PREDOMINANT VARIABLE REGION GENE USAGE BY γ/δ T CELL RECEPTOR-BEARING CELLS IN THE ADULT THYMUS

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Most T lymphocytes express a clonally distributed cell surface antigen receptor composed of disulfide-linked α and β subunits, noncovalently associated with several subunits called CD3 proteins. T cells that express the α/β form of the T cell receptor (TCR) recognize antigens in the context of cell surface proteins encoded by class I and class II genes of the major histocompatibility complex (MHC). A small distinct subset of peripheral T lymphocytes (<5%) and thymocytes expresses an alternative CD3-associated receptor composed of disulfide-linked γ and δ subunits; most of these so-called γ/δ cells are also distinguished from most α/β-expressing T cells by their $CD4^-$, $CD8^-$ (double-negative) phenotype (1–7). The function and specificity of γ/δ receptors and the cells that express them, while unknown, is of great interest, because they may mediate a previously unappreciated immune function and/or represent an intermediate stage in T cell development. A crucial issue in evaluating the functional capabilities of these cells concerns the potential diversity of ligands that can be recognized by the γ/δ receptor. Although these ligands have not been identified, their potential diversity should be related to the expressed diversity of the γ/δ receptor itself. We have begun to analyze the diversity of γ and δ chains used by T cells specifically selected for expression of a γ/δ heterodimer on their surface. This approach also allows us to assess the coexpression of particular Vγ and Vδ chain pairs by these cells. This approach contrasts that of isolation of γ and δ cDNA clones from populations of cells in which many cells do not express a γ/δ T cell receptor, and where isolation of in-frame cDNAs does not necessarily imply functional expression.

Like other T cell receptor and immunoglobulin subunits, the γ subunit is encoded by distinct variable (V), joining (J), and constant (C) region gene segments that have characteristic similarities to immunoglobulin amino acid sequence and undergo

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somatic rearrangements (of V to J) to generate a complete functional gene (8–11). In the mouse, three functional C genes, each with an associated J segment, and seven potentially functional V genes have been detected (Fig. 4 *A*) (9–13). Therefore, the potential V-region diversity of the γ chain is limited compared with that of the immunoglobulin heavy or light chains, and the α and β subunits of the α/β TCR.

Recently, a fourth rearranging TCR-like gene, called "x", was isolated by Chien et al. (14). More recently, serological evidence (15–18), and amino acid sequence analysis of a δ chain from a hybridoma (19), have conclusively shown that the x gene encodes the δ chain. The Cδ gene is located just 5′ to the known Jα gene segments in the mouse, and is therefore embedded between the Vα gene segments and the Jα-Cα gene segments (Fig. 3 *A*). Two Dδ segments, two Jδ segments, and five Vδ sequences have thus far been reported (20). Of particular interest is whether the δ and α chains use the same pool of germline V gene segments. Use of the same V segment pool would not only indicate potential similarities in the specificity repertoire of α/β and γ/δ cells, but would indicate that the potential diversity of δ chains (and therefore of the γ/δ receptor) is quite large; there are estimated to be over 50 Vα genes in the mouse (21). Of five Vδ sequences reported by Chien et al. (14, 20), two are quite similar to the Vα7 gene, whereas the others are only distantly related in sequence to other known V gene sequences.

To evaluate the diversity of γ and δ chains used by murine γ/δ cells, we have analyzed a panel of five γ/δ receptor expressing hybridomas produced by fusion of a TCR β-loss variant of the tumor cell line BW5147 with an enriched population of γ/δ cells from the young adult thymus of C57BL/6 (B6) mice (22). A previous protein analysis of the receptor on four of these cell lines suggested that at least two electrophoretically distinct γ chains and at least three δ chains are represented (22). In addition, we have analyzed the enriched population of γ/δ cells with various δ probes to arrive at an estimate of the diversity of expressed Vδ gene segments in the adult thymus.

Materials and Methods

Cell Lines. The DN7.1, DN12.1, DN7.3, and DN2.3 T hybridomas were described by Marusic et al. (22). The DN1.1 hybridoma was isolated in the same fusion (S. Marusic-Galesic, T. Saito, L. Tentori, J. Zuniga-Pflucker, D. H. Raulet, J. P. Allison, and A. M. Kruisbeek, manuscript submitted for publication). The T cell hybridomas were grown in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum and 50 μM 2-ME. The enriched population of thymic γ/δ cells was prepared as described (4) by culturing CD4$^-$, CD8$^-$ thymocytes (~5% γ/δ cells) from young B6 mice in a lymphokine cocktail for 3 d. The resultant population was analyzed by immunofluorescent staining and immunoprecipitation with anti-CD3 antibodies, and is estimated as 50–80% γ/δ-bearing, CD3$^+$ cells.

cDNA Cloning. RNA was isolated from freshly harvested cells by lysis in guanidinium isothiocyanate (23). Poly(A)$^+$ mRNA was purified by two cycles of oligo(dT) cellulose chromatography.

Double-stranded (ds) cDNA from DN 7.3, DN 7.1, and DN2.3 mRNAs was synthesized by the procedure of Gubler and Hoffman (24) using Moloney Leukemia Virus reverse transcriptase and RNase H. The dscDNA was treated with T4 DNA polymerase and Eco RI methylase before ligation of Eco RI linkers. The dscDNA was digested with Eco RI and size fractionated by agarose gel electrophoresis. dscDNA 1–4 kb in length was isolated by binding to glass beads (25) and ligated to λgt 10. Recombinant phage were packaged using Gigapack (Stratagene Cloning Systems, San Diego, CA), and libraries were screened without amplification. The DN 7.3 library was probed with an oligonucleotide corresponding to the

Cx sequence of Chien et al. (14) (5'CCAGCCTCCGGCCAAACCATCTG). The DN7.1 and DN2.3 libraries were probed with a 900 bp Eco RI fragment encoding Cδ and 3' untranslated (3'UT) sequences, which was excised from a full-length δ7.3 cDNA clone.

DNA was sequenced by a combination of chemical cleavage (26) and dideoxy chain termination on dsDNA using a modified form of T7 DNA polymerase (Sequenase; USB Biochemical Corp., Cleveland, OH). For sequence and Southern analysis of Jδ2 genomic sequences, two oligonucleotides corresponding to the 5' (5'CCCGACAGATGTTTTTGGAACTGGC) and 3' (5'GGGGCTCCACAAAGAGCTCTATGCC) halves of the Jδ2 coding region were synthesized.

Blot Hybridization. The filters were hybridized as described (27) with 3×10^7 dpm of each probe, labelled by random hexamer priming (28); filters were washed at 68°C with 2× SSC, 0.05% SDS, and exposed to preflashed Kodak XAR film.

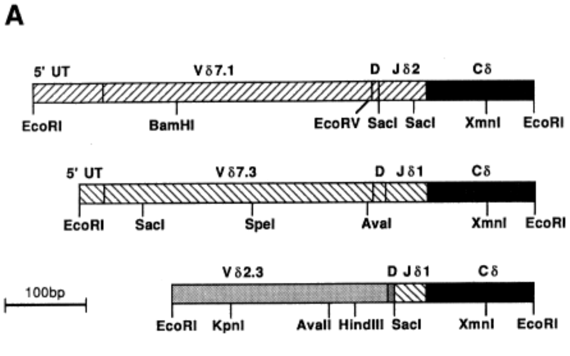
Results

New Vδ and Jδ Gene Segments. To examine the δ chain diversity in the hybridomas, δ cDNA clones were isolated from three of the cell lines, DN7.3, DN7.1 and DN2.3 (Fig. 1). The deduced amino acid sequences of the 5' portions of these cDNA clones have several features common to other V regions, including numerous conserved residues (Fig. 1 *B*). One of the cDNAs, δ7.1, is identical to the V_{M21} V region described by Chien et al. (20). Two of the cDNAs, δ7.3 and δ2.3 are novel Vδ genes. Of the three cDNAs, two (Vδ7.1 and Vδ7.3) are only distantly related to known Vα gene segments (<29 and 37% amino acid identity, respectively). The third cDNA, δ2.3, is very similar to Vα4 gene segments, with 95.3% amino acid identity and 97% nucleotide identity to the TA65 V region reported by Arden et al. (29). This finding documents the functional expression of a Vα-like gene as part of a δ chain on a γ/δ cell line.

Assuming that the NH_2 termini of the mature δ chains are as indicated in Fig. 1, the δ7.1 and δ7.3 cDNAs encode polypeptides of 29,966 and 30,477 daltons, respectively (note that the δ2.3 cDNA does not extend to the NH_2 terminus). The sizes of the δ chains of DN7.1, DN7.3, and DN2.3 determined by SDS-PAGE analysis after enzymatic removal of NH_2-linked glycans, are 34, 35, and 36 kD, respectively (22). As has been noted previously, the discrepancies between the calculated molecular weights and determined molecular masses of δ chains may be due to *O*-linked glycosylation or other posttranslational modifications of the polypeptide, or may reflect aberrant gel mobilities of δ chains (14).

