Basophils (Bsp-1⁺) Derive From the Leukemic Clone in Human Myeloid Leukemias Involving the Chromosome Breakpoint 9q34

By Michael P. Bodger, Christine M. Morris, Martin A. Kennedy, Jacqueline A. Bowen, Johanna M. Hilton, and Peter H. Fitzgerald

The monoclonal antibody (MoAb) Bsp-1 was used to purify basophilic cells from leukemic blood of five patients with Philadelphia chromosome (Ph') positive chronic myeloid leukemia (CML) and two patients with acute myeloid leukemia (AML) characterized by the chromosomal translocation t(6;9)(p23;q34). When cultured, Bsp-1 positive cells from all CML and AML patients showed the same clonal characteristics and with toluidine blue-positive basophils in chronic phase CML and two patients with acute myeloid leukemia (AML) characterized by the granulocytic, erythroid, and B-lymphoid cell lineages. The evidence that basophils derived from the leukemic clone. Bsp-1 staining correlated with toluidine blue-positive basophils in chronic phase CML and with toluidine blue-negative blast cells expressing an immature myeloid phenotype in blast crisis CML and AML. Chromosome in situ hybridization showed that the ABL oncogene was translocated from chromosome 9 to chromosome 22 in CML patients but remained on chromosome 9 in the AML patients. These results indicate that the breakpoint at 9q34 in CML is 5' of ABL whereas the breakpoint at 9q34 in AML is 3' of ABL. Field inversion gel electrophoresis showed that the 9q34 breakpoint was not within 200 kb 3' of ABL in one of the AML patients, nor was there any rearrangement of the PIM oncogene locus at 6p21.

Peripheral blood basophilia is frequently observed in myeloproliferative disorders and chronic myeloid leukemia (CML) and often heralds the blast crisis of CML. Bone marrow basophilia is also found consistently in a subgroup of acute myeloid leukemia (AML) characterized by the chromosomal translocation t(6;9)(p23;q34). CML originates in the pluripotent stem cell, and subsequent differentiation involves the granulocytic, erythroid, and B-lymphoid cell lineages. The evidence that basophils in CML are of leukemic origin is circumstantial, whereas the relationship between the leukemic clone and basophils in AML is unknown. We have recently described a monoclonal antibody (MoAb), Bsp-1, that reacts specifically with human basophils, and we have purified basophils from normal individuals and CML patients in numbers sufficient for cytochemical and immunophenotypic analysis. We use Bsp-1 to purify basophilic cells from CML and AML and show that cultured Bsp-1⁺ cells exhibit the same cyogenetic characteristics as the leukemic clone.

MATERIALS AND METHODS

Patient details. Patients 1 through 5 were all Ph' positive and showed the characteristic hematologic and clinical picture of CML. All patients had significant basophil counts (Table 1). Patients 6 and 7 had the t(6;9)(p23;q34) in leukemic cells and were diagnosed as having AML-M2. Patient 6 was referred with a WBC of 153 x 10⁹/L and a bone marrow differential of 34% myelocytes/neutrophils, 6% erythroblasts, and 15% lymphocytes. When leukemia was diagnosed, patient 7 had a WBC of 23 x 10⁹/L and bone marrow differential of 51% myeloblasts, 3% promyelocytes, 34% myelocytes/neutrophils, 6% erythroblasts, and 6% lymphocytes.

Basophil and T-cell isolation. Peripheral blood from five patients with CML and two patients with AML was separated by centrifugation on 60% Percoll density gradients. Mononuclear cells were recovered from the interface, and residual red cells were removed by lysis. Bsp-1⁺ cells were purified from the mononuclear cell fraction using cell-sorting techniques.

T lymphocytes were isolated as E rosette-positive cells after centrifugation on 60% Percoll gradients. The cells were cultured for three days in RPMI medium supplemented with 10% fetal calf serum (FCS) and 1% phytohemagglutinin (PHA) (GIBCO, Grand Island, NY) at 37°C and then for a further 18 days in medium supplemented with 50% PHA-lymphocyte-conditioned medium (PHA-LCM).

Immunophenotyping and cytochemistry. Bsp-1⁺ cells were enumerated by indirect immunofluorescence. Two-color immunofluorescence for the simultaneous detection of two-surface antigens on a single cell was performed as previously described. Cell smears were prepared using a cytocentrifuge (Shandon Southern Products Ltd., Cheshire, England) and stained with May-Grunwald-Giemsa and 1% toluidine blue in methanol to identify metachromatic staining granules in basophils. Sudan black, alkaline phosphatase, acid phosphatase, nonspecific esterase, peroxidase, periodic acid Schiff, and chloroacetate esterase staining were performed according to published methods.

Cytogenetic preparations. Buffy coat cells from bone marrow and/or blood samples and Bsp-1⁺ cells were cultured for 96 to 120 hours in 10% FCS/RPMI supplemented with 10% conditioned medium from the bladder tumor cell line 5637. The T-cell cultures were synchronized with methotrexate and harvested by standard cytogenetic techniques. The metaphase chromosome preparations were trypsin-Giemsa banded.

