T Cell Receptor Gene Trans-Rearrangements: Chimeric γ-δ Genes in Normal Lymphoid Tissues

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Joining of V-, D-, and J-region gene segments during DNA rearrangements within all antigen receptor genes involves recognition of the same highly conserved heptamer-nonamer sequences flanking each segment. In order to investigate the possibility that recognition of these conserved sequences may sometimes permit intergenic joining of segments among different antigen receptor genes, DNA of normal human lymphoid tissues was examined by polymerase chain reaction amplification for the presence of chimeric γ-δ T cell receptor gene rearrangements. These studies detected V_γ-(D_δ)-J_δ and V_δ-(D_δ)-J_γ rearrangements in thymus, peripheral blood, and tonsil. Analysis of thymus RNA indicated that many of these rearrangements are expressed as V_γ-(D_δ)-J_δ-C_δ and V_δ-(D_δ)-J_γ-C_γ transcripts. Most transcripts (19 of 20 complementary DNA clones studied) are appropriately spliced and show correct open translational reading frames across the V-(D)-J junctions. Thus, chimeric antigen receptor genes are generated in a subset of normal lymphoid cells, probably as a result of chromosomal translocations, and such genes may possibly contribute to increased diversity within the antigen receptor repertoire.

ANTIGEN RECEPTOR GENE REARrangement has generally been considered to occur during normal lymphocyte development exclusively as a cis process, possibly through scanning of the lymphocyte recombinase along antigen receptor DNA in search of individual V-, D-, and J-region gene segments accessible for recombination (*1*). Nevertheless, several lines of evidence suggest that intergenic or trans-rearrangement, involving two separate antigen receptor genes, might also normally occur. For example, each of the different immunoglobulin and T cell receptor (TCR) genes is thought to rearrange by a shared mechanism in which the recombinase recognizes highly conserved heptamer-nonamer nucleotide sequences flanking all known rearranging gene segments (*1*). Also, studies of DNA surrounding the breakpoints of certain chromosomal rearrangements in lymphoid tumors have led to speculation that recombination between antigen receptor loci and putative oncogenic loci may sometimes proceed by recognition of genuine heptamer-nonamer sequences adjacent to antigen receptor loci and ectopic heptamer-like sequences near the oncogenic loci (*2*). Furthermore, chromosomal rearrangements with breakpoints in chromosomal bands containing antigen receptor loci have occasionally been observed in cytogenetic studies of phytohemagglutinin-stimulated lymphocytes from normal human peripheral blood (*3*).

To detect possible interchromosomal rearrangements between different antigen receptor genes in normal lymphoid tissues, we focused initially on γ- and δ-TCR genes for two reasons. First, both genes have relatively simple structures, facilitating the molecular analysis of potential interchromosomal rearrangement, and second, these genes are believed to undergo rearrangement at about the same stage of thymocyte development, often in the same cell (*4*). Polymerase chain reaction (PCR) was used in parallel on samples of DNA from normal human juvenile thymus and human germline (sperm), with a PCR primer pair consisting of a 5′ γ-TCR V-region oligonucleotide and a 3′ δ-TCR J-region oligonucleotide. The V_γ primer anneals to a 21-bp conserved sequence in the 5′ leader segment of each of the eight V regions of the V_γ-subgroup I cluster (*5*) and directs synthesis downstream from this site. The J_δ primer anneals to a 20-bp sequence at the extreme 3′ end of the $J_\delta 1$ segment (*6*) and directs synthesis upstream from this site. After 30 to 60 cycles of amplification, reaction products were analyzed by DNA blot hybridization with a ^{32}P-labeled V_γI DNA probe. The resulting autoradiogram showed a broad band corresponding to DNA fragments of about 480 to 510 bp in the thymus reaction (Fig. 1A), whereas no corresponding band was seen in the germline control reaction. In this size range, the PCR product was extracted from a preparative agarose gel of amplified thymus DNA and ligated into DNA of the M13 phage sequencing vector. The phage library produced from this ligation was screened by hybridization with the V_γI probe, and the DNA inserts from the positive plaques were sequenced.

All M13 clones contained inserts with the same basic structure consisting of a bona fide V_γ sequence joined to a variable junctional sequence, often containing one or two recognizable D_δ motifs, which in turn was joined to $J_\delta 1$ sequence (Table 1). Of the eight possible V_γI gene segments, four ($V_\gamma 2$, $V_\gamma 3$, $V_\gamma 4$, and $V_\gamma 8$) are represented in this limited series of clones. The absence of clones containing $V_\gamma 1$ and $V_\gamma 6$ gene segments is consistent with the fact that these V regions are flanked by altered re-