Each of the cDNAs include productive (in frame) Vδ-Dδ-Jδ joints (Fig. 1 *C*). The Vδ7.3 gene segment is juxtaposed to Jδ1. Vδ2.3 is also rearranged to Jδ1; however this joining event results in the deletion of three amino acids encoded by the 5' end of Jδ1. In contrast, Vδ7.1 is juxtaposed to the Jδ2 gene segment, which is only moderately homologous to Jδ1. This the first report of a functional rearrangement to Jδ2, indicating that the δ locus includes at least two functional Jδ gene segments.

For further analysis of Jδ2, we cloned the germline Jδ2 gene segment. Because Southern hybridization analysis indicated that Jδ2 lies between Jδ1 and Cδ (see below) we isolated genomic clones from a BALB/c liver library that hybridized with Cδ and analyzed the region upstream of Cδ for sequences that hybridized with an oligonucleotide probe corresponding to the Jδ2 sequence. The determined Jδ2 germline sequence (which is identical to that recently reported by Chien et al., [20]), and Dδ and Jδ1 gene segment sequences reported by Chien et al. (20), were compared with δ cDNA clones to assess junctional diversity. This comparison reveals



B

δ7.1

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                                                                          AGTGAGGCTCAGCAGGGCAGCCCAGGAAAGCACCAGCCGATTACAAACTCATCCTCAGTGAAACT
                                                                           |+1       V
ATG CTT TGG AGA TGT CCA GTC CTC TGC ATA TTC ATC TTC AGT ACA GGG ACC TCT TTG GAT GTA TAT TTG GAA CCA GTT GCC AAA ACT TTT ACT GTT GTA GCT GGG
 M   L   W   R   C   P   V   L   C   I   F   I   F   S   T   G   T   S   L   D   V   Y   L   E   P   V   A   K   T   F   T   V   V   A   G
             20          Δ                                                                   40 ┌CHO
GAT CCT GCC TCC TTC TAC TGC ACT GTA ACA GGA GGG GAC ATG AAG AAT TAT CAT ATG AGC TGG TAT AAG AAG AAT GGA ACT AAT GCT CTG TTT TTA GTA TAC AAG
 D   P   A   S   F   Y   C   T   V   T   G   G   D   M   K   N   Y   H   M   S   W   Y   K   K   N   G   T   N   A   L   F   L   V   Y   K
            ┌CHO                 60                                 ┌CHO                                         80
CTA AAT AGC AAT TCT ACT GAT GGT GGA AAG AGC AAC CTC AAA GGG AAA ATT AAC ATT TCA AAA AAT CAG TTT ATA CTC GAC ATT CAG AAG GCA ACA ATG AAA GAC
 L   N   S   N   S   T   D   G   G   K   S   N   L   K   G   K   I   N   I   S   K   N   Q   F   I   L   D   I   Q   K   A   T   M   K   D
                     Δ             |     D         | 100     J                                                                     | 120 C
GCT GGT ACG TAC TAC TGT GGG TCA GAT ATC GGA GGG AGC TCC TGG GAC ACC CGA CAG ATG TTT TTT GGA ACT GGC ATA GAG CTC TTT GTG GAG CCC CAA AGC CAG
 A   G   T   Y   Y   C   G   S   D   I   G   G   S   S   W   D   T   R   Q   M   F   F   G   T   G   I   E   L   F   V   E   P   Q   S   Q
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δ7.3

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                                                                                                   TGAGCTGGTCAGTGTCTGGG ATG CAG ACG CTA CTA
                                                                                                                         M   Q   T   L   L
                                                +1       V                                                                   20          Δ
TGG CCT CCT TTC TTC TTC ACA GAC AAG GAT GTG CTG TGC ATC ACG CTG ACC CAG AGC TCC ACT GAC CAG ACA GTG GCA AGC GGC ACT GAA GTA ACA CTG CTC TGC
 W   P   P   F   F   F   T   D   K   D   V   L   C   I   T   L   T   Q   S   S   T   D   Q   T   V   A   S   G   T   E   V   T   L   L   C
                                                                 40
ACG TAC AAT GCG GAT TCT CCA AAC CCA GAT TTA TTT TGG TAT CGC AAA AGG CCA GAC AGA TCC TTC CAG TTC ATC CTT TAT AGG GAC GAC ACT AGT TCC CAT GAT
 T   Y   N   A   D   S   P   N   P   D   L   F   W   Y   R   K   R   P   D   R   S   F   Q   F   I   L   Y   R   D   D   T   S   S   H   D
     60                                                     ┌CHO                     80
GCA GAT TTT GTT CAA GGT CGA TTT TCT GTG AAG CAC AGC AAG GCC AAC AGA ACC TTC CAT CTG GTG ATC TCT CCA GTG AGC CTT GAA GAC AGC GCT ACT TAT TAC
 A   D   F   V   Q   G   R   F   S   V   K   H   S   K   A   N   R   T   F   H   L   V   I   S   P   V   S   L   E   D   S   A   T   Y   Y
 Δ                 |     100     D         |     J                                                       120   |             C
TGT GCC TCG GGG TAT TAT ACC GGA GGG ATA CGA GCT ACC GAC AAA CTC GTC TTT GGA CAA GGA ACC CAA GTG ACT GTG GAA CCA AAA AGC CAG CCT CCG
 C   A   S   G   Y   Y   T   G   G   I   R   A   T   D   K   L   V   F   G   Q   G   T   Q   V   T   V   E   P   K   S   Q   P   P
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δ2.3

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                                 V    20             Δ  ┌CHO                                                         40
CAA GTG GCC CTC TCA GAA GAG GAC TTT CTT ACG ATA CAC TGC AAC TAC TCA GCC TCA GGG TAC CCA ACC CTG TTC TGG TAT GTG CAG TAT CCC GGA GAA GGG CCA
 Q   V   A   L   S   E   E   D   F   L   T   I   H   C   N   Y   S   A   S   G   Y   P   T   L   F   W   Y   V   Q   Y   P   G   E   G   P
                                                         60                                                                              80
CAG CTC CTC TTT AGA GCC TCA AGG GAC AAA GAG AAA GGA AGC AGC AGA GGT TTT GAA GCT ACA TAT GAT AAA GGG ACC ACC TCC TTC CAC TTG CGG AAA GCT TCA
 Q   L   L   F   R   A   S   R   D   K   E   K   G   S   S   R   G   F   E   A   T   Y   D   K   G   T   T   S   F   H   L   R   K   A   S
                                         Δ             |     D         |     100             J                             |     C
GTG CAA GAG TCA GAC TCG GCT GTG TAC TAC TGT GCT CTG AGT GAG GGA TAC GAG CTC GTC TTT GGA CAA GGA ACC CAA GTG ACT GTG GAA CCA AAA AGC CAG CCT
 V   Q   E   S   D   S   A   V   Y   Y   C   A   L   S   E   G   Y   E   L   V   F   G   Q   G   T   Q   V   T   V   E   P   K   S   Q   P
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FIGURE 1. (*A*) Restriction maps of 5′ Eco RI fragments of δ7.1, δ7.3, and δ2.3 cDNAs indicating location of 5′UT, V, D, J, and Cδ sequences. (*B*) DNA sequence and deduced amino acid sequences of δ7.1 and δ7.3, and δ2.3 cDNAs. Conserved cysteine residues thought to participate in disulfide bond formation are indicated by Δ. Conserved residues common to other V regions are numerous, including C(23), C(94), W(36), Y(37), Y(92), and Y(93). Potential carbohydrate addition sites (N-X-S/T) are indicated by*CHO* over the asparagine residue. The amino terminus of δ7.1 is indicated as +1 by comparison to protein sequence derived by Born et al. (19). The amino terminus of δ7.3 has been approximated by analogy to δ7.1.

