Rearrangement and Expression of T Cell Antigen Receptor Genes in B Cell Chronic Lymphocytic Leukemia


Fifty-nine patients with B cell chronic lymphocytic leukemia (B-CLL) were screened for clonal rearrangement of T cell receptor (TCR) β and γ chain genes. Four were found with rearranged TCRβ genes, but none had detectable rearrangement of TCRγ genes. One typical patient with B-CLL had a TCRβ gene structure consistent with a variable-diversity-joining rearrangement into the Cβ2 gene on one allele. An apparently identical rearrangement pattern was seen in a second patient, which suggested that there may be a restriction on the repertoire of possible TCRβ gene recombinations in mature B cells. Two further patients had a simple deletion of sequences, consistent with a diversity-joining rearrangement into Cβ2 on one allele. All four patients had rearrangements of immuno-globulin heavy- and light-chain genes typical of mature B cell malignancies. However, on review of clinical, morphological, and immunophenotype data, two had features consistent with B cell prolymphocytic leukemia or B lymphoma, and a third had progressed to a prolymphocytic transformation. Low-level expression of a predominantly 1.0- to 1.2-kilobase germ line TCRβ gene transcript was detected in several B-CLLs and at a comparable level in the four with rearranged TCRβ genes. This, together with the low frequency of TCR gene rearrangement, suggests that most B-CLL cases arise at a developmental stage when factors required for TCR gene activity are not operative.

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ized peripheral blood was used for analysis of DNA (and subsequently RNA). Additional blood samples in those patients identified as having TCR gene rearrangements were immunophenotyped at the Royal Free Hospital.2223

Analysis of TCR gene structure. High-molecular weight DNA was prepared from total peripheral blood samples by standard procedures of proteinase K digestion and phenol extraction and then further purified by extraction with methoxyethanol in 0.5 mol/L K phosphate, pH 6.8. After overnight dialysis against 10 mmol/L Tris, pH 7.5, and 0.5 mmol/L EDTA, DNA was sequential ethanol, then washed in 70% ethanol, and finally dried and redissolved in 1 mmol/L Tris, pH 7.5, and 0.05 mmol/L EDTA. DNA samples (5 to 8 μg) were digested with an excess of restriction enzymes under conditions recommended by the suppliers and, after the addition of sodium dodecyl sulfate to 0.5%, were electrophoresed on agarose gels of concentrations 0.5% to 1.0%. In all experiments a control DNA from normal peripheral blood white cells (pooled from five individuals) was used to define germline configuration of TCR genes. Denaturation and gel neutralization was performed as described elsewhere25 and the DNA blotted onto nitrocellulose filters using 20× SSC (3 mol/L sodium chloride, 300 mmol/L sodium citrate). Five different gene probes were used in these studies. Three of these were Ig gene probes, one for the heavy-chain joining region (JH)24 and one for each of the light-chain constant-region gene classes λ (CA)27 and κ (Ce). Figure 1 shows the structural relationship of the TCRγ and γ gene probes to their respective loci. The βT probe was synthesized from a subclone representing the constant region of the cDNA clone YT35,20 whereas a TCRγ probe was made from a genomic clone encompassing the joining region of the TCRγ gene locus.4 Purified insert fragments were 32P labeled to a specific activity of approximately 106 cpm/μg by using a modification of the oligopriming method32 in a reaction buffer comprising 10 mmol/L Tris, 50 mmol/L sodium chloride, 10 mmol/L magnesium chloride, 2 mmol/L β-mercaptoethanol, and 100 μg/mL bovine serum albumin. Hybridization of nitrocellulose filters with probe (0.5 to 2.0× 106 cpm/mL) was performed overnight at 65°C in a Denhardt’s reagents supplemented with carrier nucleic acid and 9% (w/v) dextran sulphate. Filters were washed (four times for 15 minutes) in hybridization buffer without dextran sulphate and then in 0.1× SSC at 65°C (twice for 15 minutes), dried, and autoradiographed at −70°C for several days by using a Cronex Lightning Plus intensifying screen (DuPont, Wilmington, DE). In several experiments filters were reprobed after a period of time when the initial signal was lost through radioactive decay.

