During the development of functional T lymphocytes, a variety of genes involved in antigen recognition undergo somatic rearrangement. These include the alpha, beta, and gamma chain genes. Recently a fourth rearranging gene, the delta chain gene, embedded in the alpha chain locus, has been described. We have determined the structure of the delta chain locus in relation to the alpha chain locus.25,26 Delta mRNA is expressed in human normal thymocytes and phytohemagglutinin (PHA) stimulated T cells.14,16,24 In leukemic T-cell lines that express delta chain mRNA, the delta chain genes are rearranged.14,16,19,21,24 To determine the structure of the delta chain locus in relationship to the alpha, beta, and gamma locus in T-cell acute lymphoblastic leukemia (T-ALL) we have studied DNA from 15 patients with this diagnosis. T-ALL has proven to be a useful model of normal T-cell development. As such our findings indicate that delta chain gene rearrangement occurs early during T-cell differentiation. Our findings of a restricted pattern of rearrangements support the suggestion of a limited repertoire for the delta chain gene.

**MATERIALS AND METHODS**

**Patient samples.** Bone marrow or peripheral blood was obtained from pediatric patients with a diagnosis of T-ALL following informed consent. Mononuclear cells were isolated by ficoll-hypaque centrifugation. At this point the samples contained >80% blast cells. These cells were used for phenotype and DNA analysis.

**Phenotype analysis.** The diagnosis of T-ALL was based on the expression of the T-cell associated antigens CD7 (WT1), CD5 (T1), CD2 (T11), CD1 (T6), CD3 (T3), and the focal positivity of acid phosphatase.22 Nuclear TdT was determined by fixing cytospin-cultured cells in cold methanol for 30 minutes, then transferring the slides to cold PBS for 30 minutes; cells were stained with a rabbit anti-TdT antibody and a positive reaction detected with FITC-F(ab')2 goat anti-rabbit IgG (both from Bethesda Research Laboratories, Gaithersburg, MD).

**DNA analysis.** High molecular weight DNA was extracted from the mononuclear cells, digested to completion with BamHI, EcoRI, or HindIII (BRL, Gaithersburg, MD), size fractionated by electrophoresis through a 0.8% agarose gel and transferred to nylon membranes (GeneScreen Plus, New England Nuclear, Boston). Prehybridization and hybridization was performed for 16 to 24 hours at 42°C in a solution containing 1 mol/L NaCl, 1% SDS, 10% dextran sulfate, and 50% deionized formamide; hybridization solution also contained 100 µg/mL salmon sperm DNA and 1 x 106 cpm/mL of labeled probe. Membranes were washed for 30 minutes successively in 2 x SSC/1% SDS at 65°C and 0.1% SSC at room temperature and exposed to XAR-5 film (Eastman Kodak, Rochester, NY) with an intensifying screen at −70°C for 24 hours.

**DNA probes.** The probes used in this study were a 3-kb EcoRI-HindIII fragment that contains the Ig heavy chain J region (provided by Dr P. Leder, Boston), a 720-bp EcoRI fragment that hybridizes to TcR β1 and C beta 2 (provided by Dr T. Mak, Toronto) and a 0.7-kb HindIII-EcoRI fragment that contains the J gamma 1 region (provided by Dr T. Rabbitts, London). The delta chain probes used in this study were MH6, a 5-kb EcoRI fragment that lies between J delta 1 and J delta 2, and C delta a 1.3-kb EcoRI cDNA fragment that contains the delta constant coding region. J delta 1 and 5' untranslated sequence lying immediately 5' of J delta...
1. Probes were labeled to a high specific activity with $^{32}$P using the random primer method.

RESULTS

Phenotypic characterization. The phenotype of the 15 patients is summarized in Table 1. According to the development scheme proposed by Reinherz, 11 of 15 patients had the phenotype of precursor cortical thymocytes group I (CD7+, CD1-, CD3-, TdT+) and four of 15 had the phenotype of immature cortical thymocytes group II (CD7+, CD1+, CD3-, TdT+). The cells of one patient (no. 9) showed only reactivity with CD7.

Ig gene rearrangement. Ig heavy chain gene rearrangement was determined by probing EcoRI DNA with the Ig J region probe. Three patients (nos. 1, 7, 8) showed a single rearranged band; the size of the rearranged bands suggest that they are the product of D-J rearrangement.

TcR beta and gamma rearrangement. TcR beta rearrangement was determined by probing BamHI or EcoRI cut DNA with a cDNA probe containing the TcR beta constant region. Rearrangements of TcR beta were found in 12 of the 15 cases and involved both C beta 1 (ten alleles) and C beta 2 (14 alleles). Rearrangement of the gamma chain was determined by probing BamHI cut DNA with the J gamma probe. The gamma chain was rearranged in 14 of 15 cases. In all but one case (no. 9), the rearrangements were biallelic. This case was CD7+, CD2− and lacked beta chain rearrangement suggesting that it represents a very early stage in T-cell development.