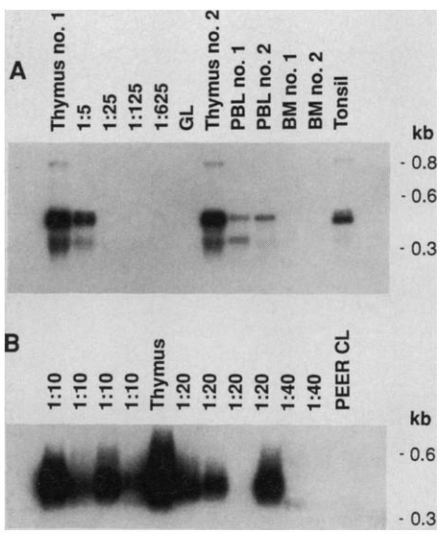

Fig. 1. Analysis of V_γ-(D_δ)-J_δ trans-rearrangements by blot hybridization of PCR products. (**A**) PCR was performed on 2 μg of high molecular weight DNA from normal juvenile thymus glands of two individuals (obtained during cardiac surgery), normal tonsil, peripheral blood mononuclear cells of two normal volunteers (PBL), total bone marrow from two normal donors (BM), human sperm (GL), or the human T cell leukemia cell line PEER with the V_γ,J_δ primer pair as described in Table 1, but for only 30 cycles. The reaction mixture (15 μl) was then separated by electrophoresis through a 1.2% agarose gel, transferred to nylon membranes, and hybridized with a mixture of three ^{32}P end-labeled oligonucleotides complementary to the $V_\gamma 2$, $V_\gamma 3$, and $V_\gamma 4$ gene segments at positions 254 to 273 of the described sequences. Hybridization was performed at 54°C in 6× saline sodium citrate (SSC) containing 5× Denhardt's solution, 1 m*M* sodium pyrophosphate, and denatured salmon sperm DNA (50 μg/ml) for 3 hours. The membranes were washed in 6× SSC at room temperature for 15 min and then again at 54°C for 15 min; they were then autoradiographed on Kodak XAR5 film without intensification for 12 to 24 hours. Serial 1:5 dilutions of thymus DNA were made in human sperm DNA to a total of 2 μg of DNA. (**B**) Dilutions of thymus DNA were made with human sperm DNA to a total of 2 μg of DNA. The 1:20 dilution therefore corresponds to 0.1 μg of thymus DNA or ~10^4 cell equivalents. PCR products were generated and analyzed as in (A).

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combination signals and are therefore probably incapable of rearrangement (*5*).

Because of the manner in which the PCR products were cloned in the M13 vector, the portion of V_γ regions present in each of the V_γ-(D_δ)-J_δ clones extends downstream from a common Kpn I site to include nucleotides 284 to 480 of the previously described V_γ sequences (*5*). Within this region there are few deviations from the known V_γ sequences. Ten of the thymus-derived clones exactly match the known sequences, whereas four show from one to four base changes. Whether these few changes represent somatic mutations, inherited polymorphisms, or errors introduced during the PCR amplification cannot be determined. The clones also contain from 10 to 27 nucleotides of J_δ1 sequence extending upstream of the J_δ primer. In each case, the DNA in this region exactly matches the described J_δ1 sequence (*6*).

The general structure of the junctional regions between the V_γ and J_δ segments in the series of thymus-derived clones is similar to that observed in standard intragenic δ-TCR gene rearrangements (*6*). The clones show small deletions of varying lengths (ranging from 0 to 12 bp) at the 3′ end of the V_γ segments. These are followed by so-called N-insertions, consisting of variably sized stretches of apparently random nucleotides, which display a bias toward increased G+C content. As mentioned above, most of the clones show one or two recognizable D_δ motifs (*6*) interspersed between N-insertions. At the 5′ end of J_δ1, there are again small deletions (ranging from 0 to 17 bp).

Comparison of previously described V_γ sequences (*5*) to that of the PEER V_δ sequence (*6, 7*) indicates limited sequence homology near the 3′ end of these gene segments. We therefore considered the remote possibility that heteroduplex formation might occur between single strands of incomplete V_γ and full-length V_δ-(D_δ)-J_δ products during annealing steps in early PCR cycles, with subsequent "filling-in" and amplification to yield spurious V_γ-(D_δ)-J_δ products. Although the absence of identifiable 3′ V_δ sequence from any of the chimeric products would argue against this explanation for the observations, a control experiment was carried out by performing PCR with the V_γ,J_δ primer pair on DNA extracted from the PEER cell line, which contains standard γ- and δ-TCR gene rearrangements (*6–8*). If the chimeric products generated from thymus DNA were actually a PCR artifact resulting from homology between V_γ and V_δ sequences, then PCR on PEER DNA might be expected to yield spurious products. However, no V_γ-(D_δ)-J_δ product could be detected by PCR on PEER DNA (Fig. 1B).