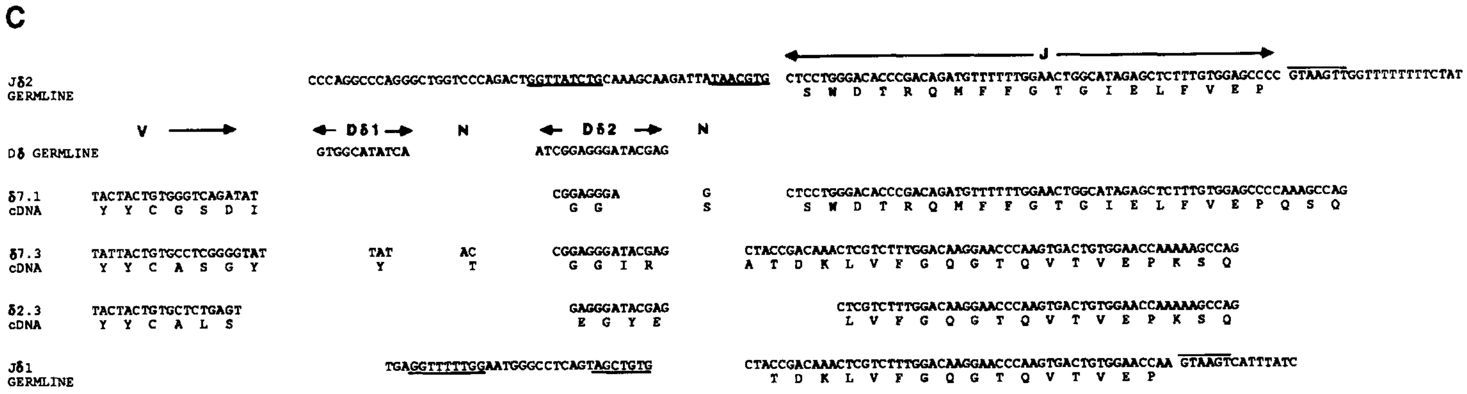
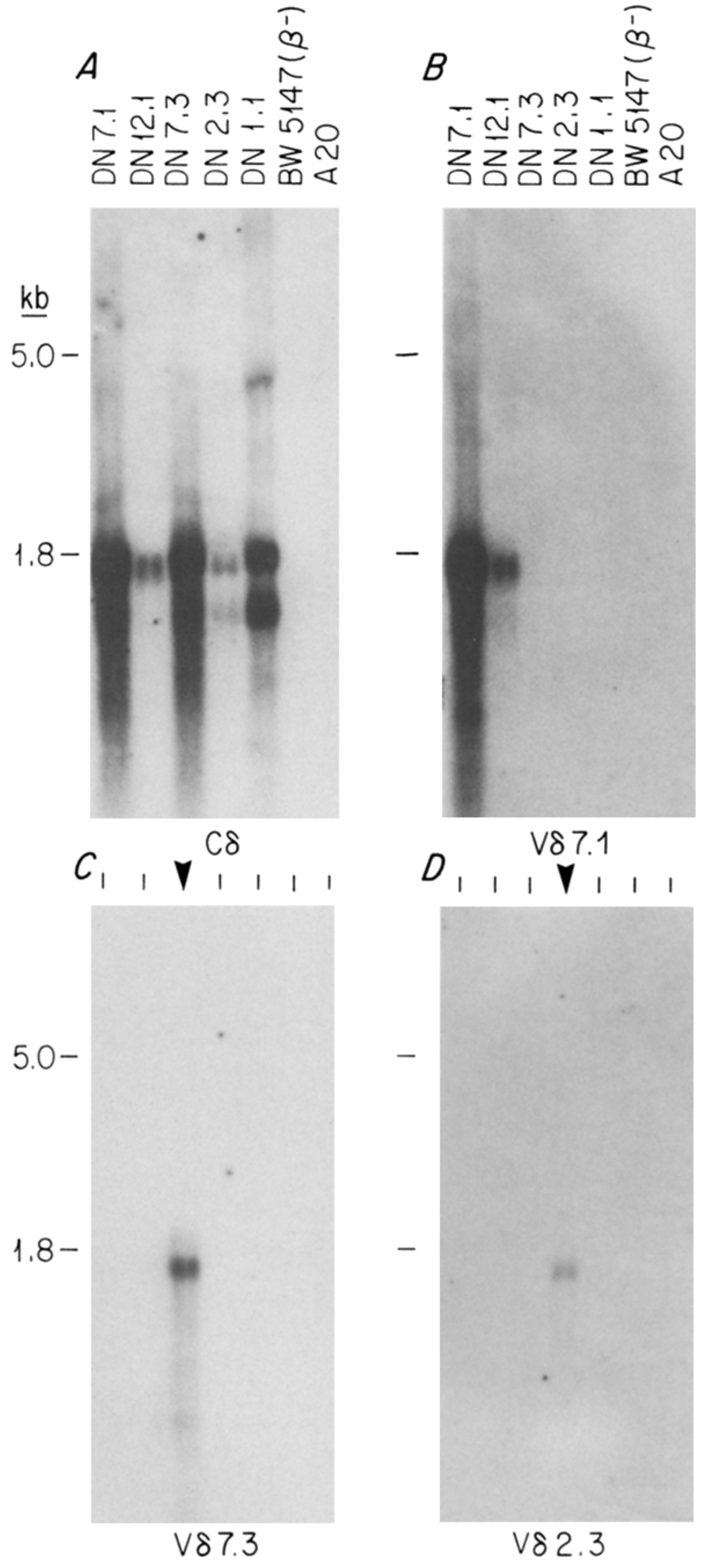


FIGURE 1. (*C*) Sequence comparison of V-D-J junctions of δ7.1, δ7.3, and δ2.3 cDNAs and germline Dδ1 and Dδ2 and germline Jδ1 and Jδ2 sequences (14, 20, and this report). Also indicated are putative *N*-regions (junctional nucleotide sequences not encoded in germline DNA, thought to result from the addition of nucleotides at recombination joints catalyzed by the enzyme terminal deoxynucleotidyl transferase). The sequence ascribed to Dδ1 in the δ7.3 sequence is not derived from the germline Vδ7.3 gene (our unpublished data), and may therefore be derived from Dδ1 as indicated. Heptamer and nonamer recombination signals in Jδ1 and Jδ2 have been underlined. Splice donor sequences at the 3′ side of Jδ1 and Jδ2 have been indicated by a line over the sequence.


A
DN 7.1
DN 12.1
DN 7.3
DN 2.3
DN 1.1
BW 5147 (β⁻)
A 20
kb
5.0
1.8
Cδ
B
DN 7.1
DN 12.1
DN 7.3
DN 2.3
DN 1.1
BW 5147 (β⁻)
A 20
Vδ 7.1
C
5.0
1.8
Vδ 7.3
D
Vδ 2.3

that each of the cDNAs use Dδ2 gene segments. δ7.3 may use Dδ1 sequences, since germline Vδ7.3 sequences do not account for the observed additional nucleotides (unpublished data).

Four Distinct δ Genes Are Expressed in a Panel of Five γ/δ T Hybridomas. Northern hybridization analysis with a Cδ probe detected a transcript of ~1.8 kb in all five hybridomas, although DN12.1 and DN2.3 have considerably lower levels of this Cδ transcript (Fig. 2). A smaller Cδ transcript, possibly representing a DJC-transcript, was apparent in some of the lines. Neither transcript was detectable in BW5147, the fusion partner of the hybridomas. A Vδ7.3 probe detected a 1.8 kb transcript only in the DN7.3 hybridoma. Therefore, of the five hybridomas, only the DN7.3 cell line expresses detectable levels of a Vδ7.3 transcript.

A Vδ7.1 probe detected 1.8 kb transcripts in two cell lines (DN7.1 and DN12.1). The levels of this transcript are roughly concordant with the levels of the 1.8 kb Cδ transcript in these cells. Hence the DN7.1 cell line and possibly the DN12.1 cell line express functional Vδ7.1 transcripts. A Vδ2.3 probe detects a transcript only in the DN2.3 cell line (Fig. 2).

To assess the δ rearrangements in the hybridomas, Southern hybridization analysis was performed with Vδ and Jδ probes (Fig. 3). A single band of 7.0 kb hybridized with the Vδ7.1 probe in an Eco RI digest of liver DNA, suggesting that this V segment represents a single-membered subfamily (Fig. 3 *B*). Both copies of Vδ7.1 are deleted in the BW5147 cell line. In the DN7.1 cell line, there are two rearrangements of the Vδ7.1 gene segment: to Jδ2 on one chromosome (a 4.5 kb Eco RI fragment) (Fig. 3 *B* and *C*) and to Jδ1 on the other (a 9.0 kb Eco RI fragment) (Fig. 3 *B* and *E*). Because the cDNA clone isolated from DN7.1 corresponded to a productive Vδ7.1-Jδ2Cδ rearrangement, the 4.5 kb Eco RI fragment presumably encodes the expressed δ chain in the DN7.1 cell line. In the DN12.1 cell line the Vδ7.1 gene is also rearranged to Jδ2, detected as a 4.5 kb Eco RI fragment with both probes. The intensity of hybridization to this fragment compared with that in the DN7.1 hybridoma suggests that both chromosomes have undergone Vδ7.1-Jδ2 rearrangements. Both copies of Jδ1 are deleted in the DN12.1 cell line, as a result of the Vδ-Jδ2 rearrangements. The data indicate that the expressed δ chain in the DN12.1 cell line is the product of a Vδ7.1-Jδ2 rearranged gene, consistent with the earlier observation that the δ chains of DN7.1 and DN12.1 are indistinguishable by nonequilibrium pH gradient electrophoretic analysis (29a). The independent origin of these two cell lines is indicated by the presence of presumably nonfunctional γ and δ rearrangements specific to each cell line (Figs. 3 and 4, see below).