Analysis of TCR gene expression. Total mononuclear cells were prepared from fresh peripheral blood by Ficoll-Hypaque centrifugation (Nyegaard, Oslo) and cellular RNA extracted by the guanidium isothiocyanate–cesium chloride method.33 RNA was redissolved in water at approximately 100 to 500 μg/mL (determined by ethidium bromide fluorescence) and analyzed by Northern blotting, essentially as described previously, after electrophoresis on 1% agarose formamide gels.33 P-labeled probes were synthesized by oligopriming as described earlier by using a βT template (Fig 1A) and an additional IgH clone encompassing a 1.3-kilobase (kb) fragment containing the Cγ heavy-chain region.34 Hybridization and posthybridization washing and autoradiography were performed as described previously.35

RESULTS

Rearrangement of βT genes. By using the βT probe depicted in Fig 1A, three different enzymes, EcoRI, HindIII, and BamHI were used to screen all B-CLL DNA samples for rearrangement of βT genes. In all but four of the 59 cases no evidence was found, either from the appearance of additional bands or from reduction in intensity of hybridization signal to germline fragments, for any structural alteration to the TCRβ genes. Figure 2 shows the results of Southern blot analysis using the βT probe for the four patients showing TCR genes rearrangements, and Table 1 summarizes the predicted structure of rearranged genes in these cases.

Patient 16. After digestion of patient 16 DNA with EcoRI and hybridization with βT probe, a reduction in the intensity of the 10.5-kb germline fragment encompassing Cβ1 relative to that of the 3.7-kb fragment containing Cβ2 was observed (Fig 2A). A digest of normal peripheral blood DNA (containing predominantly polyclonally rearranged TCRβ genes) and that from patient no. 25 (germline βT genes), is shown in parallel for comparison. Because there is still a germline 10.5-kb fragment present in patient no. 16, we can infer that only one Cβ1 allele has been deleted, with a possible rearrangement into a Cβ2 gene (Fig 1A). When analyzed with HindIII (Fig 2, panel B), an additional rearranged fragment slightly larger than the 8.2-kb germline fragment containing Cβ2 was seen, which confirmed that the deletion of upstream Cβ1 sequences is accompanied by a change in sequence extending beyond the 5′ HindIII site bordering the 8.2-kb Cβ2 fragment. Because this rearranged fragment is larger than the germline Cβ2 fragment, it follows that a simple deletion of sequences alone, as often occurs in a simple D-J rearrangement, is not involved. To further investigate the structural alteration 5′ to the Cβ2 gene, an additional enzyme, XbaI, was used in combination with HindIII. In normal DNA the XbaI site 5′ to the Cβ2 gene reduces the size of the 8.2-kb HindIII fragment and can thus be used to delineate the location of the presumptive breakpoint 5′ to the Cβ2 gene in patient 16 (see Fig 1A and control digest, Fig 2, panel C). A rearranged XbaI/HindIII fragment larger than the 6.0-kb germline fragment was again seen, thereby suggesting that the 5′ breakpoint maps close to the diversity region on Cβ2. We also note that the increased size of rearranged fragments, compared with the germline, are different in the HindIII and XbaI × HindIII analysis. This is not obviously compatible with a rare genetic

Fig 1. Restriction maps of TCRβ (a) and TCRγ (b) C-J region loci redrawn from Toyonaga et al,32 Greenberg et al,12 and Lefranc and Rabbits,5 respectively. Not all known joining regions are shown in a. R. EcoRI; H. HindIII; B. BamHI; X. XbaI. Not all restriction sites are shown in B and only relevant XbaI sites are shown in A. The HindIII site in brackets in B is polymorphic.5
polymorphism or somatic variant Cβ2 locus such as that arising from an insertion element.