Chromosome in situ hybridization. The probe pAB1 sub 9 was nick-translated with 3H-nucleotides (Amersham, Buckinghamshire, England) to a specific activity of 1 to 2 x 10¹¹ dpm/μg and hybridized in situ to metaphase chromosomes, as previously described.

Field inversion gel electrophoresis (FIGE). DNA for FIGE was prepared by embedding intact cells in low-gelling temperature agarose and treating by standard methods. Yeast chromosomes and phage lambda oligomers were used as size markers. FIGE was

From the Cytogenetic and Molecular Oncology Unit, Christchurch Hospital, Christchurch, New Zealand.

Submitted March 7, 1988; accepted October 10, 1988.

Supported by grants from The Cancer Society of New Zealand and its Canterbury-Westland Division.

Address reprint requests to M. P. Bodger, PhD, Cancer Society of New Zealand Cytogenetics Unit, Christchurch Hospital, Christchurch, New Zealand.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1989 by Grune & Stratton, Inc.

Blood, Vol 73, No 3 (February 15), 1989: pp 777-781
carried out with a constant ratio of forward to backward pulse lengths of 3:1, starting at 9 seconds forward and increasing to 114 seconds forward during a 50-hour run. Gels were UV blotted to Genescreen Plus in a 50-hour run. Gels were UV blotted to Genescreen Plus.

Table 1. Basophil Analyses of Leukemic Samples.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sample</th>
<th>Diagnosis</th>
<th>WBC (x 10^3/L)</th>
<th>Bsp-1&lt;sup&gt;+&lt;/sup&gt; Cells&lt;sup&gt;†&lt;/sup&gt; (%)</th>
<th>Toluidine Blue Cells&lt;sup&gt;‡&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BM</td>
<td>CML</td>
<td>255</td>
<td>35</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>BM</td>
<td>CML</td>
<td>224</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>BM</td>
<td>CML</td>
<td>85</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>BM</td>
<td>CML</td>
<td>308</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>BM</td>
<td>CML</td>
<td>124</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>BL AML</td>
<td></td>
<td>153</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>BM</td>
<td>AML</td>
<td>23</td>
<td>0</td>
<td>43</td>
</tr>
</tbody>
</table>

Abbreviations: BM, bone marrow; BL, leukemic blood.

*Mononuclear cell fraction.

†Myeloid blast crisis (25% circulating blast cells).

Table 2. Immunophenotype and Cytoc hemistry of Bsp-1<sup>+</sup> Cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>MoAb</th>
<th>OTK11 (CD2)</th>
<th>FMCB (CD9)</th>
<th>J5 (CD10)</th>
<th>WM15 (CD13)</th>
<th>CMRF7 (CD15)</th>
<th>B4 (CD19)</th>
<th>My9 (CD33)</th>
<th>My10 (CD34)</th>
<th>RFB-1</th>
<th>CA-2 (HLA-DR)</th>
<th>W6/32 (HLA-ABC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (+)</td>
<td>0</td>
<td>70 (+)</td>
<td>90 (+)</td>
<td>95 (+)</td>
<td>20 (+)</td>
<td>85 (+)</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15 (+)</td>
<td>5 (+)</td>
<td>0</td>
<td>80 (+)</td>
<td>40 (+)</td>
<td>70 (+)</td>
<td>20 (+)</td>
<td>85 (+)</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20 (+)</td>
<td>20 (+)</td>
<td>20 (+)</td>
<td>20 (+)</td>
<td>20 (+)</td>
</tr>
</tbody>
</table>

Cytochemical stain†

<table>
<thead>
<tr>
<th>Toluidine blue</th>
<th>Acid phosphatase</th>
<th>Alkaline phosphatase</th>
<th>Sudan black</th>
<th>Nonspecific esterase</th>
<th>Peroxidase</th>
<th>Periodic acid Schiff</th>
<th>Chloroacetate esterase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>85</td>
<td>2</td>
<td>73</td>
<td>0</td>
<td>83</td>
</tr>
</tbody>
</table>

Abbreviation: nd, not determined.

‡Myeloid blast crisis (25% circulating blast cells).

Table 3. Karyotypic Analyses of Leukemic Samples and of Bsp-1<sup>+</sup> Cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sample</th>
<th>Karyotype</th>
<th>Leukemic Cells</th>
<th>Bsp-1&lt;sup&gt;+&lt;/sup&gt; Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BM</td>
<td>46,XY,t(9;22)(q34; q11)</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>BM</td>
<td>46,XY,t(9;22)(q34; q11)</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>BM</td>
<td>46,XX,t(9;22)(q34; q11)</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>BM</td>
<td>46,XX,t(9;22)(15) (q34; q11;q11)</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>BM</td>
<td>46,XY, -9;9(22) (q34; q11), +der(9), t(2;9)(p15;q24)</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>BI</td>
<td>46,XY, +8, +13,t (6;9)(p23;q34)</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>BM</td>
<td>46,XY,t(6;9)(p23; q34)</td>
<td>35</td>
<td>12</td>
</tr>
</tbody>
</table>

Abbreviations: BM, bone marrow; BL, leukemic blood.