A Vδ7.3 probe hybridized to a single 10.5 kb Eco RI fragment in B6 liver DNA suggesting that, like Vδ7.1, Vδ7.3 is a member of a single-membered V gene subfamily (Fig. 3 *D*). A rearranged 9.4 kb Eco RI fragment in the DN7.3 hybridoma hybridized with both the Vδ7.3 and Jδ1 probes, indicating that this rearranged gene

FIGURE 2. Expression of Cδ and Vδ gene sequences in RNA from DN hybridomas. 10 μg of total RNA was electrophoresed on denaturing formaldehyde agarose (1.1%) gels. (*A*) Hybridization with Cδ, a 900 bp Eco RI fragment from δ7.3 cDNA containing Cδ coding and 3'UT sequences. (*B*) Hybridization with Vδ7.1, a 240 bp Bam HI-Eco RV fragment from δ7.1 cDNA. (*C*) Hybridization with Vδ7.3, a 370 bp Eco RI-Ava I fragment from δ7.3 cDNA. (*D*) Hybridization with Vδ2.3, a 210 bp Eco RI-Hind III fragment from δ2.3 cDNA.

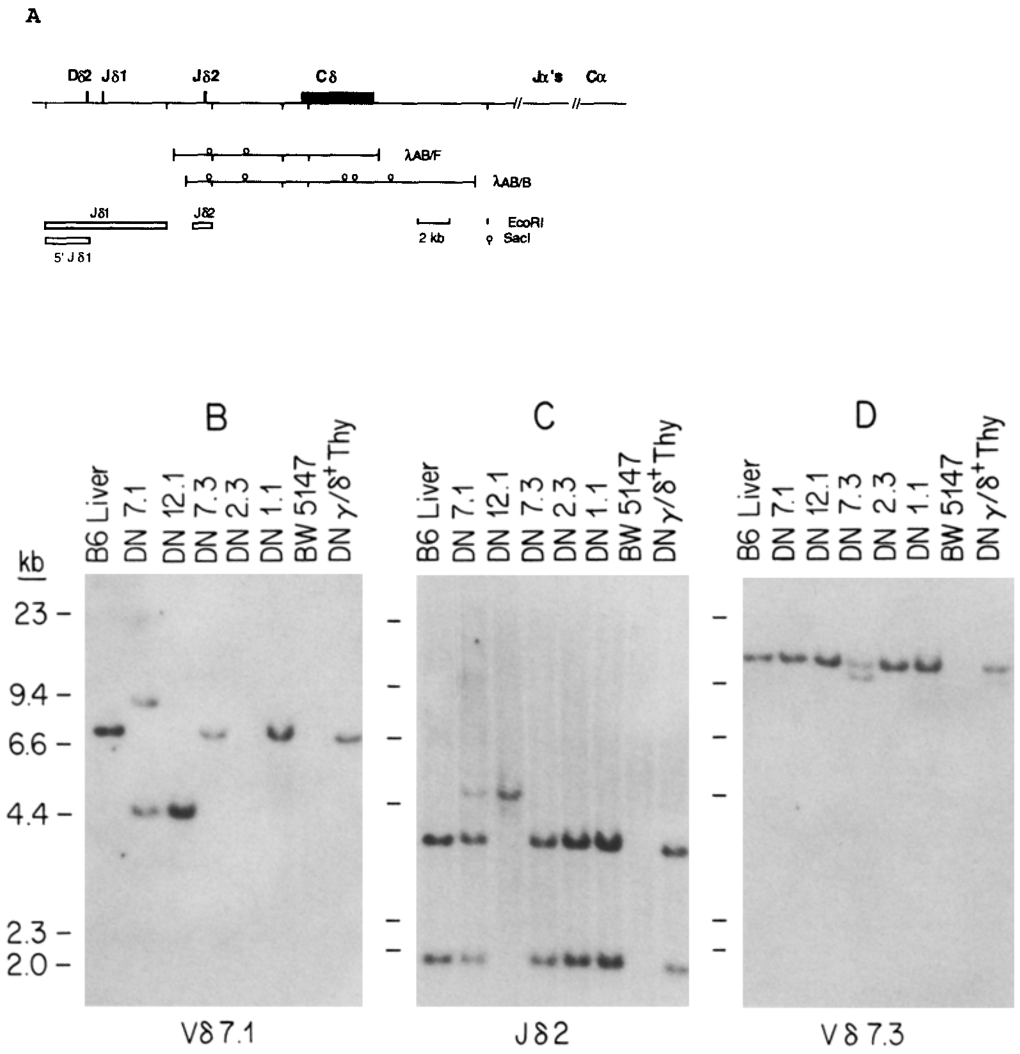
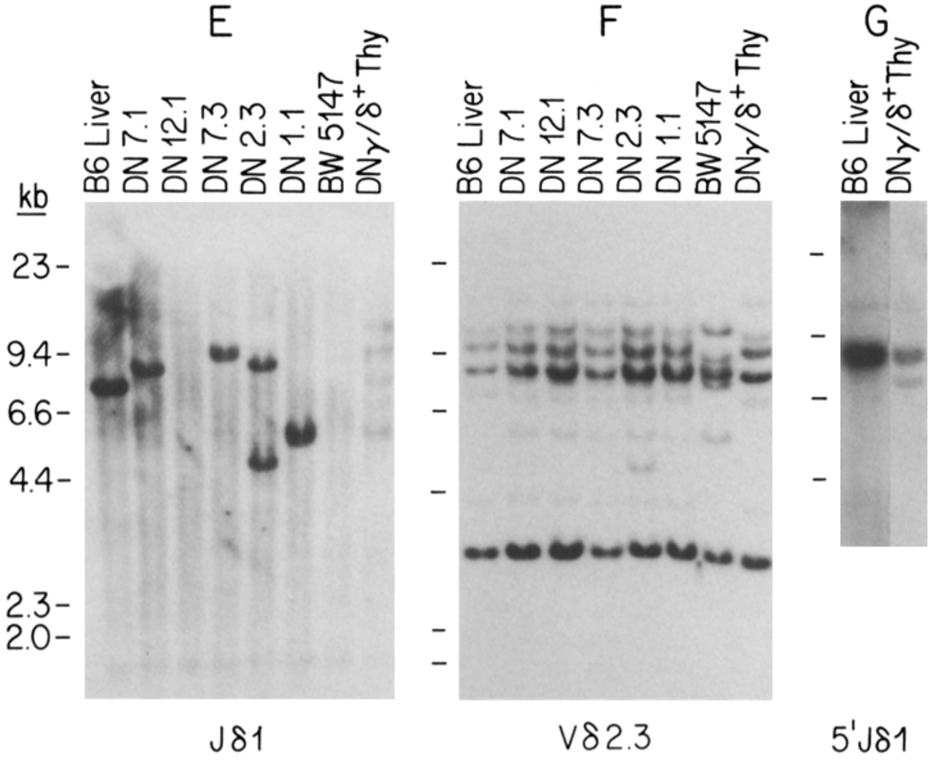


FIGURE 3. (*A*) Organization of the TCR δ chain locus in the BALB/c mouse indicating the location of Jδ1, Jδ2, and Cδ gene segments (from Chien et al. [14, 20] and this study). A genomic library of BALB/c liver DNA (kindly provided by Dr. André Bernards, Whitehead Institute) was screened with a 900 bp Eco RI fragment containing Cδ coding and 3'UT region sequences. Two phage clones, λAB/F and λAB/B, were isolated and mapped as shown. DNA probes isolated from genomic DNA and used for Southern hybridization are indicated (Jδ1, 5'Jδ1, and Jδ2, described below). (*B–F*) Southern analysis of Vδ and Jδ rearrangements in DNA from DN hybridomas and γ/δ-enriched double negative C57BL/6 thymocytes. All DNAs were digested with Eco RI, electrophoresed on 0.7% agarose gels and transferred to nitrocellulose. Hind III-digested λ DNA size markers are indicated as shown. (*B*) Hybridization with Vδ 7.1 probe (see Fig. 2). (*C*) Hybridization with Jδ2 probe (a 1.2 kb Hind III-Eco RI fragment isolated from λ AB/F). The Jδ2 probe which is contained within a 2.85 kb Eco RI fragment in BALB/c DNA hybridizes to two Eco RI fragments in C57BL/6 DNA as shown here and similarly in Lindsten et al. (43). (*D*) Hybridization with Vδ 7.3 probe (see Fig. 2). (*E*) Hybridization with Jδ1 probe (kindly provided by Dr. Astar Winoto, Whitehead Institute), a 7.5 Eco RI fragment isolated from C3H DNA. The bands in DN7.3 and DN1.1 are visible as doublets in the original autoradiograph. (*F*) Hybridization with Vδ2.3 probe (see Fig. 2). (*G*) Hybridization with 5' Jδ1, a 2.6 kb Eco RI-Hinc II fragment from the 5' side of the 7.5 kb Jδ1-containing Eco RI fragment.



represents the expressed δ gene. Rearrangements of the Vδ7.3 gene were not detected in any of the other hybridomas. An additional rearranged Eco RI fragment of 9.5 kb was detected in the DN7.3 cell line with the Jδ1 probe, presumably corresponding to a nonfunctional δ rearrangement.