As a second control to exclude a possible artifactual origin for V_γ-(D_δ)-J_δ trans-rearrangements, the PCR with the V_γ,J_δ primer pair was carried out on fragments of thymus DNA, 6.6 to 9.4 kb in size, prepared by digestion with Hind III restriction enzyme and size-selection in agarose gels. On the basis of the known positions of Hind III cleavage sites flanking V_γ (*5*) and J_δ (*9*) sequences in germline DNA, fragments in this size range would be expected to contain

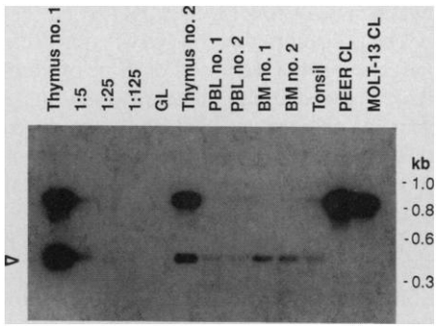

Fig. 2. Analysis of V_δ-(D_δ)-J_γ trans-rearrangements by blot hybridization of PCR products. PCR was performed on 2 μg of DNA from the indicated tissues with a 5′ V_δ1 primer and a 3′ J_γ primer, for 30 cycles, according to the conditions described in Table 2. The PCR product was detected with an internal V_δ1 oligonucleotide probe, GTATGAAACAAGTTGGTGG. The band at ~400 bp corresponds to the predicted product from V_δ-J_γ trans-rearrangements. (A band at about 875 kb has not yet been characterized.) The serial dilutions of thymus DNA were made as in Fig. 1.

V_γ-(D_δ)-J_δ trans-rearrangements, but to lack unrearranged V_γ segments or rearranged V_γ-J_γ segments, all of which lie within Hind III fragments of less than 4.5 kb (*5, 10*). The M13 library constructed from the products of the PCR on the 6.6- to 9.4-kb DNA fragments was found to be highly enriched in V_γ-(D_δ)-J_δ clones (a 20-fold excess of positive plaques derived from a single PCR reaction compared to the number of positive plaques derived from unfractionated thymus DNA). These results are again consistent with trans-rearrangement clones representing true in vivo recombination products

Table 1. Junctional regions of V_γ-(D_δ)-J_δ trans-rearrangements. PCR amplification was performed in a reaction buffer consisting of 50 m*M* KCl, 35 m*M* tris (*p*H 8.3), 2.5 m*M* $MgCl_2$, bovine serum albumin (100 μg/ml), each deoxyribonucleotide (dNTP) at 0.2 m*M* 2 μg of normal human thymus DNA or tonsil DNA, 300 ng of two oligonucleotide primers, and 1 to 2 U of *Taq* DNA polymerase, in a total volume of 100 μl. The V_γI primer, representing the conserved 5′ leader sequence of the V_γI family, has the sequence GAAGCTTCTAGCTTTCCTGTCTC. The J_δ primer, hybidizing to the extreme 3′ end of the J_δ1 segment, has the sequence CGAATTCCACACACGGGTTC and includes an Eco RI restriction site at its 5′ end. The reactions were carried out for 30 cycles of denaturation (1 min at 94°C), primer annealing (2.5 min at 54°C), and primer extension (1.5 min at 70°C, with a final extension period of 6 min on the last cycle), in a Perkin-Elmer Cetus automated thermal cycler. A portion (15 μl) of the reaction mixture was then diluted into 100 μl of fresh reagents and subjected to an additional 30 cycles of amplification. The PCR products were separated by electrophoresis through a 1.2% agarose gel, and DNA in the size range of 350 to 500 bp was eluted. The DNA was digested with Kpn I and Eco RI and ligated into DNA of phage M13mp19. Phage libraries were screened with a full-length V_γI DNA probe generated by PCR of thymus DNA with V_γI,J_γ primers (see below), and positive clones were sequenced by dideoxy chain termination (*15*). The specific V_γ segment in each clone is indicated by the first number in the clone designation. Clones designated gd2.1, 2.2, 3.1, 3.2, 4.1, 4.2, and 8.1 are derived from unfractionated thymus DNA. Clones designated gd2.3, 3.3, 4.3, 4.4, 4.5, 4.6, and 4.7 are derived by PCR on 6.6- to 9.4-kb fragments of thymus DNA prepared by digestion with Hind III restriction enzyme and gel electrophoresis. The clone designated gd2.4 is derived from tonsil DNA. There were no deviations from described V_γ sequences in clones gd2.3, 3.2, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, and 8.1. The remaining clones showed from one to four base changes in various locations. All clones showed J_δ1 sequence exactly matching the described sequence (*7*). Apparent D_δ sequences are underlined.

