CONCISE REPORT

Clonal Rearrangement and Expression of the T Cell Receptor β Gene and Involvement of the Breakpoint Cluster Region in Blast Crisis of CGL

By L.C. Chan, A.J. Furley, A.M. Ford, D.A. Yardumian, and M.F. Greaves

A case of lymphoid blast crisis of Ph1-positive CGL is described in which the blast cells had an immature T cell phenotype, clonal rearrangement and expression of the T cell receptor β gene, and a rearrangement of the breakpoint cluster region (bcr) on chromosome 22. This case therefore provides definite evidence for transformation involving a common myeloid–T lineage progenitor, prominence of the Ph1 molecular alteration into the T cell lineage, and clonal selection in blast crisis at the level of a committed T lineage precursor.

C HRONIC granulocytic leukemia (CGL) originates in a multipotential, hematopoietic stem cell.1-3 Progeny of this transformed cell include granulocytic, erythroid, megakaryocytic, and monocytic cells, eosinophils, and B lymphocytes.4,4 An involvement of the T cell lineage in Philadelphia (Ph1)-positive CGL has, however, been difficult to establish; attempts to demonstrate the Ph1 chromosome or monoclonality with respect to glucose 6PD in T cells from patients in chronic phase have yielded variable or equivocal results.5,5 Immunophenotyping studies of CGL in blast crisis have shown a preponderance of myeloid and/or B cell precursor phenotypes16-3 with only a few isolated cases of possible T cell lineage involvement.9-12 Similarly, de novo ALL with Ph1 chromosome have common ALL or B cell precursor phenotypes,13 whereas Ph1-positive T-ALL, though recorded,11,14,15 is very rare. Two recent cases,16,17 one of childhood T lymphoblastic lymphoma and the other of T-ALL, have provided evidence that the Ph1 chromosome may arise as a secondary event in the evolution of these disorders.

Selective growth advantage of single B cell precursors in blast crisis of CGL has been demonstrated by clonal rearrangements of immunoglobulin genes.18,19 The recent availability of molecular probes for the T cell receptor (TCR) β gene now provides a similar opportunity to explore T cell selection in CGL since this gene rearranges in a similar fashion to immunoglobulin20 and is shown to be clonally rearranged and transcribed in T cell malignancies.21 In addition, the cloning of a DNA probe that specifically recognizes rearrangements in the breakpoint cluster region (bcr) on chromosome 22 in CGL22 provides a new and unique molecular marker to analyze particular cell types including T cells for their involvement in CGL. We recently identified a rare case of blast crisis of Ph1-CGL in which the blast cells had the composite phenotype of thymic leukemia cells (cf T-ALL). We report here the detailed immunophenotype of this case and results for probing for rearrangement and expression of TCR β gene and also for involvement of bcr.

MATERIALS AND METHODS

Restriction enzymes BamHI, EcoRI, HindIII, and BglII were supplied from Anglian Biotechnology Laboratories and used according to the manufacturer’s specifications; [α-32P]dCTP (specific activity 300 Ci/mmol) was purchased from Amersham International. Two probes were used for the study: (1) a 300-bp BglII/StuI constant region cut from Jurkat cDNA of TCR β gene23 was kindly provided by Dr Tak Mak (Ontario Cancer Institute), and (2) a 1.2-kilobase BglII/HindIII bcr cloned fragment was kindly supplied by J. Groffen (Oncogene Scientific, Inc, New York).

RESULTS

The clinical and laboratory data of patient J.H. are shown in Table I. The composite phenotypes of the leukemic blasts WT1(CD-7)*, T11a(CD-2)*, TdT*, MY9*, B4*, and SmIg- indicates this case to be of early T cell precursor origin.

Hybridization of J.H.’s DNA with the TCR β gene probe shows clonal rearrangement of a TCR β gene allele of 25.0 kb coexisting with the remaining 22.7 kb germ line allele in the BamHI digest (Fig 1A). The autoradiograph pattern of the EcoRI digest (Fig 1B) confirms that the rearranged allele derives from Cβ1; this is shown by the creation of a new band of 11.4 kb coexisting with the remaining 10.5-kb germ line Cβ1 allele. The smaller band of 4.5 kb in the EcoRI track represents Cβ1 alleles, rearrangement of which would not be detectable as there exist EcoRI restriction sites both 5' and 3’ of the Cβ1 locus. The HindIII digest (Fig 1C) confirms noninvolvement of the Cβ1 locus as it shows a single germ line of 7.5 kb; similarly, the smaller band of 3.4 kb represents Cβ1 alleles, rearrangement of which would not be apparent as HindIII cuts 5’ and 3’ of the C locus. The status of the immunoglobulin heavy-chain locus was similarly examined using a human μ constant region probe (Ch). No rearrangement was observed when a BamHI digest of the patient’s DNA was probed with the Cμ probe (data not shown). The results from these analyses therefore reinforce the lineage affiliation of the leukemic blasts to be of T cell origin. Finally, the T cell lineage of the blast crisis was further

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Table 1. Clinical and Laboratory Data of CGL-T Cell Blast Crisis