In this group of T-ALL, it is interesting to note that in cases in which the Ig genes were rearranged, only a single allele was involved, while in cases in which the beta chain was rearranged, both alleles were affected. The use of gene rearrangement to determine cell lineage is somewhat weakened by the finding of both Ig and TcR rearrangements in a clonal population of cells. This ambiguity, however, may be resolved if biallelic and uniallelic rearrangements are distinguished.

TcR delta rearrangement. The D and J segments are located at least 11 kb 5′ of the delta constant gene. At least two D segments and two J segments have been identified in the mouse13 and more recently in humans.23 We used two probes to determine the structure of the delta locus (Fig 1). The C delta probe that contains the constant region, J delta 1 and 5′ untranslated sequences immediately 5′ of J delta 1, detects in EcoRI digested DNA a 6.4 kb band that contains the 5′ untranslated region, and 3 bands of 4.4, 1.7, and 1.6 kb that contain constant region sequences.20 MH6 detects a 21 kb BamHI fragment that contains D delta 2, J delta 1 and J delta 2; HindIII cut DNA probed with MH6 produces a 6 kb fragment that contains D delta 2 and J delta 1, and a 3 kb fragment. The 3 kb fragment contains J delta 2. However, the MH6 hybridizing sequences are 5′ of J delta 2 and so do not detect rearrangements to this J.

The pattern of delta chain gene rearrangement is summarized in Table 1 and representative Southern blots are shown in Figs 1 and 2. In three cases (nos. 12, 13, 14), C delta constant hybridizing sequences were absent, indicating biallelic rearrangement to J alpha sequences. Rearrangements of the delta locus were detected in 11 of the 15 patients.

With the C delta probe, there was deletion of the 6.4-kb EcoRI band in the 11 cases that showed involvement of the delta locus.20 In two cases (nos. 5 and 9), in association with the deletion was the presence of a new band. The rearrangement in these cases involves sequences 5′ of J delta 1 (data not shown).

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>TdT</th>
<th>CD7 (Tomp1)</th>
<th>CD1</th>
<th>CD3</th>
<th>CD2</th>
<th>CD5</th>
<th>Surface Markers</th>
<th>Immunoglobulin Gene</th>
<th>TcR Receptor Genes</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: R, rearranged; G, germline; D, deleted; ND, not determined; d, deletion of the 6.4 kb band.

*Patterns of bands corresponding to $\delta$21 locus.
Fig 1. Rearrangement patterns of TcR delta chain gene (MH6 probe) in patients with T-ALL. Lane numbers refer to individual patients (see Table 1). Lane G shows the germline control. DNAs were digested with HindIII (Panels A and B) or with BamHI (Panel C). As shown in the map in panel C, in germline DNA, the MH6 probe detects, after HindIII digestion, a 6 kb and a 3 kb band (containing D delta 2, J delta 1 and J delta 2, respectively) and, after BamHI digestion, a 21 kb band (containing D delta 2, J delta 1, and J delta 2). Faint bands, which can be noted in patient nos. 1, 12, 13, 14, presumably correspond to DNA from contaminating non-leukemic cells.

DNA cut with HindIII and probed with MH6 revealed rearranged bands in ten of the 15 cases (Fig 1 A and B). In the ten cases studied, there were 14 rearranged bands. These 14 bands fell into four different sizes of 13 kb, 11 kb, 8.5 kb, and 5 kb.

DNA cut with BamHI and probed with MH6 demonstrated rearranged bands in the same ten cases (Fig 1 C). With this combination of probe and restriction enzyme, 17 rearranged bands were observed. Although it is difficult to accurately determine the size of large fragments over 20 kb, it appears that the 17 rearranged bands fall into seven size classes: 30 kb, 23 kb, 18 kb, 17 kb, 16 kb, 15 kb, and 12 kb.

Four patients had uniallelic (nos. 1, 3, 4) or biallelic (no. 11) deletions of MH6 recognizing sequences both in HindIII and BamHI digests, suggesting either a V-J alpha or a V-J delta rearrangement that involved the J delta 2 segment. In order to discriminate between the two possibilities, DNA analysis with the C delta probe was performed (Fig 2). As shown in Fig 2, in all four patients constant region sequences were present in association with the deletion of the 6.4-kb EcoRI band.