Clone	V_γ	N + D_δ	J_δ
gd2.1	ACTGTGCCACCTGGGACGGG	*CCGCGGCGGTGACCGT*	ACACCGATAAACTCATCTTTGGAAAAG
gd2.2	ACTGTGCCACCTGGGA	*GCCCCTTCCTACTGCTCGGGGGATGTTTTG*	CACCGATAAACTCATCTTTGGAAAAG
gd2.3	ACTGTGCCACCTGGGACGGG	*AGGGTTCTTCACCTTACTGGGGGATACGCGGT*	ACACCGATAAACTCATCTTTGGAAAAG
gd2.4	ACT	*TTCCGGGGGATGGAGACTGGG*	TTTGGAAAAG
gd3.1	ACTGTGCCACCTGGGAC	*GTACCTTCCCGACACGTTCGGGGGATGTGGTTGAGGT*	GATAAACTCATCTTTGGAAAAG
gd3.2	ACTGTGCCACCTGGG	*TCCCACCTTCCTCCGTCCTGAGGTGAAGTGGGGGATTTCTA*	CGATAAACTCATCTTTGGAAAAG
gd3.3	ACTGTGCCACCTGGGACAG	*ATAAACTAGAGGTTCCTATCGGTGGGGGCCT*	CCGATAAACTCATCTTTGGAAAAG
gd4.1	ACTGTGCCACCTG	*ACCCG*	TTTGGAAAAG
gd4.2	ACTGTGCCACCTGGGATG	*AGTCTGAGGACAGGGGGACTGGGGGATACGCGAAGCCC*	CACCGATAAACTCATCTTTGGAAAAG
gd4.3	ACTGTGCCACCTGGGATGGG	*CTCTGTACCGGACGCTTCCGGGG*	TCTTTGGAAAAG
gd4.4	ACTGTGCCAC	*GGTTTGGGGGACTCCTCTG*	ACCGATAAACTCATCTTTGGAAAAG
gd4.5	ACTGTGCCACCTGGGATGG	*AAAC*	ATAAACTCATCTTTGGAAAAG
gd4.6	ACTGTGCCACCTGGGATG	*AAGAGCCCTTCCTACCCTGGGGGATACACGT*	TAAACTCATCTTTGGAAAAG
gd4.7	ACTGTGCCACCTGGGATGGG	*CCTTCCCCTACTTACTGGGGGCCTTATCGT*	ACACCGATAAACTCATCTTTGGAAAAG
gd8.1	ACTGTGCC	*GCTTCATCGAACGC*	ACACCGATAAACTCATCTTTGGAAAAG

rather than PCR artifacts.

The possibility that V_δ-(D_δ)-J_γ trans-rearrangements, the reciprocal of those products described above, might be generated in developing thymocytes by a similar (and conceivably coupled) mechanism of intergenic recombination was investigated by repeating the PCR amplification procedure with a $V_\delta 1$,J_γ primer pair (Fig. 2). Amplification of normal human thymus DNA yielded a reaction product of the predicted size (~400 bp), which was detected by blot hybridization with an internal $V_\delta 1$ oligonucleotide probe. This product was absent when PCR was carried out on germline DNA and on DNAs of PEER and MOLT 13 cell lines, both of which contain standard γ- and δ-TCR gene rearrangements (*6–8*). The 400-bp product from thymus DNA was cloned in M13 phage, and the DNA inserts from multiple phage isolates were sequenced. Each of nine M13 clones analyzed contain a bona fide $V_\delta 1$ segment exactly matching the previously described sequence (*6, 7*), joined to J_γ (Table 2). In a given clone there are from 11 to 26 nucleotides of J_γ sequence that exactly match the described sequence (*5*) extending upstream from the J_γ primer. As in the series of V_γ-(D_δ)-J_δ clones, features of the junctional regions of the V_δ-(D_δ)-J_γ clones are entirely analogous to those seen in standard gene rearrangements, with variable 3′ deletions of $V_\delta 1$ and 5′ deletions of J_γ, random G+C–rich N-insertions of variable sizes, and, in two clones, recognizable D_δ motifs.

The presence of D_δ sequences in both the V_γ-(D_δ)-J_δ and the reciprocal V_δ-(D_δ)-J_γ clones is consistent mechanistically with previous observations of both V_δ-D_δ and D_δ-J_δ partial gene rearrangements in lymphoid cells (*11*). A V_δ-D_δ-J_γ trans-rearrangement could result from recombination between a previously formed V_δ-D_δ partial gene rearrangement and a J_γ gene segment, while a V_γ-(D_δ)-J_δ trans-rearrangment could result from recombination between a V_γ gene segment and a partial D_δ-J_δ gene rearrangement. Lack of recognizable D_δ sequence from some of the V_γ-(D_δ)-J_δ and V_δ-(D_δ)-J_γ clones is also consistent with the absence of apparent D_δ sequences from some standard δ-TCR rearrangements (*6*).

The frequency of cells bearing γ-δ trans-rearrangements in normal juvenile human thymus was estimated by performing PCR with V_γI,$J_\delta 1$ or $V_\delta 1$,J_γ primer pairs on samples of thymus DNA serially diluted into germline DNA, and the specific PCR products were detected by blot hybridization

Table 2. Junctional regions of V_δ-(D_δ)-J_γ trans-rearrangements. PCR amplification was performed on normal thymus DNA according to the above conditions with a 5′ $V_\delta 1$ primer, GCTGCAGTCATCAGTATCCATGCC, corresponding to nucleotide positions (+)21 to 40 of the described $V_\delta 1$ sequence (*6*) and including a Pst I restriction site at its 3′ end, and a 3′ J_γ primer, CGTCGACAACAAGTGTTGTTCCAC, corresponding to the extreme 3′ end of the $J_\gamma 1$ and $J_\gamma 2$ sequences (*5*) and including a Sal I restriction site at its 5′ end. The PCR product was in the size range of 350 to 450 bp, was digested with Pst I and Sal I restriction enzymes, and cloned in phage M13ml18. The resulting phage library was screened with an internal $V_\delta 1$ oligonucleotide probe, GTATGAAACAAGTTGGTGG (positions 65 to 83 of the described sequence) and positive clones were sequenced. In every clone, the complete $V_\delta 1$ sequence matched the published sequence, as did the J_γ sequence upstream of the primer site. Sequences identifiable as D_δ motifs are underlined.

