Cloning of the gene encoding the δ subunit of the human T-cell receptor reveals its physical organization within the α -subunit locus and its involvement in chromosome translocations in T-cell malignancy

(mechanisms of leukemogenesis/T-cell leukemia)

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ABSTRACT By taking advantage of “chromosomal walking” techniques, we have obtained clones that encompass the T-cell receptor (TCR) δ -chain gene. We analyzed clones spanning the entire J_α region extending 115 kilobases 5' of the TCR α -chain constant region and have shown that the TCR δ -chain gene is located over 80 kilobases 5' of C_α . TCR δ -chain gene is rearranged in the γ/δ -expressing T-cell line Peer and is deleted in α/β -expressing T-cell lines. Sequence analysis of portions of this genomic region demonstrates its identity with previously described cDNA clones corresponding to the C_δ and J_δ segments. Furthermore, we have analyzed a t(8;14)(q24;q11) chromosome translocation from a T-cell leukemia and have shown that the J_δ segment is rearranged in cells deriving from this tumor and probably directly involved in the translocation. Thus, the newly cloned TCR δ chain is implicated in the genesis of chromosome translocations in T-cell malignancies carrying cytogenetic abnormalities of band 14q11.

The salient feature of the functional capacity of the T cell is its ability to recognize antigenic and major histocompatibility complex molecules. Any potential effector capability displayed by a given T cell follows from this recognition. Thus, a major goal of cellular immunology has been to obtain an understanding in molecular terms of the mechanisms whereby recognition occurs. This goal has been greatly facilitated by the molecular cloning of receptor structures involved in antigen-specific interactions. One of these structures is borne on the surface of most peripheral T cells. It comprises two chains, denoted α and β , each having a mass of 40–50 kDa, linked to one another by means of disulfide bonds. This heterodimer is in turn noncovalently associated with the T3 protein complex (1). Studies on the genetics of the α/β receptor have demonstrated that these genes are organized in a manner similar to the immunoglobulin genes; variable (V), diversity (D), joining (J), and constant (C) segments are components of each gene, and these recombine combinatorially during T-cell ontogeny to encode fully functional α and β molecules (2–7).

It has become apparent that some T cells express on their surfaces a second antigen-specific receptor complex. Molecular genetic studies initially predicted the presence of a third T-cell receptor (TCR) gene, denoted γ (5). It was subsequently demonstrated that the γ protein was one subunit of a heterodimer that associated with T3 proteins on the surface of some peripheral blood T cells, thymocytes, and other cells (8–10). The second component of the heterodimer was designated the TCR δ chain (11). In the mouse, a DNA

segment, TCR $_\delta$, was identified in the 5' portion of the α -chain J region that displayed two properties that might be expected of the δ chain: expression early in T-cell maturation and deletion during the course of ontogeny (12, 13). Recently, human cDNA clones corresponding to the δ chain have been obtained and characterized (14). These have been shown to code for the expected proteins and to derive from genes that rearrange during T-cell development as do other TCR and immunoglobulin genes.

Aside from their inherent importance for our understanding of the function of the normal cellular immune response, these studies also have significant bearing on our comprehension of the basis of tumor-specific chromosome translocations in human lymphoid neoplasms. Nonrandom chromosome translocations are a nearly constant feature of human leukemias and lymphomas (15). In T-cell malignancies, the chromosomal region most frequently involved in translocations is chromosome 14 band q11 (16–19). In addition, T cells from patients with the immunodeficiency disease ataxia telangiectasia often carry abnormalities of chromosome 7q35 and 7p15 (20). To these regions, we and others have mapped the genes for the TCR α -, β -, and γ -chain genes (refs. 7, 21, 22, respectively). Previous studies from our laboratory have attempted to characterize the most prevalent translocations, those involving 14q11, in detail. Investigations employing somatic cell hybrid cell lines have demonstrated the molecular involvement of the TCR α -chain gene in a number of T-cell translocations. Specifically, the TCR α locus is split by the t(11;14)(p13;q11) translocation (23), the t(8;14)(q24;q11) translocation (24, 25), and the t(10;14)(q24;q11) translocation (26). In each instance, the V_α segments remain on the derivative (14) chromosome, whereas the C_α region is translocated to the reciprocal partner. In one case of T-cell leukemia carrying the t(8;14), it was demonstrated directly that chromosome 8 sequences had been aberrantly joined to a J_α segment on chromosome 14 (25). The further elucidation of the molecular structures of the aforementioned translocations initially provided the impetus for our more complete delineation of the J_α region.