<table>
<thead>
<tr>
<th>Patient:</th>
<th>J.H.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age:</td>
<td>66</td>
</tr>
<tr>
<td>Sex:</td>
<td>Male</td>
</tr>
<tr>
<td>WBC count:</td>
<td>$109 \times 10^9/L$</td>
</tr>
<tr>
<td>% blasts:</td>
<td>PB, 11.0 BM, 71.0</td>
</tr>
<tr>
<td>Hematologic diagnosis:</td>
<td>CGL blast crisis (patient presented 3 years previously with classical Ph' CML)</td>
</tr>
<tr>
<td>Cytogenetics:</td>
<td>46, XY, t(9;22)</td>
</tr>
<tr>
<td>Surface markers:</td>
<td>Bone marrow</td>
</tr>
<tr>
<td>(1) T cell:</td>
<td>WT1(CD-7)* 66% T11a(CD-2)* 74%</td>
</tr>
<tr>
<td></td>
<td>OKT4(CD-4)* 0% C3(CD-8)* 0%</td>
</tr>
<tr>
<td></td>
<td>Na134(CD-1)* 0% Smlg 0%</td>
</tr>
<tr>
<td>(2) B cell:</td>
<td>B4(CD-19)* 0%</td>
</tr>
<tr>
<td>(3) Myeloid:</td>
<td>MY9 0%</td>
</tr>
<tr>
<td>(4) anti-CALLA:</td>
<td>AL2(CD-10)* 75%</td>
</tr>
<tr>
<td>(5) anti-HLA-DR:</td>
<td>DA2 21%</td>
</tr>
<tr>
<td>(6) Terminal transferase:</td>
<td>(Tdt) 75%</td>
</tr>
<tr>
<td>Double staining:</td>
<td>WT1/TdT 78%</td>
</tr>
<tr>
<td>Immunophenotype:</td>
<td>Immature thymic</td>
</tr>
</tbody>
</table>

*CD, cluster of differentiation with arbitrary number assigned (see Immunology Today 5:158-159, 1984).

supported by the evidence of TCR β gene transcripts (Fig 2) from an RNA blot; however, the size of the transcripts lies between the 1.3-kb-size message representing complete VDJC sequences and the 1.1-kb-size transcripts from DJC rearrangement as described in various T cell lines and fresh T-ALL leukemic samples.

To find out whether the same breakpoint cluster region in CMLs was involved in this case of T cell blast crisis, DNA of J.H. was analyzed by hybridization to a bcr probe. The involvement of the bcr locus on chromosome 22q is shown by the existence of a new rearranged BglII band of 3.2 kb together with a germ line BglIII band of 5.0 kb representing the normal chromosome 22 (Fig 3); similarly, there is a new EcoRI band of 26.0 kb together with the normal 18.5-kb bcr allele. No rearrangement of bcr locus was seen in the BamHI and HindIII digests, suggesting that the breakpoint in bcr on chromosome 22 lies upstream of the BamHI site, as shown in Fig 4. The fact that only a single new band was detected in the EcoRI and BglII digests emphasizes that clonal rearrangement of bcr locus has occurred.

**DISCUSSION**

The involvement of T cells in CGL blast crisis phase of Ph'-positive CGL is confirmed in the study. Although the composite immunophenotype of the leukemic cells in this case indicates that they were of thymic type, as observed in T-ALL, the demonstration of rearrangements in the TCR β

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Fig 1. Autoradiographs of DNA digests hybridized with a TCR β constant region probe. B, BamHI; E, EcoRI; H, HindIII. c, control (placental) DNA; p, patient's DNA. Sizes of DNA fragments are given in kilobases (kb). Clonally rearranged bands arrowed.

Fig 2. Autoradiography of RNA transcripts hybridized with a TCR β constant region probe. T, thymocyte RNA; P, patient RNA. Sizes of RNA transcripts are given in kilobases (kb).

Fig 3. Autoradiograph of DNA digests hybridized with a bcr probe. B, BamHI; E, EcoRI; Bg, BglII; H, HindIII. c, control DNA; p, patient's DNA. Sizes of DNA fragments are given in kilobases (kb). Clonally rearranged bands arrowed.
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gene and the expression of TCR β gene transcripts reinforces this lineage affiliation. The intermediate-sized TCR β gene transcripts between 1.3 kb and 1.1 kb lead us to speculate that they may arise from an incomplete rearrangement. Furthermore since the TCR β gene rearrangements were clearly monoclonal, we conclude that blast crises in this patient involved clonal selection at the level of T cell precursors that had already rearranged the TCR β gene. This, then, parallels blast crisis with a common ALL phenotype in which lymphoblasts have clonal rearrangements of the immunoglobulin gene.4,19 In addition, we found that bcr on chromosome 22 was rearranged, thus proving that the T lymphoblasts in this case were members of the Ph' leukemic clone.

We take these data as providing definite evidence for the involvement, albeit rare, of the T lineage in Ph'-positive CML and presume that, in these cases, at least, the initial target cells for transformation descended from a common progenitor of the T and myeloid lineages. Additional proof of the existence of such a common progenitor comes from a recent report by Fauser et al27 that demonstrates the presence of the Ph' chromosome in T lymphocytes within multilineage hematopoietic colonies (CFU-GEMM T) generated from bone marrow cells from a patient with Ph' CML.

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REFERENCES

27. Furley A: unpublished observations
Clonal rearrangement and expression of the T cell receptor beta gene and involvement of the breakpoint cluster region in blast crisis of CGL

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