501–530.
 44. Sleckman, B.P., C.H. Bassing, C.G. Bardon, A. Okada, B. Khor, J.-C. Bories, R. Monroe, and F.W. Alt. 1998. Accessibility control of variable region gene assembly during T cell development. *Immunol. Rev.* In press.
 45. Krangel, M.S., H. Yssel, C. Brocklehurst, and H. Spits. 1990. A distinct wave of human T cell receptor γ/δ lymphocytes in the early fetal thymus: evidence for controlled gene rearrangement and cytokine production. *J. Exp. Med.* 172:847–859.
 46. Livak, F., A. Wilson, H.R. MacDonald, and D. Schatz. 1997. $\alpha\beta$ lineage-committed thymocytes can be rescued by the $\gamma\delta$ T cell receptor (TCR) in the absence of TCR β chain. *Eur. J. Immunol.* 27:2948–2958.