Southern analysis using a Vδ2.3 probe detects seven Eco RI fragments in B6 liver DNA, presumably corresponding to multiple members of the Vα4 subfamily (Fig. 3 *F*). In the DN2.3 cell line, a rearranged band of 5.0 kb is detected with both Vδ2.3 and Jδ1 probes. DN2.3 also contains a second rearrangement (a 9 kb Eco RI fragment) detected with a Jδ1 probe.

The DN1.1 cell line expresses a γ/δ receptor, as demonstrated by immunoprecipitation analysis with an anti-δ chain antiserum (S. Marusic-Galesic, T. Saito, L. Tentori, J. Zuniga-Pflucker, D. H. Raulet, J. P. Allison, and A. M. Kruisbeek, manuscript submitted for publication). Two rearrangements of the Jδ1 gene segment were detected in this cell line, which are not detected with Vδ7.1, Vδ7.3, or Vδ2.3 gene segments (Fig. 3 *E*). The sizes of the rearranged fragments detected with a Jδ1 probe in DN1.1 (a doublet at ~6 kb) are different from those in the other cell lines, indicating that DN1.1 expresses a distinct Vδ-Jδ1 rearranged gene. Therefore four distinct Vδ genes are used in a panel of five γ/δ cell lines.

Three Different γ Chains Are Expressed in a Panel of Five γ/δ T Hybridomas. To determine the identity of the γ chains expressed by the hybridomas, Southern analysis of gene rearrangements and RNA analysis were performed. Interpretation of the

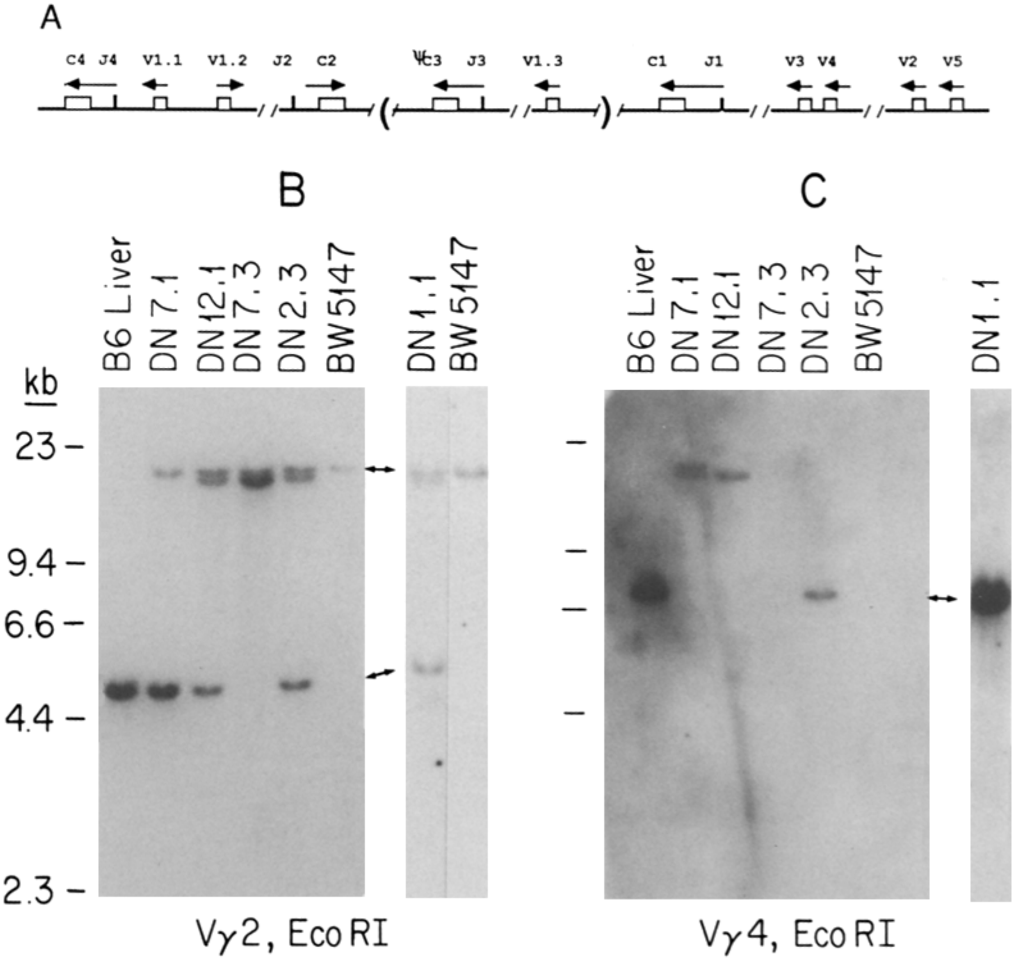
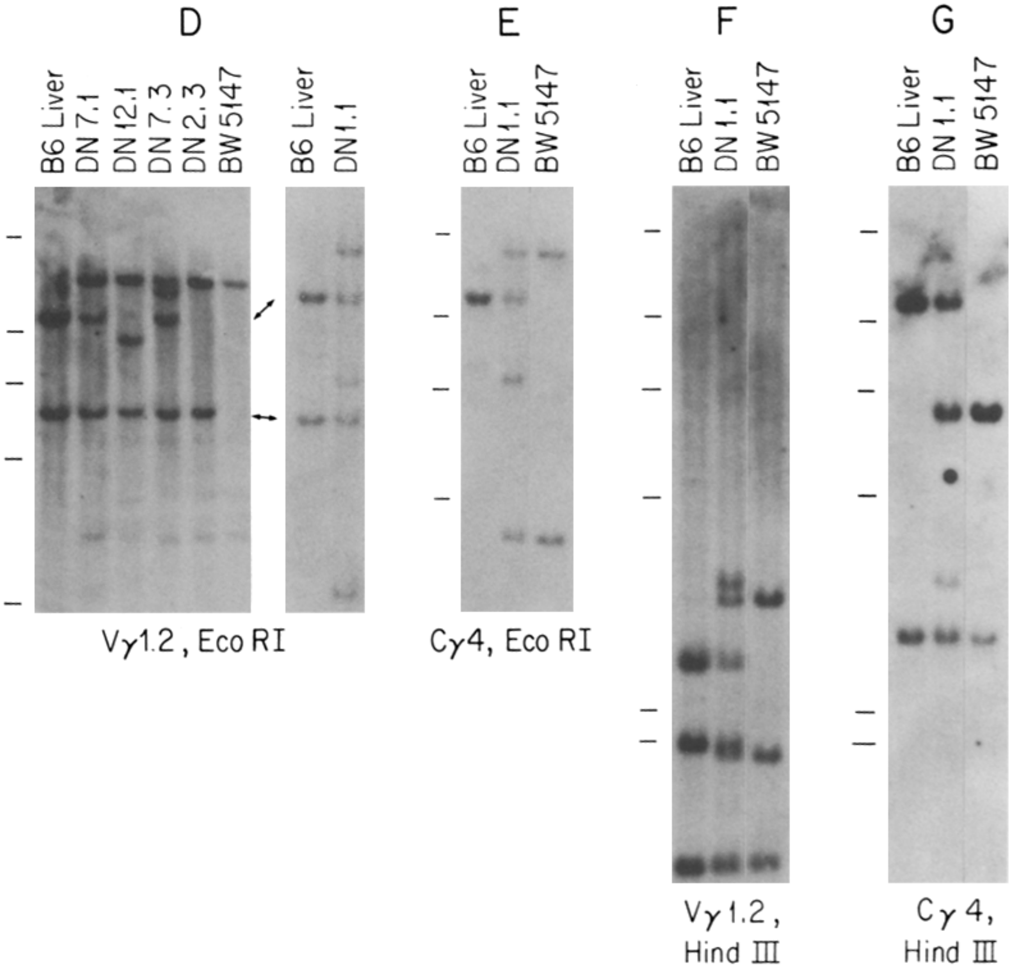