In four cases (nos. 5, 7, 8, 9) rearrangements of both alleles in BamHI digests, but only of one allele in the HindIII digests were observed.
Of the 22 alleles that could be rearranged, all seemed to be rearranged in the delta locus. The results of the analysis with the C delta probe indicated that rearrangements of both alpha and delta were not seen in this patient population. Thirteen of the 22 rearrangements involved J delta 1, five of the rearrangements presumably involved J delta 2 (nos. 1, 3, 4, 11), and four rearrangements occurred 5' of the J delta 1 (nos. 5, 7, 8, 9).

Both the HindIII and BamHI patterns of rearrangement suggest that there are only a limited number of possible rearrangements. For example, on HindIII digestion, patient nos. 1, 2, 3, 4, 6, 7, and 8 have similar 13 kb bands. On a BamHI digest, patient nos. 1, 2, 3, 4, 6, and 8 had similar 30 kb bands. This suggests that in patient nos. 1, 2, 3, 4, 6, and 8, the MH6 probe is detecting the same rearrangement. Using a similar argument, the 16-kb band detected following BamHI digestion of DNA from patient nos. 6 and 9 represents the same rearrangement indicated by the 5-kb band on the HindIII digestion.

In one patient (no. 15) no rearrangements were detected with any of the probes.

**DISCUSSION**

During the development of functional T cell, genes responsible for antigen recognition undergo somatic rearrangement, which ultimately results in the expression of an antigen receptor on the surface of the T cell. In the majority of T cells, the receptor contains the proteins encoded for by the alpha and beta chains of the T-cell antigen receptor genes. One percent to 10% of mature T cells express on their surface an alternate gamma-delta T-cell antigen receptor. During thymic development, cells bearing the gamma-delta receptor appear before those bearing the alpha-beta heterodimer.

In order to determine the stage at which the delta chain undergoes rearrangement in relationship to the alpha, beta, and gamma chain genes, and to determine the variability in delta chain rearrangements, we analyzed the structure of the delta locus in 15 cases of T-ALL representing early stages of T-cell differentiation.

The Ig, beta, gamma, and delta genes were not rearranged in one case (no. 15). The cells of this patient expressed CD7 and CD5 but not markers of B cell (CD19 and CD24) or myeloid (MY7 and MY9) differentiation. This phenotype may represent a very early stage of lymphoid differentiation or a yet unrecognized cell type. Recently, Weiss et al reported a number of cases with similar phenotype and genotype. A third possibility is that the expression of cell surface proteins is aberrant, reflecting the malignant nature of the leukemic cells, and does not correlate with any normal cell type.

In the present study, 11 of the 15 patients had rearrangement of the delta locus. In the majority of the cases the rearrangement involved J delta 1. This is similar to findings in the mouse. In the four cases (nos. 1, 3, 4, 11) where deletions of the MH6 hybridizing sequences were observed both in HindIII and BamHI digests, a rearrangement within the delta locus involving the J delta 2 segment possibly occurred. This interpretation is supported by the finding that the delta constant region sequences were detected when DNA was hybridized to the C delta probe, as shown in Fig 2.

In four patients (nos. 5, 7, 8, 9), analysis of BamHI digests revealed a biallelic rearrangement of the delta locus, whereas upon HindIII digestion one of the two alleles apparently retained the germline configuration. Although not formally proven, the rearranged bands in the HindIII digests may have been of the same size as the germline bands. For instance, it has been shown that rearrangement including V
delta 2 and J delta 1 results in a HindIII rearrangement band of the same size as the germline one (Migone N, unpublished data, November 1988).

We have recognized in this group of patients a limited number of rearrangements. Of the 20 rearrangements involving the delta locus, 7 appear to be identical. Three other patterns of rearrangement that involved two or more alleles were also recognized.

Though we have likely not identified all the possible forms of rearrangement, our findings suggest that the pattern of rearrangement is restricted in a manner similar to that found in the gamma locus. It is intriguing that both chains of the gamma-delta heterodimer have restricted heterogeneity; the function of this receptor is still unknown.

In the present series of 15 T-ALL representative of early stages of human T-cell development, we found two patients with rearranged delta and gamma genes and the beta locus in a germline configuration. In 12 patients the gamma, delta-alpha, and beta loci had undergone rearrangement. These observations are consistent with the view that the delta gene rearrangement is an early event in T-cell differentiation occurring contemporarily to gamma gene rearrangement.

REFERENCES

28. Feinberg AP, Vogelstein B, A technique for radiolabelling
DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6, 1983


T-cell receptor delta gene rearrangement in childhood T-cell acute lymphoblastic leukemia

A Biondi, E Champagne, V Rossi, G Giudici, A Cantu-Rajnoldi, G Masera, A Mantovani, TW Mak and MD Minden