In light of recent data on the genomic structure of the murine TCR $_\delta$ -chain gene and the isolation of human δ cDNAs, it seemed likely to us that this member of the TCR gene family would prove to be involved in human chromosomal rearrangements in a like manner to other TCR genes. Moreover, the possible interposition of the δ -chain gene between the C_α and V_α segments provided a potentially intriguing explanation for the involvement of this chromosomal region in various T-cell translocations. Thus, we undertook the characterization of the genomic organization

FIG. 1. Genomic organization of the α -chain and δ -chain locus. A series of cosmid clones extending 105 kb 5' to the C_α region were isolated by chromosomal walking. Restriction sites are given for *Bam*HI (B), *Eco*RI (E), *Sst* I (S), and *Xba* I (X). Structural genes are shown by closed boxes at the top line. Locations of J_α and C_α genes are from refs. 25 and 34–38. Ranges covered with isolated clones are shown by horizontal bars with abbreviated names. Polymorphic restriction sites are indicated by an *.

of the human TCR δ -chain gene. In this study we report the physical relationship of the human TCR δ - and α -chain genes and demonstrate the involvement of the δ -chain gene in T-cell-specific chromosome translocations.*

MATERIALS AND METHODS

Vector Preparation. The cosmid vector pMFG1 was constructed by introducing a *Not* I linker into the *Sma* I site of the pHSG274 vector obtained from the American Type Culture Collection (27). The introduction of this rare restriction site enables the linearization of a cosmid clone without digestion of the insert for rapid restriction mapping. Cosmid clones were completely digested with *Not* I and then partially digested with the desired restriction enzyme. Digested DNAs were then separated by electrophoresis through a 0.2% agarose gel, denatured, neutralized, and transferred to nitrocellulose membranes. Following fixation of the DNA by baking at 80°C for 60 min, filters were probed with either a 0.4-kilobase (kb) *Not* I/*Hind*III vector fragment or a 1.0-kb *Eco*RI/*Not* I vector fragment that had been labeled with 32 P. After autoradiography, restriction maps were calculated from the indirect end-labeled partial digest patterns.

In addition to its convenient *Not* I site, this vector has two unique features: (i) a relatively small size, enabling cloning of 35- to 48-kb inserts; (ii) HSV-TK and pBR322 P1 promoters, facilitating *Escherichia coli* or eukaryotic selection with kanamycin or G418.

Construction of the Cosmid Library. The cosmid library was constructed from partially digested human placental DNA and cosmid vector pMFG1 according to the techniques described by Grosveld *et al.* (28) and Ish-Horowitz and Burke (29). Equal amounts of pMFG1 were digested separately with *Eco*RI and *Sal* I and were extracted with phenol. The digests were then treated with bacterial alkaline phosphatase at 65°C for 1 hr. The bacterial alkaline phosphatase treatment was followed by a *Bam*HI digestion. The resulting *Eco*RI–*Bam*HI or *Sal* I–*Bam*HI oligonucleotides were removed from the vector preparations by isopropyl alcohol precipitation. A 10-fold molar excess of equimolar amounts of the vector arms (*Eco*RI–*Bam*HI arm and a *Sal* I–*Bam*HI arm) was then ligated with partially digested *Sau*3A fragments of human placental DNA 35–50 kb in length that had been isolated by size selection on a 10–40% sucrose gradient (30). Ligation with T4 ligase at 220 units/ml was in 66 mM Tris-HCl, pH 7.6/6.6 mM $MgCl_2$ /10 mM dithiothreitol/0.4 mM ATP at a DNA concentration of 300 μ g/ml for 15 hr at