FIGURE 4. (*A*) Organization of TCR γ chain locus (based on refs. 9–11, 13). Relative location and transcriptional orientation of Vγ and JγCγ sequences are shown. The orientation of the Vγ1.3, Jγ3Cγ3 genes relative to the others has not been established. (*B–G*) Southern analysis of γ rearrangements in DNA from DN hybridomas. All DNAs were digested with Eco RI, electrophoresed on 0.7% agarose gels and transferred to nitrocellulose. Hind III-digested λ DNA size markers are indicated as shown. The Vγ2, Vγ4, and Vγ1.2 probes were those described in Garman et al. (10). The Cγ4 probe is a 1.6 kb Eco RI–Bam HI genomic fragment that includes the first exon of Cγ4 and ∼700 bp of DNA in the Jγ4-Cγ4 intron. The following information is helpful in analysis of the blot hybridization data: (*a*) Donor B6 thymocyte-derived Vγ2-Cγ1Cγ1 rearrangements can be distinguished from the BW5147-derived Vγ2-Jγ1Cγ1 rearrangement due to a polymorphism between AKR (strain of origin BW5147) and B6 in an Eco RI site located 3′ of the Cγ1 gene; the B6- and AKR-derived rearranged Vγ2-Jγ1Cγ1 genes reside on 17 and 19 kb Eco RI fragments, respectively (*B*). (*b*) Because Vγ4 and Vγ3 are closely linked on the same germline Eco RI fragment, the Vγ4 probe detects rearrangement of both genes; rearrangement of Vγ3 or Vγ4 to Jγ1 results in an 18 or 17 kb Eco RI fragment, respectively (*C*). (*c*) Donor B6-derived rearrangements to Jγ4 were detected in three cell lines, DN1.1 (*G*), DN12.1 and DN7.3 (not shown), by probing Hind III-digested DNAs with a Cγ4 probe. DN12.1 and DN7.3 both have Vγ1.1-Jγ4Cγ4 rearrangements (a 3 kb Hind III band). This rearrangement yields a 9.5 kb Eco RI fragment in DN12.1 which is visible by hybridization with Vγ1.2 (*D*). The 14 kb Eco RI band in DN7.3 is the result of Vγ1.1-Jγ4Cγ4 and Vγ1.2-Jγ2Cγ2 rearrangements on the same chromosome (Vγ1.1, Vγ1.2, and Jγ4Cγ4 are all on the same germline Eco RI fragment (9, 11).



In DN1.1, the rearrangement of Vγ1.3 to Jγ4Cγ4 yields a 6.6 kb Eco RI fragment and a 3.5 kb Hind III fragment (*E* and *G*), consistent with the location of germline Eco RI sites (9). For comparison, rearrangement of Vγ1.1 to Jγ4 yields a 9.5 kb Eco RI fragment (see above); based on the location of Eco RI sites in germline DNA, rearrangement of Vγ1.2 to Jγ4 would yield an 11.6 kb Eco RI fragment. (*d*) Hybridization with Cγ probes (not shown) revealed no bands that cannot be accounted for by the rearrangements listed in Fig. 5.

Southern analysis is complicated by the fact that BW5147, the fusion partner, donates several rearranged γ genes to the hybridomas (13, 30) (Figs. 4 and 5): Vγ1.2-Jγ2Cγ2 (one allele), Vγ2-Jγ1Cγ1 (one allele), and Vγ5-Jγ4Cγ4 (one allele). The number of rearranged alleles of each type is based on the observation by Pelkonen et al. (13) that the Vγ5-Jγ4Cγ4 rearrangement on one of the two BW5147 chromosomes has deleted the intervening DNA (which includes the V1.1-V1.3, V2-4, and C1-C3 γ-gene segments). Previous studies indicate that both the Vγ1.2-Jγ2Cγ2 and Vγ5-Jγ4Cγ4 rearranged genes of BW5147 are nonproductive (13, 30).

	REARRANGEMENTS								RNA						PROTEIN			
	DONOR					BW										Mr		
	V1.1-J4C4	V1.3-J4C4	V2-J1C1	V3-J1C1	V4-J1C1	V1.2-J2C2	V2-J1C1	V5-J4C4	C1, C2, C3	V1.1, V1.2, V1.3	V2	V3	V4	C4	ANTI-C1,2 REACTIVE	NO TREATMENT	N-GLYCONASE	ASSIGNMENT
BW5147						+	+	+	–	–	–	–	–	–				
DN7.1	–	–	–	+	+	+	+	+	+	+	–	+	+	+	+	34	31	V4-J1C1
DN12.1	+	–	+	–	+	+	+	+	+	+	+	–	+	+	+	34	31	V4-J1C1
DN7.3	+	–	+	–	–	+	+	+	+	+	+	–	–	+	+	35	32	V2-J1C1
DN2.3	–	–	+	–	–	+	+	+	+	+	+	–	–	+	+	35	32	V2-J1C1
DN1.1	–	+	–	–	–	+	+	+	+	+	–	–	–	+	–	38	ND	V1.3-J4C4

FIGURE 5. Summary of γ gene rearrangements and transcripts present in the five γ/δ T hybridomas. The rearrangements are based largely on the data in Fig. 4. The Vγ1.1-Jγ4Cγ4 rearrangements were assessed by Southern blot analysis of Hind III-digested DNAs, probed with Cγ4 and Vγ1.2 probes (not shown). The presence of the indicated transcripts were determined by ribonuclease protection assays of total RNA (not shown). A Cγ2 probe was used to detect Cγ2 and the crosshybridizing Cγ1 and Cγ3 transcripts, and a Vγ1.2 probe was used to detect Vγ1.1, Vγ1.2, and Vγ1.3 transcripts. Where a negative is indicated in the table, no band was visible even in overexposures of the autoradiograph. The protein relative molecular masses are from references 22 and 29a.

The γ chains of the DN7.3 and DN2.3 cell lines are encoded by Vγ2-Jγ1Cγ1 rearranged genes. B6-derived Vγ2-Jγ1Cγ1 rearrangements are present in both cell lines, and can be distinguished from the BW5147-derived Vγ2-Jγ1Cγ1 rearrangement (Fig. 4 *B*). Although other rearranged γ genes are present (Fig. 5), they cannot account for the properties of the γ chains on these cell lines, which are *N*-glycosylated (unlike the Vγ1.2-Jγ2Cγ2-encoded chain [8]) and react with an anti-Cγ antiserum, which does not detect Cγ4 (22). Consistent with this assignment, the γ-chains of DN7.3 and DN2.3 are indistinguishable from each other by size, glycosylation pattern and isoelectric point (22, 29a). Because it is not known whether the BW5147-derived Vγ2-Jγ1Cγ1 rearranged gene is functional, it is not possible to definitively assign the γ chains in DN7.3 and DN2.3 as of B6-thymocyte origin. Of note in this context, however, is that the BW5147-derived Vγ2-Jγ1Cγ1 gene is not expressed as mRNA in BW5147, nor in a hybridoma, DN7.1, that uses a distinct γ gene (see below) and has no B6-donor-derived Vγ2 gene rearrangement (Fig. 5). Thus the BW5147 allele may be specifically inactivated in the hybridomas.

The γ chain of the DN7.1 cell line is encoded by a gene other than Vγ2, because this cell line lacks Vγ2 transcripts as well as donor-derived Vγ2 gene rearrangements. Instead, Vγ3-Jγ1Cγ1 and Vγ4-Jγ1Cγ1 rearranged genes are present in this cell line. Because the γ chains of DN7.1 and another cell line, DN12.1, are indistinguishable by SDS-PAGE and NEPHGE analyses, and DN12.1 has a rearranged Vγ4-Jγ1Cγ1 gene but no Vγ3-Jγ1Cγ1 gene, we conclude that the γ chains on both cell lines are encoded by Vγ4-Jγ1Cγ1 rearranged genes. Consistent with this assignment, the γ chains on both cells are N-glycosylated and react with the anti-Cγ antiserum that is specific for Cγ1 and Cγ2 but not Cγ4. In addition, the size of the γ chains on these hybridomas (34 kD) is significantly less than that of the Vγ3-Jγ1Cγ1-encoded γ chain (42 kD) expressed by a dendritic epidermal cell line (31), probably due in part to the presence of an acceptor site for *N*-glycosylation in Vγ3 that is absent in Vγ4 (10). Furthermore, the DN12.1 and DN7.1 γ chains are smaller than the Vγ2-Jγ1Cγ1 chains expressed by DN7.3 and DN2.3 (35 kD).