Clone	$V_\delta 1$	N + D_δ	J_γ
dg 1	TTTTGTGCTCTTGGGGA	*ATG*	TTATAAGAAACTCTTTGGC
dg 2	TTTTGTGCTCTTGGGGA	*ACTACTCGCGCACCT*	ATTATAAGAAACTCTTTGGC
dg 3	TTTTGTGCTCTTGGGGA	*ACGACCTACCCCTACTGACCG*	CTTTGGC
dg 4	TTTTGTGCTCTTGGGGAA	*GGGTATGCACCTCCTGGGGGATACCGCTGGTAT*	TTATAAGAAACTCTTTGGC
dg 5	TTTTGTGCTCTTGGGGAA	*AGGG*	TATAAGAAACTCTTTGGC
dg 6	TTTTGTGCTCTTGGGGAA	*GAGGC*	ATTATAAGAAACTCTTTGGC
dg 7	TTTTGTGCTCTTGGGGAACT	*TGTCGTA*	AAGAAACTCTTTGGC
dg 8	TTTTGTGCTCTTGGGGAAC	*AACCCTTCCTCTGGGGGATACGACT*	GAATTATAAGAAACTCTTTGGC
dg 9	TTTTGTGCTCTTGGGGAACT	*ATGCTGT*	AAGAAACTCTTTGGC

Table 3. Junctional regions of V_γ-(D_δ)-J_δ-C_δ cDNA clones. Total RNA was prepared from normal human thymus by the guanidinium/hot phenol method (*16*). Reverse transcription was performed on 10 μg of RNA with a C_δ oligonucleotide primer,CAAGCTTGACAGCATTGTAC [positions +543 to 558 of the described C_δ sequence (*6*)], in PCR reaction buffer containing dNTPs (Table 1), 100 ng of primer and 200 U of murine leukemia virus reverse transcriptase, in a total volume of 50 μl at 42°C for 45 min. Ten microliters of the reaction mixture was then diluted to 100 μl final volume in PCR buffer containing dNTPs, 300 ng of both V_γI and C_δ primers, and 2.5 U of *Taq* polymerase. PCR was performed under standard conditions (Table 1) for 30 cycles. PCR product (90 ng of 350- to 550-bp product) size-selected by gel electrophoresis was subjected to an additional 30 cycles of PCR with a primer pair consisting of either (i) the original C_δ oligonucleotide and an internal V_γI oligonucleotide with the sequence C(T/G)(T/G)CTACATCCACTGGTACCT, corresponding to positions 270 to 290 of the described sequences or (ii) the original 5′ V_γI primer and the J_δ primer. The resulting products were digested with either (i) Kpn I and Hind III or (ii) Hind III and Sal I and cloned in phage M13mp19. Phage libraries were screened with either (i) J_γ or (ii) V_γI internal oligonucleotide probes, and positive clones were selected for sequence analysis. The clones designated gdcDNA 2.8, 2.9, and 2.10 were obtained with the V_γI,J_δ primer pair in the second round of PCR and therefore include the V_γI leader sequence and splice junction, while the remaining clones were obtained using the V_γI(internal),C_δ primer pair in the second round of PCR and therefore include the portion of V_γ sequence extending downstream from position 270, the J_δ segment, the J_δ-C_δ splice junction, and the C_δ first and second exons and their splice junction. Within these regions, clones gdcDNA 2.1, 2.4, 2.7, and 4.1 showed no deviations from the described sequences. The remaining clones showed from one to two base changes per clone, located at various positions. Clone gdcDNA 2.6 shows an apparent insertion of nine nucleotides at the 3′ end of the V_γ segment, possibly representing a PCR artifact. However, none of the other clones showed such insertions. The first number in the clone designations corresponds to the specific V_γ segment represented in each clone. Each of the clone contains the $J_\delta 1$ segment with the exception of clone gdcDNA 2.6, which utilizes $J_\delta 2$. The cDNA sequences surrounding and including the junctional regions are illustrated as triplet codons to show the preservation of the correct open reading frames in all clones except gdcDNA 2.8. Apparent D_δ sequences are underlined.