16°C. *In vitro* packaging was carried out by the procedures of Hohn and Murray (31) using 4 μ l of the ligation mixture with 10 μ l of freeze-thaw lysate and 15 μ l of sonic extract. Transduction was performed by Grosveld *et al.* (28) using HB101 cells as recipients.

With 1.5 μ g of size-selected human placental DNA fragments, we constructed a library containing about 4×10^5 colonies distributed on 30 nitrocellulose filters.

Oligonucleotide Synthesis. Oligonucleotides were synthesized with the phosphoramidite method (32) on an Applied Biosystems synthesizer model 381A. The oligonucleotides 5' AAACCATCCGTTTTTGTTCATG 3', 5' ATTCTCTGGCC-TGGGACTTTG 3', and 5' TGGTTCCACAGTCAC 3' were used for probes and sequencing primers of C_δ , C_δ untranslated (UT) region, and J_δ .

Sequencing. Sequencing was carried out by the dideoxy chain-termination method (33) on fragments of genomic DNA that had been subcloned into M13.

RESULTS

Molecular Cloning of the Entire J_α Region. To define the structure of the TCR J_α region, we employed the technique of "chromosome walking" utilizing cosmid libraries prepared from genomic DNAs. Initially, clones corresponding to the C_α region were isolated with an α -chain cDNA isolated from the Jurkat T-cell line as described (7). These clones were characterized by restriction enzyme digestion and Southern blotting. Thereafter, new probes were generated from overlapping cosmid clones for continued walking. These probes will be described in detail elsewhere. The results of this iterative cloning operation are illustrated in Fig. 1. Over 115 kb of the TCR α locus extending 5' of the C_α region is illustrated. A panel of probes generated from this region detects rearrangement in most T-cell lines we have tested; furthermore, the 5'-most probe of our panel, pMI19 (Fig. 2), is deleted in α/β -expressing lines. For example, the mature T-cell leukemia cell line Buc7 shows no hybridization with this probe. On the contrary, the γ/δ -expressing cell line Peer shows one 12-kb rearranged fragment (Fig. 3) and lacks the 6.8-kb *Hind*III germ-line fragments that correspond to the 5' end of pMI19 (Figs. 2 and 3). This indicates one chromosome 14 allele is deleted and the other is rearranged in this region. It is thus likely that the 5' side of this probe corresponds to the J_δ locus.

Characterization of TCR δ Clones. Having cloned a substantial portion of the J_α region, we next sought to determine whether this region also encompasses the δ -chain gene. To this end, we synthesized oligonucleotide probes corresponding to the sequence of the human C_δ and J_δ segments, as published by Hata and colleagues (14). Southern blotting experiments employing these oligonucleotide probes demon-

*The sequences reported here are being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03784 for C_δ and accession no. J03785 for J_δ).

FIG. 2. Restriction map of the human δ -chain locus. Restriction sites are given for *Bam*HI (B), *Eco*RI (E), *Sst* I (S), *Hind*III (H), and *Xba* I (X). The regions subcloned into M13 are shown by a, b, c, and d. Oligonucleotides corresponding to J_δ , 5' C_δ , and the C_δ 3' UT region were subsequently hybridized to the regions a, b, and d. Arrows illustrate our sequence strategy. pMI19 indicates the location of the probe used to detect rearrangements associated with J_δ . Open boxes show the location of J_δ and C_δ . Polymorphic restriction sites are indicated by an *.

strated hybridization of the 5' C_δ probe to a region ≈ 84 kb 5' of C_α , of the C_δ 3' UT probe to 81 kb 5' of C_α , and of the J_δ probe to a region ≈ 95 kb 5' of C_α (Fig. 2).