The DN1.1 cell line expresses yet a third γ chain, because it has no donor-derived Vγ2, Vγ3, or Vγ4 rearrangements. The putative γ chain of DN1.1 is not reactive with anti-Cγ1,2 antiserum (S. Marusic-Galesic, T. Saito, L. Tentori, J. Zuniga-Pflucker, D. H. Raulet, J. P. Allison, and A. M. Kruisbeek, manuscript submitted for publication), and therefore is likely a product of the Cγ4 gene. Consistent with this assignment, a heretofore unreported B6 donor-derived rearrangement of the Cγ4 gene on a 6.6 kb fragment in an Eco RI digest (Fig. 4 *E*) and a 3.5 kb fragment in a Hind III digest (Fig. 4 *G*) was present in this cell line. Because a Vγ1.2 probe hybridized to fragments of identical size in both digests (Fig. 4 *D* and *F*), we conclude that one of the three-members of the Vγ1 gene subfamily has rearranged to Jγ4 in the DN1.1 cell line. The sizes of the rearranged fragments are those expected from rearrangement of Vγ1.3 to Jγ4, and are inconsistent with rearrangements of either Vγ1.2 or Vγ1.1 to Jγ4 (Fig. 4). Although a BW5147-derived Vγ5-Jγ4Cγ4 rearranged gene is also present in this cell line, an earlier study found that this gene is nonfunctional (13). Donor-derived Vγ5-Jγ4Cγ4 rearrangements are apparently not present in DN1.1, because a donor-derived Vγ1.2 gene, in germline configuration, is retained. Rearrangement to Jγ4 of both Vγ1.3 and Vγ5 in the same cell should delete both intervening Vγ1.2 genes. Therefore, this cell line contains only one rearrangement involving Jγ4Cγ4 that can encode the expressed γ chain: Vγ1.3-Jγ4Cγ4 (B6 thymocyte derived).

As expected, each hybridoma expressed the transcript corresponding to the gene determined to be functional in the analysis described above (summarized in Fig. 5). In addition, however, each hybridoma expressed other γ transcripts, which may be nonfunctional. The expression of nonproductive γ transcripts in T cells has been frequently observed (12, 30, 32–34). Taken together, our data suggest that three distinct γ chains encoded by Vγ2-Jγ1Cγ1, Vγ4-Jγ1Cγ1, and Vγ1.3-Jγ4Cγ4 rearranged genes, are represented among five γ/δ T hybridomas.

Restricted Use of δ and γ Chains in an Enriched Population of Thymic γ/δ Cells. To determine the approximate frequency of δ rearrangements in thymic γ/δ cells, we analyzed the rearrangements present in DNA from an enriched population of such cells (50–80% γ/δ cells) isolated by short-term (3 d) culture in a lymphokine cocktail (4). Because most of these cells express a γ/δ receptor, quantitation of various δ rearrangements represents an approach to determining the usage and diversity of Vδ genes in the population. Detection of such rearrangements by Southern blot analysis suffers from the fact that rearrangements that are relatively rare in the population will escape detection; if there are many such rearrangements, a good deal of the diversity of δ gene rearrangements will be overlooked. On the other hand, if most of the alleles can be quantitatively accounted for by visible rearrangements, some estimate can be made of the diversity of δ rearrangements in the population. Although Southern analysis does not allow determination as to whether a rearrangement is functional (in frame), the fact that the majority of cells in the population express a δ chain means that most of the cells have at least one functional rearrangement (see below).

Five distinct rearrangements of Jδ1 were detected in the population with a Jδ1 probe (Fig. 3 *E*). One fragment of 9.4 kb corresponds to a rearrangement of Vδ7.3 to Jδ1, as shown by hybridization with a Vδ7.3 probe (Fig. 3 *D*). Two predominant ~6 kb rearrangements in the population correspond in size to rearrangements in the DN1.1 cell line. One of these corresponds to a Dδ1-Jδ1 rearrangement, based on our recent finding that this band hybridizes with a 5′ Dδ1 probe (not shown). The other 6 kb fragment presumably corresponds to the productively rearranged Vδ gene in DN1.1, suggesting that this V gene is commonly rearranged by cells in the population. Of the two remaining rearranged bands detected in the population with a Jδ1 probe (~6.6 and 13 kb), the 6.6 kb band represents a nonfunctional Dδ2-Jδ1 rearrangement; we found that a 2.5 kb Eco RI–Hinc II genomic DNA fragment that includes sequences 5′ of Dδ2 in germline DNA hybridizes to the 6.6 kb Eco RI fragment in DNA from the enriched population (Fig. 3 *F*). Because Dδ2 is 0.9 kb upstream of Jδ1 in germline DNA (20), the size of this rearranged fragment is consistent with a Dδ2-Jδ1 rearrangement. Significantly, none of the visible rearranged bands correspond to rearrangements of Vδ7.1 and Vδ2.3, as revealed by hybridization with these probes (Fig. 3 *B* and *F*).

To quantitate the visible rearrangements, we reprobed the Southern shown in Fig. 3 *E* with a single-copy reference probe (a murine IL-6 cDNA), and subjected the autoradiographs to densitometric analysis (data not shown). By comparing the lanes containing liver DNA with the lanes containing γ/δ cell DNA, and normalizing for amount of DNA by reference to the single-copy probe, we found that the sum of DNA present in visible Jδ1 rearrangements corresponds to ~45% of the potential δ alleles in the population. It was therefore important to determine whether the remaining δ alleles are present as diverse, undetectable rearrangements or are deleted from the cells. A replicate filter was therefore hybridized with a mixture of a Cδ cDNA probe and the IL-6 reference probe. Because the restriction enzyme used for these Southerns, Eco RI, cuts between Cδ and both Jδ segments, the Cδ alleles should not be obscurred by rearrangements. Analysis of the autoradiographs by densitometry (data not shown) revealed that ~50% of the δ alleles are deleted from the population, perhaps by deletional rearrangement of Vα gene segments to Jαs (14). The data indicate that nearly all of the δ rearrangements in the population can be accounted for by the visible Jδ1 rearrangements described above. Because half the δ alleles are deleted, and most cells in the population express a δ chain, it follows that many or most of the rearrangements observed must be functional. The limited number of rearrangements observed indicate that most thymic γ/δ cells use one of a few Vδ genes.

One commonly rearranged Vδ gene is Vδ7.3. Densitometric analysis of Southerns hybridized with Jδ1 and Vδ7.3 revealed that Vδ7.3 to Jδ1 rearrangements represent ~16–30% of the rearranged δ genes in the population. Since half the alleles are deleted from the population, these data suggest that a significant fraction of the cells express a Vδ7.3-Jδ1Cδ encoded δ chain.

Rearrangements of the Jδ2 gene were undetectable in DNA from the thymic γ/δ cell population (Fig. 3 *C*). Densitometric analysis of the autoradiograph, in comparison with the same blot reprobed with the single-copy reference probe (data not shown), revealed that the unrearranged Jδ2 bands account for the large majority (~90%) of the δ alleles still present in the population, as expected from our finding (above) that most of these δ alleles have undergone rearrangement to Jδ1 or are in germline configuration. These data suggest that Jδ2 rearrangements are rare in the population.

Taken together, our results suggest that most cells in the adult thymic γ/δ cell population express one of a few Vδ genes rearranged to Jδ1. The abundance of the Vδ7.3-Jδ1 rearrangement in the population (16–30% of the δ alleles) suggests that this gene segment is one of the commonly expressed Vδ gene segments; in contrast, the Vδ7.1 and Vδ2.3 gene segments appear to be only rarely used. Separate studies (34a, 34b) have found that the large majority of cells in the population express a γ chain encoded by a Vγ2-Jγ1Cγ1 rearranged gene. Therefore, most cells in this population appear to use receptors composed of a small subset of the available γ and δ subunits.

Nevertheless, rare cells in the population express other Vδ-Jδ and Vγ-Jγ genes, as indicated by the isolation of the DN7.1, DN12.1, and DN2.3 T hybridomas from the same population. We have not determined why the panel of hybridomas is apparently not numerically representative of the parent population, although it is possible that the short-term culture of the cells in the lymphokine cocktail before fusion preferentially stimulates a subset of cells, thus enhancing their fusion efficiency.

Discussion

Restricted Use of Vγ and Vδ Genes. Earlier studies have shown that the potential diversity of the γ chain in the mouse is relatively limited, with only seven (known) V gene segments, three functional J segments, and no known D segments (9–13). While there is clear evidence of junctional diversity created at Vγ-Jγ joints, presumably due in part to the addition of random nucleotides (*N* regions), the extent of *N* diversity in γ chains may be relatively limited (9–12). Moreover, γ gene use in the adult thymic population of γ/δ cells is strikingly nonrandom, with most cells expressing the product of a single rearranged gene, Vγ2-Jγ1Cγ1 (34a, 34b). Interestingly, γ gene use may depend on anatomical site and developmental stage, since protein and mRNA expression studies suggest that fetal thymocytes and the peripheral dendritic epidermal cells (DEC) commonly express γ chains other than Vγ2-Jγ1Cγ1 (10, 12, 31).