Clone	V_γ	N + D_δ	J_δ
gdcDNA 2.1	TAC TGT GCC	*ACG*	ACC GAT AAA CTC ATC
gdcDNA 2.2	TAC TGT GCC ACC TGG GAC	*GAG GGA CAC CGG GGG ATA CGC TTG TAC*	ACC GAT AAA CTC ATC
gdcDNA 2.3	TAC TGT GCC ACC TGG GAC GGG	*CTA TGT TCC TAC GGG AGA CCC*	ACC GAT AAA CTC ATC
gdcDNA 2.4	TAC TGT GCC ACC	*CCC AAC ACG AGT AAC GAG GCC CTA TTT AAC*	AAA CTC ATC
gdcDNA 2.5	TAC TGT GCC ACC TGG GAC GGG	*GGG CCT GAG AAA GTC CGG GGC CAG AAA TAC*	ACC GAT AAA CTC ATC
gdcDNA 2.6	TAC TGT [GGG CAG GGT]CCA CCT GGG ACG	*GCA AGA AAT GAG*	GAC ACC CGA CAG ATG TTT
gdcDNA 2.7	TAC TGT GCC ACC TGG GAC	*GAG*	ACC GAT AAA CTC ATC
gdcDNA 2.8	TAC TGT GCC ACC TGG	*CAA CTT CGG TCC CTA CTG GGG GAT AGG ATG G*	GAC ACC GAT AAA CTC ATC
gdcDNA 2.9	TAC TGT GCC ACC TGG GAC GGG	*GGG*	AAA CTC ATC
gdcDNA 2.10	TAC TGT GCC ACC TGG GAC GGG	*CGT CGC*	ACC GAT AAA CTC ATC
gdcDNA 4.1	TAC TGT GCC	*ACG AAT CAA CGC CGG CTC CCG ATC*	ACC GAT AAA CTC ATC

Table 4. Junctional regions of V_δ-(D_δ)-J_γ-C_γ cDNA clones. RNA-PCR was performed as described above, except with different oligonucleotide primers. The primer for reverse transcription was a C_γ oligonucleotide with the sequence GGAGCTCTTCTTTTCTTGCCA, corresponding to the extreme 3′ end of the C_γ coding sequence (*17*) and including a Sac I restriction site at its 5′ end, and the primer pair for the first PCR step was V_δ1,C_γ. The second PCR step was carried out either with the original V_δ1,C_γ primer pair (generating the M13 phage library from which clones dgcDNA 1 and 2 were derived) or with a V_δ1 (internal),C_γ primer pair (generating the library from which the remaining clones were derived). Sequences of the two V_δ1 primers are in the legend to Table 2. PCR products were cloned in phage M13mp19 after digestion with the appropriate restriction enzymes and positive phage were isolated by screening of the resulting libraries with the J_γ oligonucleotide probe. Each of the clones showed V_δ1,J_γ and C_γ sequences identical to those previously described. The C_γ sequence was in every case the "γRγ" isotype (*17*). The cDNA sequences surrounding and including the junctional region are illustrated as triplet codons to show preservation of the correct open reading frame in each clone. An apparent D_δ motif is underlined.

Clone	V_δ1	N + D_δ	J_γ
dgcDNA 1	TTT TGT GCT CTT GGG	GAA TTC GCG ACG	AAT TAT TAT AAG AAA CTC
dgcDNA 2	TTT TGT GCT CTT GGG	GAA TCG CAA CCA CCT CGA ATA CTG GGG GAT ACG GGG	TAT TAT AAG AAA CTC
dgcDNA 3	TTT TGT GCT CTT GGG	GAA AAG GGG	AAT TAT TAT AAG AAA CTC
dgcDNA 4	TTT TGT GCT CTT GGG GAC	GGG TTT CCA	TAT AAG AAA CTC
dgcDNA 5	TTT TGT GCT CTT GGG GAC	CGG GGT	TAT AAG AAA CTC
dgcDNA 6	TTT TGT GCT CTT GGG	GCA ATT TCC CCA AAG TGG ACC ACG AAG	AAT TAT TAT AAG AAA CTC
dgcDNA 7	TTT TGT GCT CTT GGG	GAA CTA GAG TCT CAG	AAA CTC
dgcDNA 8	TTT TGT GCT CTT GGG		TAT TAT AAG AAA CTC
dgcDNA 9	TTT TGT GCT CTT GGG GAC	ACT CCG	AAT TAT TAT AAG CTC

with appropriate internal V-region oligonucleotide probes. Both types of trans-rearrangement were detected at dilutions of 1:5 but were not detected at a dilution of 1:125 (Figs. 1B and 2), while at intermediate dilutions of 1:20 or 1:25, the specific PCR product was sometimes present. These results indicate that the single-cell limit, yielding a Poisson distribution of PCR product, is reached for both V_γI-(D_δ)-J_δ1 and V_δ1-(D_δ)-J_γ trans-rearrangements in the range of 1:20 to 1:25 dilution. Since all reactions contained 2 μg, or 2×10^5 genome equivalents of DNA, the frequency of both types of trans-rearrangements among normal thymocytes can be estimated as 1×10^{-4}, a frequency that coincides with the five to nine different clones obtained in representative but not exhaustive screenings of M13 libraries derived from 2 μg of thymus DNA. The estimated frequency of 10^{-4}, however, necessarily represents a lower limit on the number of thymocytes bearing γ-δ trans-rearrangements, since we have only carried out PCR with a restricted set of V- and J-region primers.