The enzyme BamHI generates a 22.6-kb germline fragment encompassing both Cβ1 and Cβ2 genes (Fig 1A). Analysis of patient no. 16 DNA with this enzyme gave a single major rearranged fragment in addition to a germline-sized fragment as shown in Fig 2, panel E. Owing to the large size of fragments and the nonspecific degradation of this DNA sample (evidenced by the high background smearing), the 22.6-kb germline fragment is considerably underrepresented in this digest. Also, we note the presence of several additional substoichiometric diffuse bands against the background smear in this digest (also visible in the XbaI × HindIII digest in Fig 2C). The origin of these additional hybridizing fragments is not clear. However, the predominant hybridization pattern obtained with different enzymes would appear to be internally consistent and supports a structure in which one Cβ1 gene is deleted and the Cβ2 region on the same allele has undergone recombination with noncontiguous sequences. This pattern of TCRβ gene struc-
T RECEPTOR GENE REARRANGEMENT IN B-CLL

Table 1. Rearrangement of Ig and TCR Genes in B-CLL

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<th>Igγ</th>
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<th>Cγ1</th>
<th>Cγ2</th>
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<td>GD</td>
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<td>GR</td>
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<td>GG</td>
<td>GR</td>
<td>RG</td>
<td>(D-J)</td>
<td>GG</td>
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</tbody>
</table>

Abbreviations: G, germ line; D, deletion; R, rearranged.

*βT rearrangements are identical in these patients.

Discussion:

- **Fig 2**: Shows the results of analysis of patient no. 23 DNA for βT gene rearrangement. All four enzymes, EcoRI, HindIII, XbaI, and BamHI, showed evidence for clonal rearrangement of βT genes. Somewhat surprisingly, the sizes of rearranged fragments detected were identical to those in patient 16 (Fig 2), which suggests that βT genes had undergone an identical V-D-J-like rearrangement (Table 1). Reprobing of the Southern blots from patients no. 16 and 23 with an Ig probe confirmed that these were from different CLL patients (data not shown).

- **Patient no. 23**: Figure 2 shows the results of analysis of patient no. 23 DNA for βT gene rearrangement. All four enzymes, EcoRI, HindIII, XbaI, and BamHI, showed evidence for clonal rearrangement of βT genes. Somewhat surprisingly, the sizes of rearranged fragments detected were identical to those in patient 16 (Fig 2), which suggests that βT genes had undergone an identical V-D-J-like rearrangement (Table 1). Reprobing of the Southern blots from patients no. 16 and 23 with an Ig probe confirmed that these were from different CLL patients (data not shown).

- **Patient no. 23**: Figure 2 shows the results of analysis of patient no. 23 DNA for βT gene rearrangement. All four enzymes, EcoRI, HindIII, XbaI, and BamHI, showed evidence for clonal rearrangement of βT genes. Somewhat surprisingly, the sizes of rearranged fragments detected were identical to those in patient 16 (Fig 2), which suggests that βT genes had undergone an identical V-D-J-like rearrangement (Table 1). Reprobing of the Southern blots from patients no. 16 and 23 with an Ig probe confirmed that these were from different CLL patients (data not shown).

**Patient no. 52**: Figure 2 shows the results of analysis of patient no. 52 DNA for βT gene rearrangement. All four enzymes, EcoRI, HindIII, XbaI, and BamHI, showed evidence for clonal rearrangement of βT genes. Somewhat surprisingly, the sizes of rearranged fragments detected were identical to those in patient 16 (Fig 2), which suggests that βT genes had undergone an identical V-D-J-like rearrangement (Table 1). Reprobing of the Southern blots from patients no. 16 and 23 with an Ig probe confirmed that these were from different CLL patients (data not shown).

**Patient no. 34**: EcoRI analysis of patient no. 34 DNA showed an apparently germline configuration of both Cβ1 genes (Fig 2A). As with patient 52, both HindIII and XbaI Cβ2-containing germline fragments were reduced in size by an equivalent amount to generate a rearranged fragment, whereas no rearranged BamHI fragment was resolvable from the germline. These data strongly support a simple Cβ2 D-J deletion on one allele without loss of the Cβ1 gene.

**Rearrangement of γT genes**: All 59 patients with B-CLL were screened for γT gene rearrangement by using restriction enzyme digests as follows: A, EcoRI; B, HindIII; and C, BamHI.