One potential explanation for our observations of oligonucleotide hybridization to genomic J_α clones is cross-hybridization between related immunoglobulin superfamily sequences. Thus, we undertook to obtain the nucleotide sequences of the relevant portions of these clones to establish their identity with the published δ transcript sequences. Our sequencing strategy is illustrated in Fig. 2.

The identity of our genomic C_δ sequences with the published cDNA sequences is shown in Fig. 4. Immediately 5' of the coding sequence, consensus splice acceptor signals are located. These are the characteristics expected of the gene coding for the δ -chain transcript previously described.

Complete identity between our J_δ clones and the cDNA J_δ segment was also observed, as illustrated in Fig. 5. Also included for comparison is the murine J_δ segment. Consensus heptamer and nonamer recombination signals are apposed immediately upstream of J_δ . Presumably these facilitate V-J joining, as do other TCR and immunoglobulin recombination signals. At the 3' end of the J_δ segment, consensus splice donor signals are found. We conclude that we have isolated a genomic counterpart of the J_δ segment in its entirety. Thus, as has been suggested in the murine genome, the δ -chain gene is embedded in the 5' portion of the J_α region.

Involvement of the TCR δ -Chain Gene in the T-Cell t(8;14)(q24;q11) Translocation. Two converging lines of evidence led us to suspect that the TCR δ chain might be

implicated in the genesis of T-cell-specific translocations. (i) Each of the immunoglobulin and TCR genes previously enumerated has been demonstrated to be involved in translocations at the molecular (the immunoglobulin and TCR α and β) or cytogenetic (for TCR γ) level. (ii) Our somatic cell hybrid analyses had localized the 14q11 break points of several tumors to the region between C_α and V_α . We tested the possibility of TCR δ involvement in several tumors carrying translocations that had been previously shown to carry a truncated TCR α locus.

One of these tumors was derived from a young patient (DeF) with an acute lymphoblastic leukemia of a T-cell phenotype carrying a t(8;14) translocation. A previous study from our laboratory characterized somatic cell hybrids between leukemia cells from this patient and rodent fusion partners and demonstrated deregulation of the *c-myc* gene consequent to the translocation (24). It was also shown that although the 14q- chromosome retained V_α sequences, the C_α region was translocated to the 8q+ chromosome (24). A summary of the results is shown in Table 1. Fig. 6 illustrates the results obtained from a Southern blot experiment utilizing DNA from this patient's leukemia and from hybrid cells digested with *Bam*HI and probed with the TCR δ probe pMI19 (Fig. 2). The patient DNA sample demonstrates a single rearranged band when compared with the placental DNA. Thus, one chromosome 14 δ allele is deleted, and the other is rearranged. Hybrid 563 BA5-BC10 is a somatic cell hybrid between DeF tumor cells and the mouse T-cell line BW5147. It contains only the 8q+ chromosome but neither the normal 14 nor the 14q-. It also retains C_α but not V_α . The filter illustrates that this hybrid also retains the rearranged TCR δ band. We conclude that the rearrangement occurred on the *Bam*HI fragment detected by pMI19 5' of C_δ and that the probe is translocated to the 8q+. It is highly unlikely that the rearrangement detected by the δ probe is unrelated to the translocation, since this would necessitate a translocation-associated rearrangement situated closely nearby and, in fact, between J_δ and V_α . In sum, these findings strongly suggest that the translocation occurred at J_δ and provide a strategy for the molecular characterization of the TCR δ -associated translocation.