The detection of γ-δ trans-rearrangements in thymus DNA raises the question of whether cells containing these rearrangements are developmentally end-stage and destined to die in the thymus or are capable of emigration to peripheral lymphoid organs. To examine this issue, PCR was performed on DNA from normal human tonsil, peripheral blood mononuclear cells, and bone marrow, with the V_γ,J_δ and V_δ,J_γ primer pairs. Specific PCR products corresponding to both types of trans-rearrangements were detected in the DNA of normal tonsil and in the peripheral blood mononuclear cells from two different individuals (Fig. 1A and Fig. 2). The identity of the PCR signal seen in peripheral lymphoid tissues was confirmed by sequencing the tonsil-derived product (Table 1, clone gd 2.4). V_δ-(D_δ)-J_γ rearrangements were also detected in normal bone marrow, whereas V_γ-(D_δ)-J_δ rearrangements were not detected. However, this finding may not represent a real difference in tissue distribution of the two types of trans-rearrangements since products were present in these peripheral tissues at frequencies near the Poisson limit.

As in standard gene rearrangements, the random addition and deletion of nucleotides at the junctional regions of γ-δ trans-rearrangements should, on average, lead to preservation of open translational reading frames in about one-third of cases. In fact, potential open reading frames are maintained in 4 of 14 V_γI-(D_δ)-J_δ1 and 5 of 9 V_δ1-(D_δ)-J_γ thymus-derived clones. To explore the possibility that such in-frame trans-rearrangements might give rise to translatable mRNA transcripts, we carried out a two-stage RNA-PCR procedure in which reverse transcription of thymus RNA, initiated from a C_δ (or C_γ) oligonucleotide primer, was followed by PCR amplification of the resulting cDNA with a V_γ,C_δ (or V_δ,C_γ) primer pair. When 10 μg of total RNA from normal human thymus was reverse-transcribed from the C_δ oligonucleotide and amplified with the use of V_γ,C_δ primer pairs, a specific PCR product of the predicted size was detected by blot hybridization with a J_δ oligonucleotide probe (Fig. 3A). In contrast, parallel PCR on control RNA samples from PEER, SUP-T3, and FL18 cell lines failed to yield a detectable product. Analogous results were obtained in the reciprocal experiment, in which PCR was carried out by reverse transcription of RNA from a C_γ oligonucleotide, followed by amplification with V_δ,C_γ primers and detection of the product with a J_γ oligonucleotide probe (Fig. 3B). PCR products obtained with thymus RNA were eluted from preparative gels and cloned in M13 phage. Phage libraries were screened with the appropriate J-region or internal V-region oligonucleotide probes, and positive clones were selected for sequence analysis.

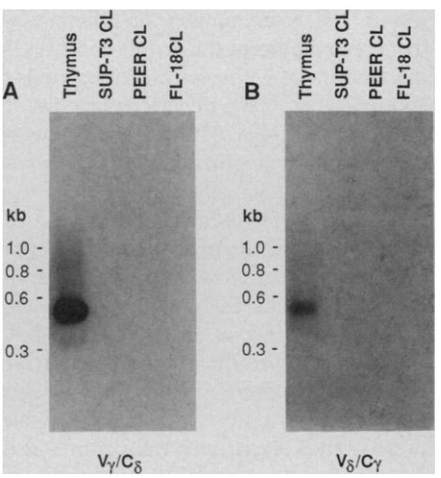

Fig. 3. Analysis of chimeric V_γ-(D_δ)-J_δ-C_δ and V_δ-(D_δ)-J_γ-C_γ mRNA transcripts by blot hybridization of RNA-PCR products. (**A**) Reverse transcription was performed on 10 μg of total RNA from the C_δ primer, followed by 30 cycles of PCR with the V_γI,C_δ primer pair, size selection of product, and an additional 30 cycles of PCR with the V_γI (internal),C_δ primer pair, as described in Table 3. Reaction products were analyzed by electrophoresis through a 1.1% agarose gel, blotting, and hybridization with the J_δ oligonucleotide probe. Cell line controls are PEER(6–8); SUP-T3, a T cell leukemia line (*18*) expressing α- and β-TCR transcripts; and FL18, a B cell lymphoma line (*19*). (**B**) Reverse transcription was performed as in (A) from the C_γ primer, followed by 30 cycles of PCR with the V_δ1,C_γ primer pair, size selection of product, and an additional 30 cycles of PCR with the V_δ1(internal),C_γ primer pair, as described in Table 4. Reaction products were analyzed as in (A) except that hybridization was performed with the J_γ oligonucleotide probe.