![Fig 3](image-url)
three enzymes: EcoRI, HindIII, and BamHI. No evidence was found for any structural change at the γT locus in any of these patients, including the four containing rearranged βT genes. To illustrate some of the data obtained, Fig 3 shows the results of γT probe analysis in the four patients who had rearranged βT genes. The diversity of rearrangements that occur at the γT locus in both T and non-T cells is limited\(^1\),\(^2\) so that monoclonal rearrangements are detected as only a small number of different-sized restriction fragments with the γT probe. Similarly, in polyclonal T cell populations, a small number of substoichiometric rearranged fragments can be detected in addition to the predominant germline fragments. This is illustrated in the control (normal peripheral blood) DNA in Fig 3A and B for the enzymes EcoRI and HindIII, which are most useful for detecting γT rearrangements. It should be noted that there is a polymorphic HindIII site adjacent to the Cγ2 joining region (Fig 1B) so that only some individuals generate the 5-kb germline fragment (Fig 3B). The enzyme BamHI is potentially less useful for detecting γT gene rearrangement because of the relatively large size of germline (and rearranged) fragments. This also poses a problem when there is significant non-specific degradation of the DNA. This is evident in patients no. 16 and 23 (Fig 3C) where the 15-kb germline fragment is underrepresented.

**Rearrangement of Ig heavy- and light-chain genes.** To determine whether the four cases showing rearrangement of βT genes had Ig gene rearrangements characteristic of mature B cell malignancies, DNA samples were analyzed with IgH (JH) and λ and κ light-chain gene probes as shown in Fig 4. On HindIII digestion, all four had rearrangement of one or both IgH genes (Fig 4A), also demonstrable with other enzymes (data not shown). Three had rearranged κ light-chain genes detectable with BamHI (Fig 4B), whereas the third (patient no. 52) had either two germline alleles intact or one deleted. Consistent with the latter possibility, this patient had a detectable rearrangement of λ light-chain genes (Fig 4C), whereas the other three patients all had two or more bands corresponding to the polymorphic germline fragments present in the control. Table 1 summarizes the Ig gene configurations inferred from these data.

**Expression of TCRβ genes.** Of the four patients displaying rearranged βT genes, further blood samples were available from three (patients no. 16, 23, and 34) for analysis of RNA. Figure 5A shows a Northern blot profile after annealing with an IgH Cμ gene probe. All three cases expressed abundant transcripts corresponding to the membrane-bound (2.7 kb) and secreted (2.4 kb) Cμ mRNAs.\(^3\) When analyzed with the βT probe, these patients also expressed detectable βT transcripts (Fig 5B). However, the level of βT RNA was comparable to that in normal peripheral blood lymphocytes (predominantly T cells), which has previously been shown to be very low.\(^4\) Transcription of a functionally (V-D-J) rearranged TCRβ gene produces a mature 1.3-kb mRNA species (eg, control T cells in Fig 5B), whereas truncated transcripts (typically in the size range 1.0 to 1.2 kb) occur as "germline"
Clinical and immunologic features of CLL patients showing $\beta$T gene rearrangement. Table 2 summarizes the clinical features of all four CLL patients who had rearranged TCR$\beta$ genes together with immunophenotype data from three. Patient no. 16 showed in all respects typical B-CLL. Patient no. 23 was clinically not atypical, but two features of the immunophenotype, high SIg and FMC7 positivity, were most unusual for B-CLL, being more reminiscent of B prolymphocytic leukemia (B-PLL) or follicular lymphoma.22,24,37 Although no immunophenotype data were available on patient no. 52, this patient was again atypical in showing 30% circulating prolymphocytes. A bone marrow aspirate performed shortly before the death of this patient showed 85% prolymphocytes and 10% to 15% blasts. The clinical course of disease in this patient therefore strongly suggests prolymphocytic transformation of B-CLL.22,38 Finally, patient no. 34 was again unusual with high SIg, more characteristic of B-PLL or follicular lymphoma.22

DISCUSSION

The overall incidence of $\beta$T gene rearrangement was four of 59 (7%) and that of $\gamma$T rearrangement, zero of 59 (0%). All four patients with rearranged $\beta$T genes also had rearranged IgH and Ig light-chain genes typical of mature B cell malignancies. However, only one of these four had a normal spectrum of clinical and surface marker features. Two had features of B-PLL/B lymphoma, and one had progressed to a prolymphocytic transformation. This suggests that the frequency of TCR gene rearrangement in more typical B-CLL cases may be very low indeed despite the universal presence of the T cell–associated antigen CD5 (T1) in this disease.21 However, further studies will be required to establish whether this apparent association of $\beta$T gene rearrangement with atypical clinical/immunophenotypic features in B-CLL is significant.