It is probable that this case is not an isolated one. We have examined one case of T-cell leukemia/lymphoma carrying the t(10;14)(q24;q11) translocation and have found that a similar TCR δ rearrangement is present (data not shown). The phenotype of this case is that of an immature, TdT⁺ (terminal deoxynucleotidyltransferase-positive) and CALLA⁺ T cell

FIG. 3. Chain rearrangement in a T δ -expressing cell line. A Southern blot of *Hind*III-digested DNA from human placenta (PL), the mature T-cell leukemia cell line Buc7, and the T δ -expressing cell line Peer was probed with the pMI19 probe. Fragment size is indicated in kb.

Human cDNA AAGAAGTCAGCCTCATACCAAAC
 Human genomic CTCACCTAATAAGTTGATTATATTTGCAGGAAGTCAGCCTCATACCAAAC

Human cDNA CATCCGTTTTTGTGTCATGAAAAATGGAACAAATGTCGCTTGTCTGGTGAAGGAATTC
 Human genomic CATCCGTTTTTGTGTCATGAAAAATGGAACAAATGTCGCTTGTCTGGTGAAGGAATTC

FIG. 4. Nucleotide sequence of the 5' region of C_δ . The RNA splice acceptor site is underlined.

Human cDNA CCGATAAAC
 Human genomic AGGTAGGTGGGAGCAGCGCTGAAGCTCCTCAAGTGGTGTACACCGATAAAC
 Mouse genomic ACAGCTGCTGAAGGCTCAGTGGTGTCTACCGACAAAC

Human cDNA TCATCTTTGGAAAAGGAACCCGTGTGACTGTGGAACCAA
 Human genomic TCATCTTTGGAAAAGGAACCCGTGTGACTGTGGAACCAAGTAACTAATTTATCTGA
 Mouse genomic TCGTCTTTGGACAAGGAACCCAAGTGTGGAACCAAGTAACTAATTTATCTGA

FIG. 5. Nucleotide sequence of J_δ . Heptamer and nonamer recombination signals are indicated by open boxes. The RNA splice donor sites are underlined.

(26). As the γ/δ receptor is present on T cells early during the course of ontogeny, it is likely that the translocations involving TCR δ will be common in immature leukemias and lymphomas.

DISCUSSION

The data presented here enable us to draw several significant conclusions. (i) We have isolated genomic clones that encode the human TCR δ -chain J and C segments previously described at the mRNA transcript (cDNA) level. (ii) These clones are situated between 80 and 100 kb 5' of the TCR α -chain C region segment, and we have cloned and characterized the entire intervening region, which probably encompasses all of the J_α segments. (iii) The TCR δ -chain gene is rearranged in T-cell leukemias and lymphomas carrying the t(8;14) and t(10;14) translocations, and it is likely that this gene is the site of chromosome translocation in these malignancies.

Several lines of evidence confirm our isolation of the TCR δ -chain gene. The restriction map of the region surrounding our putative TCR δ clones corresponds to that predicted from published genomic Southern blots probed with the previously isolated cDNA clones (14). Moreover, oligonucleotide probes synthesized from the published cDNA sequences hybridized to three fragments of our clones, 81, 84, and 95 kb from C_α . Sequencing of the hybridizing fragments confirmed sequence identity between the probe and genomic fragments. Furthermore, a Southern blot of DNA from the γ/δ -expressing cell line Peer probed with pMI19 demonstrated the rearrangement of the putative δ -chain gene in this line, whereas DNAs from α/β -expressing cell lines demonstrated deletion of this region. These data, taken together, prove the

identity of the region surrounding pMI19 and the TCR δ -chain locus.

The existence of a gene, located 5' of the murine C_α gene in the J_α region and denoted TCR $_{\delta}$, has been suggested by Chien and colleagues (12). This gene has the functional properties alluded to above: rearrangement in immature T cells and deletion in α/β -expressing lines. There are several other molecular features of the human TCR δ described here and the murine TCR $_{\delta}$. C_δ and human C_α share 79% homology, and J_δ and our J_α share 87% homology, including similar heptamer-nonamer recombination and splicing signals. Also, the distance separating the human C_δ and C_α (81 kb) is similar to that separating the murine C_δ and C_α (70 kb). In both cases, the C_δ and C_α are 5' to the respective C_α segments.