The cDNA clones resulting from V_γ,C_δ PCR on thymus RNA were found to show the general structure V_γ-(D_δ)-J_δ-C_δ (Table 3). All exon splice junctions included in the cloned PCR products were found to be correctly formed. These include the 5′ V_γ leader and J_δ-C_δ splices and the splice between the first and second C_δ exons. Two

different V_γ segments ($V_\gamma 2$ and $V_\gamma 4$) and two different J_δ segments ($J_\delta 1$ and $J_\delta 2$) are represented in the series. Deviations from the reported V_γ (*5*), J_δ, and C_δ (*6, 7*) sequences were minimal, with most clones exactly matching sequences described (Table 3). Analogously, the clones resulting from V_δ,C_γ PCR on thymus RNA showed the general structure V_δ-(D_δ)-J_γ-C_γ, again with proper splice junctions (Table 4). In this series of clones there were no deviations from the previously described sequences of $V_\delta 1$ (*6, 7*), J_γ (*5*), or C_γ (*11*).

In both groups of cDNA clones, single nonexhaustive screenings of M13 libraries derived from 10 μg of total thymus RNA yielded up to seven different clones in each case. Since duplicate clones were not observed, the total number of different γ-δ trans-rearrangement transcripts contained in this amount of RNA, which corresponds to approximately 10^6 thymocytes, must be considerably higher. Therefore, it is possible that a significant portion of all trans-rearrangements are actually transcribed.

A striking feature of both groups of cDNA clones is the overwhelming preponderance of clones showing open translational reading frames. In 10 of the 11 V_γ-(D_δ)-J_δ-C_δ clones and in all 9 V_δ-(D_δ)-J_γ-C_γ clones, an open reading frame is maintained across the junctional regions. These results imply that γ-δ trans-rearrangements which preserve the correct open translational reading frame are preferentially expressed over the more numerous trans-rearrangements that lack continuous open reading frames.

Two issues raised by our findings concern the molecular mechanism of trans-rearrangement and the possible functional role of the chimeric rearrangement products. By itself, the PCR procedure used to detect these rearrangements does not reveal the mechanism by which trans-rearrangements are created. However, for several reasons, it seems most likely that TCR trans-rearrangements are the result of chromosomal translocations. For example, balanced t(7;14)(p13;q11) chromosomal translocations have been observed at frequencies of 10^{-3} to 10^{-4} in phytohemagglutinin-stimulated lymphocytes from normal human peripheral blood (*3*). The breakpoints at 7p13 and 14q11 correspond to general chromosomal locations of the γ- and δ-TCR genes, respectively, suggesting that such translocations might represent the physical correlates of human γ-δ (and possibly γ-α) trans-rearrangements. Furthermore, at the molecular level, lymphocyte recombinase has been circumstantially implicated in the generation of chromosomal translocations in lymphoid malignancies (*2*). In fact, recombination in one example of another type of chromosomal aberration, an inv(14)(q11;q32) found in the SUP-T1 human T cell leukemia line, has been shown to involve two different antigen receptor genes, joining an immunoglobulin heavy chain V-region to a J-region of the α-TCR locus (*12*).

The presence of correct, uninterrupted translational reading frames in transcripts of chimeric gene rearrangements suggests that these rearrangements may direct the synthesis of functional TCR proteins. This interpretation is strengthened by the strong bias detected toward open rather than nontranslatable reading frames among the transcripts analyzed—a particularly significant finding in view of the general principle of allelic exclusion and preferential expression of the productive rearrangement observed in standard antigen receptor gene rearrangements (*1, 13*). If γ-δ trans-rearrangements are actually functional, chimeric V_γ-C_δ and V_δ-C_γ TCR polypeptide chains produced by them would be predicted to be qualitatively distinct, especially with respect to the local protein structure surrounding the hybrid junctional regions. In this way, γ-δ trans-rearrangements might contribute an additional component to the T cell receptor repertoire.

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Activation of Bacterial Porin Gene Expression by a Chimeric Signal Transducer in Response to Aspartate

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The Tar chemoreceptor of *Escherichia coli* is a membrane-bound sensory protein that facilitates bacterial chemotaxis in response to aspartate. The EnvZ molecule has a membrane topology similar to Tar and is a putative osmosensor that is required for osmoregulation of the genes for the major outer membrane porin proteins, OmpF and OmpC. The cytoplasmic signaling domain of Tar was replaced with the carboxyl portion of EnvZ, and the resulting chimeric receptor activated transcription of the *ompC* gene in response to aspartate. The activation of *ompC* by the chimeric receptor was absolutely dependent on OmpR, a transcriptional activator for *ompF* and *ompC*.

ADAPTATION TO ENVIRONMENTAL changes is essential for the survival of free-living bacteria. *Escherichia coli* contains receptors in the cytoplasmic membrane for the detection of a variety of environmental signals (*1*). Methyl-accepting chemotaxis proteins (MCPs) are sensory transducers that monitor temporal changes of various chemoeffectors and respond to these changes by altering the swimming

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