A rather provocative finding was that $\beta$T gene rearrangements in patients 16 and 23 were indistinguishable on the basis of fragment size, generated by different restriction enzymes. It therefore seems highly likely that these two independently rearranged $\beta$T genes are identical. In early studies on T cell leukemias, examples of analogous “identical” rearranged genes have been reported,39 although this has not been substantiated in more recent reports of other workers13 or from our own laboratory. That two identically
Rearranged βT genes have been found in the present study in a class of disease in which TCR gene rearrangement is rare may be purely coincidental. An alternative explanation is that there is a restriction on the diversity of possible TCR gene rearrangements that may occur in mature B cells. Such a restriction might, for example, be imposed by the limited accessibility of germline V region genes (and possibly of D and J regions) to the "recombinase" enzyme machinery that mediates breakage and joining at appropriate recognition sequences. A precedent for this kind of restriction of "inappropriate" gene rearrangement has recently been reported where IgH rearrangement in T cell leukemias involves preferential utilization of the D segment (DQ52) nearest to the J region to generate nonfunctionally rearranged heavy-chain genes.40

Expression of βT genes has not previously been studied in B-CLL. In B cells and in tonsil B lymphocytes (the presumptive normal cell counterpart of B-CLL), low-level "germline" expression of transcripts in the 1.0- to 1.3-kb size range has recently been described.44 In our own studies, several B-CLL cases were found to express variable but low levels of these RNAs. However, there was no significant difference between those patients with germline and those with rearranged βT genes. In particular, the two patients with presumptive V-D-J rearrangements appeared to express mostly 1.0-kb transcripts. Our working hypothesis to explain this observation is that the βT V region promoter may be regulated by cis-acting sequences that are not operative in mature B cells and that germline transcription from the unrearranged βT alleles may well account for much of the 1.0-kb βT mRNA seen in patients no. 16 and 23.

One of the most striking features of TCR gene rearrangement in the four B-CLL patients was the concomitant absence of detectable TCRγ rearrangement. With few exceptions, all cases of T cell malignancy with βT rearrangements thus far studied also have rearranged γT genes. This together with other evidence supports a strictly ordered program of gene rearrangement in T cells where the γT genes rearrange first and are followed by βT and then by αT genes. The apparent absence of γT gene rearrangement in these patients makes a clear distinction between the TCR gene rearrangement patterns normally associated with T cells and argues that these βT rearrangements have not occurred as part of a normal T cell developmental program.

The results of our survey add further support to the notion that there is an inverse correlation between the frequency of inappropriate TCRβ and γ gene rearrangement and stage of differentiation arrest in terms of the degree of B cell commitment in malignancies of B cell origin. A working model consistent with these observations is that the differentiation arrest accompanying expansion of a leukemic clone arising from early B precursor cells has the effect of expanding the developmental window during which normal Ig gene rearrangement occurs. Because Ig and TCR genes use a similar if not identical recombinase enzyme machinery,45 this increases the chances of inappropriate (and presumably normally very rare) TCR gene rearrangement events. B cell malignancies such as a B-CLL, which have a very low frequency of TCR gene rearrangement, arise from more mature B cells that have largely traversed this developmental stage.

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NOTE ADDED IN PROOF

We have recently identified two further patients in our CLL series in whom we have now deleted TCR β rearrangement of a similar configuration to that seen in patient nos. 16 and 23.

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Rearrangement and expression of T cell antigen receptor genes in B cell chronic lymphocytic leukemia

JD Norton, J Pattinson, AV Hoffbrand, H Jani, JC Yaxley and BF Leber