Presently, the relationship between γ/δ and α/β expression is poorly understood—that is, it is not known whether both receptors are expressed on T cells of the same lineage. There is precedence for expression of related molecules in succession, as observed in B cells undergoing isotype switching. If successive expression does obtain, then the physical relationship of the δ and α loci has interesting functional implications. The placement of J_δ - C_δ 5' of J_α - C_α fixes the order in which expression may take place: δ must precede α . Furthermore, it appears that productive expression must then result in δ deletion. This deletion may result from the recombination of rearranged V segments from the J_δ segment to J_α segments (12) or the deletion of an entire rearranged V- J_δ - C_δ unit. The details of this genetic apparatus remain to be elucidated.

Until now, every member of the immunoglobulin supergene family that has been shown to require physiologic

Table 1. Characterization of somatic cell hybrids between DeF T-cell acute lymphoblastic leukemia and BW5147

	MYC	IgH	C_α	V_α	NP	8	8q+	14	14q-	MYC RNA
DeF	+	+	+	+	+	+	+	+	+	++
563BA5BC10	+	+	+	-	-	-	+	-	-	+
563BC5	+	+	+	+	+	+	-	+	+	-
563BD3	+	-	-	+	+	+	-	-	+	-
BW5147	-	-	-	-	-	-	-	-	-	-

+, Presence of the indicated probe or chromosome in the hybrid. NP, nucleotide phosphorylase, which maps to 14 centromere (24).

FIG. 6. δ -Chain rearrangement in association with a t(8;14)(q24;q11) translocation. A Southern blot of *Bam*HI-digested DNA from human placenta, the mouse parental cell line BW5147, DeF tumor cells carrying a t(8;14) translocation, and hybrid cell line between BW5147 and DeF carrying the 8q+ chromosome but neither the normal 14 nor the 14q- chromosome was probed with the pMI19 probe. Fragment size is indicated in kb.

rearrangement at the DNA level for expression has also been shown to be involved in chromosome translocations in lymphoid malignancies (39). We have demonstrated here the similar involvement of the TCR δ locus in T-cell chromosome translocations. Our Southern blot and somatic cell hybrid data suggest that the t(8;14) translocation in an acute T-lymphoblastic leukemia resulted from a rearrangement between chromosome 8 3' of *MYC* (24) and chromosome 14 at *J δ* . Additionally, the δ region appears to be involved in other chromosomal translocations. Significantly, the T cells demonstrating such involvement are of immature phenotype. This is in keeping with the expression of TCR δ in early T cells. There is ample precedent for a correlation between the mechanism of chromosome translocation and the differentiation state of the transformed cell. For instance, pre-B leukemia and lymphoma generally involve a mistake in an early step of immunoglobulin gene rearrangement, *V-D-J* joining (40, 41). Thus, we would expect to find TCR δ involvement in various T-cell leukemias and lymphomas of immature phenotypes, whereas we expect TCR α - and β -chain involvement to occur in more mature T-cell malignancies.

In the case of the t(8;14) translocation characterized here, our laboratory has already demonstrated deregulation of *MYC* expression (24). Thus, it is clear that translocation into the *C δ* locus engenders *MYC* deregulation. It is not clear whether this is due to operation of the *C α* enhancer (42) or other as yet uncharacterized elements.

Finally, the probes described here may have clinical and diagnostic significance. Immunoglobulin and TCR probes are routinely employed in many hospital laboratories to determine the extent of clonality of lymphoid lesions of questionable malignancy. However, only T-cell neoplasms with rearranged β genes are usually evaluable. The probes described here allow the investigation of the clonality of T-cell neoplasms with earlier, δ -expressing phenotypes. In addition, the presence of a large panel of TCR α probes should provide a more complete understanding of the patterns of rearrangements observed at this large locus.

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