In this report we identify the rearranged genes that encode three different γ chains that are represented in a panel of five γ/δ T hybridomas: Vγ2-Jγ1Cγ1, Vγ4-Jγ1Cγ1, and Vγ1.3-Jγ4Cγ4. Expression of the two latter rearranged genes as γ polypeptides has not been previously reported. In concert with the analysis of the population, these data reveal a situation in which most adult thymic γ/δ cells express one γ chain, although a minor set of cells expressing other γ chains is present in the population. This situation is in contrast to that of the Vα and Vβ chains of the α/β TCR, where predominant V gene use is not generally observed (35, 36). It seems unlikely that the predominant usage of Vγ2 by thymic γ/δ cells is due to preferential rearrangement of the Vγ2 gene in early T cells (as has been argued for the predominant usage of J_H-proximal V_H gene segments in pre-B cells [37] because fetal thymocytes preferentially rearrange the Jγ1-proximal Vγ3 and Vγ4 genes (10).

The potential diversity of δ genes is still under investigation. The δ locus includes at least two Dδ segments and two Jδ segments (20, and this report). A critical issue concerns the number of Vδ gene segments, and whether Vα and Vδ gene segments are overlapping sets. In this report we provide evidence that four different Vδ chains are represented in a panel of five hybridomas. The deduced amino acid sequences of two of these Vδ chains are very different than those of any known Vα chain. Although the sequences are slightly more similar to Vα sequences than to V sequences of the β or γ families, they differ from Vα sequences about as much as most Vα subfamilies differ from each other. A third Vδ sequence (δ2.3), however, is very similar to a member of the Vα4 subfamily. Thus, including the earlier results of Chien et al. (14, 20), the sequences of four of six Vδ subfamilies analyzed are very different from those of the known Vα genes. Two of the genes show considerable homology to known Vα gene segments, although neither are identical. These findings raise the possibility that Vδ genes and Vα genes are largely nonoverlapping sets. It remains to be determined whether this differential use of V genes is imposed by regulation of the allowable gene rearrangements to Jδs vs. Jαs, by selection for chains able to pair with a given partner (β or γ) chain, or by selection of cells bearing particular receptors, among other possibilities.

In the present analysis, we have not attempted to estimate the total number of potential Vδ genes. This number may be quite large, particularly if other Vα genes are used for δ rearrangements. Nonetheless, Southern analysis of the thymic γ/δ population with Jδ probes revealed several predominant rearrangements, suggesting that, like Vγ genes, relatively few Vδ genes are expressed by the majority of cells in this population. In the previous analyses of δ diversity (20), the majority of cells in the populations studied did not express a surface γ/δ receptor.

The Vδ7.3-Jδ1 rearrangement was a predominant rearrangement in the population, suggesting that Vδ7.3 is a commonly used Vδ gene in thymic γ/δ cells. Considering the receptor as a whole, these results suggest that a Vγ2-Jγ1Cγ1/Vδ7.3-/Jδ1Cδ receptor, like the one expressed by the DN7.3 cell line, is one of a few dominant γ/δ receptors expressed in this population. In contrast to the expression of α/β receptors in peripheral T cells, the γ/δ receptors expressed in this population are dominated by those using one or a few Vγ and Vδ chains, respectively. A similar situation may exist in humans, since the diversity of expressed human Vδ genes may also be limited (15).

Nonrandom Association of Vγ and Vδ Gene Segments. A striking finding of the present studies is that there may be nonrandom pairing of Vγ and Vδ chains. Both T hybridomas we analyzed that express a Vγ4-Jγ1Cγ1 chain also express a Vδ7.1-Jδ2Cδ chain. In addition, the protein sequencing studies of Born et al. (19) have shown that a fetal T hybridoma that expresses Vγ4 expresses a δ chain that may be identical to Vδ7.1 (based on the identity of their peptide sequences with deduced peptide sequences of Vδ7.1). Considering that Vδ7.1 and Jδ2 rearrangements are rare in the adult thymic γ/δ cell population (as shown in this study) as are Vγ4 rearrangements (34b), the association of Vδ7.1-Jδ2Cδ and Vγ4-Jγ1Cγ1 expression is unlikely to be coincidental. Considering the earlier finding that Vγ4 transcripts (10) and Vδ7.1 rearrangements (V_{M21} [20]) are most abundant in fetal thymocytes, it is possible that cells expressing Vγ4/Vδ7.1 correspond to a fetal type that remain in small numbers in the adult thymus.

Another example of preferential pairing is suggested by the finding that most cells in the population bear a Vγ2-Jγ1Cγ1 chain (34a, 34b) and many of these cells may bear a Vδ7.3-Jδ1Cδ chain, based on the abundance of the corresponding rearrangement. Because expression of both of these genes is common, the preferential pairing may be coincidental. In any case, our data do not distinguish whether these associations are due to structural constraints on chain pairing, cellular selection, or reflect the developmental origin of the cells.

Function of the γ/δ Receptor. Our studies have not determined the extent of Vδ-Dδ-Jδ junctional diversity, and therefore it is not possible to estimate the number of different receptor sequences that could be generated with limited Vγ and Vδ gene usage; this number may be quite large. The significant combinatorial and junctional diversity observed in γ and δ chains may be more consistent with a role for γ/δ receptors in recognition of foreign antigens than in recognition of predictable structures such as hormones or invariant cell surface structures. However, the limited use of Vγ and Vδ genes, and the apparent nonrandom pairing of Vγ and Vδ chains may indicate that important components of receptor chains that contribute to specificity (i.e., complementarity-determining regions 1 and 2, by analogy to immunoglobulins) are relatively nondiverse in the γ/δ receptors expressed in the adult thymus. Further studies will be required to assess γ/δ receptor diversity at other stages in ontogeny and in other tissues.

It is useful to consider the relatively limited diversity of expressed Vγ and Vδ genes in the context of a recent report demonstrating alloreactive γ/δ cells specific for an MHC-linked antigen (38). The target class I antigen was shown to be encoded by a gene in the H-2-D or linked Qa/Tla regions. The previous finding that alloreactivity to H-2 antigens is a common feature of T cells specific for foreign antigens plus self-H-2 (39, 40) raises the possibility that γ/δ cells also display restricted recognition of foreign antigens. The limited diversity of expressed Vγ and Vδ genes might then be partly explained by limited variability of the restricting elements. It is therefore attractive to speculate that Qa/Tla-encoded class I molecules (or a subset of them), which show relatively limited polymorphism, might serve as restricting elements for recognition of foreign antigens by at least some γ/δ T cells; according to this idea, the diversity of foreign antigens recognized by γ/δ cells may be related to the extent of junctional diversity in the chains. In support of the notion that Qa/Tla class I molecules may be restricting elements are their similarities in sequence to H-2 class I molecules in the α1 and α2 domains (reviewed in 41); these regions of H-2 class I molecules are believed to be involved in binding peptides of foreign antigens (42). A separate T cell receptor involved in restricted recognition might have evolved to deal with a special class of antigens.

Elliott et al. (44) recently analyzed a panel of δ cDNA clones from adult double negative thymocytes. They find extensive junctional diversity in δ chains, due to potential for V-D-D-J joining and *N*-region diversity at each of the junctions. The Vδ7.3 sequence corresponds to their Vδ5 sequence, which they find to be frequently expressed by adult double negative thymocytes.

Summary

Previous studies have indicated that the diversity of γ genes expressed by γ/δ-bearing murine T cells is limited, but comparable information concerning the expressed diversity of δ genes is lacking. In this study, we have investigated the rearrangement and expression of δ and γ genes in T cell hybridomas that express γ/δ T cell receptors. Three productive δ chain cDNA clones were isolated (δ7.3, δ7.1,

and δ2.3) that encode new variable region sequences. Two of the δ cDNAs differ significantly from those observed in the Vα repertoire. In addition, one cDNA expressed a new Jδ region (Jδ2), which was localized between Jδ1 and Cδ genes. Using these and other δ gene probes and γ gene probes, we found that five independent hybridomas expressed four different Vδs and three different Vγs. However, analysis of an enriched population of γ/δ-expressing cells from the adult thymus suggests that only a few Vδ genes and one Vγ gene are used by the majority of the cells. These results suggest that important components of receptor chain that contribute to specificity (i.e., the germline V gene sequences) are relatively nondiverse in the thymic γ/δ population.

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