RESULTS

Basophil analyses. Patients 1 through 4 had elevated basophil counts, and the percentages of Bsp-1<sup>+</sup> cells were approximately equal to the percentages of toluidine blue-positive cells (Table 1). Twenty three percent of the mononuclear cells from patient 5 expressed the Bsp-1 antigen, but only 0.4% of the cells were toluidine blue-positive. Mature basophils were not observed in samples from patients 6 and 7, but patient 6 had small numbers of Bsp-1<sup>+</sup> and toluidine blue-positive cells. Patient 7 had 43% Bsp-1<sup>+</sup> cells but no toluidine blue-positive cells (Table 1).

The discrepancy between the Bsp-1 antigen and toluidine blue results from patients 5, 6, and 7 was investigated by double-marker studies. The Bsp-1<sup>+</sup> blast cells of all three patients showed two additional clones, which evolved from the leukemic karyotype. The AML-M2 patients had the translocation t(6;9)(p23;q34) in leukemic blood (patient 6) and bone marrow (patient 7). Patient 6 had additional chromosomes 8 and 13 (Table 3). Cultured Bsp-1<sup>+</sup> cells from all seven patients exhibited the respective leukemic karyotypes (Table 3; Figs 1 and 2).

T cells isolated from patients 1, 2, 4, and 5 cultured in PHA-LCM showed normal karyotypes in every one of 20, 22, 15, and 18 metaphases examined, respectively.

Chromosome in situ hybridization and FIGE analyses. The v-abl probe hybridized significantly to the normal chromosome 9 and to the Ph' (22q−) chromosome in leukemic blasts of patients 5, 6, and 7.
leukemic metaphase cells from CML patients 4 and 5 (Table 4). In contrast, the v-abl probe hybridized significantly to the normal and derivative chromosome 9 in leukemic metaphase cells from the AML patients 6 and 7 (Table 4). These results confirm that ABL is translocated as a result of the t(9;22) but not as a result of the t(6;9).

The proximity of ABL to the 9q34 breakpoint of the t(6;9) was investigated by FIGE.

Fig 1. G-banded karyotype from cultured Bsp-1 cells from patient 3. Arrows indicate the 9q- and 22q+ (Ph1) chromosomes.

Fig 2. G-banded karyotype from cultured Bsp-1 cells from patient 6. Arrows indicate the 6p- and 9q+ chromosomes. Partial karyotypes show the 6p- and 9q+ chromosomes on the right hand side of each pair.
Table 4. In Situ Hybridization of v-abl to Leukemic Bone Marrow Cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>No. of Metaphases Analyzed</th>
<th>Total on Genome</th>
<th>No. of Labeled Sites</th>
<th>On Chromosome No.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>106</td>
<td>204</td>
<td>15</td>
<td>15q- /22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22q-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 (4)</td>
<td>9</td>
</tr>
<tr>
<td>5†</td>
<td>151</td>
<td>381</td>
<td>6 (3)</td>
<td>9q+</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>155</td>
<td>7 (0)</td>
<td>16 (16)†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14 (14)†</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>144</td>
<td>4 (1)</td>
<td>9q+</td>
</tr>
</tbody>
</table>

*Entries indicate the number of labeled sites on the chromosome and, in parentheses, the number of labeled sites at the chromosome breakpoint.
†Significant labeling, \( \chi^2, P < .001 \).
‡Analysed during the chronic phase.

DISCUSSION

Leukemic cells from the majority of CML patients are characterized by a specific cytogenetic marker, the Ph' chromosome, which is one product of a reciprocal translocation t(9;22)(q34;q11). CML is a clonal disorder of the pluripotential stem cell, and therefore individual cell lineages that exhibit the Ph' chromosome are derived from the same leukemic clone. The leukemic origin of basophils in CML has long been suspected, but purified basophils from these patients have not previously been examined for the Ph' chromosome. By using the MoAb Bsp-1, we show that basophilic cells from CML have the Ph' chromosome and are therefore derived from the leukemic clone. We have complemented this finding by demonstrating that the BCR gene is rearranged in Bsp-l+ cells from patients with CML. Clonal evolution of leukemic cells was also present in Bsp-1+ cells from patient 5. Purified Bsp-1+ cells from the two AML patients exhibited the diagnostic karyotype t(6;9)(p23;q34) and must also originate from the leukemic clone.

The translocation of the ABL gene from chromosome 9 to the Ph' chromosome in Ph'-positive CML has been widely documented. Our observation that ABL remains on chromosome 9 in t(6;9) AML confirms recent data of Westbrook et al. These results imply that the breakpoint on chromosome 9 is 3' of ABL in t(6;9) AML, whereas it is 5' of the v-abl homologous region in CML. Our FIGE results demonstrate that the 9q34 breakpoint is more than 200 kb 3' of ABL in the t(6;9) in patient 6. Furthermore, the PIM oncogene, which is localized to chromosome 6p21, did not appear to be structurally affected by the t(6;9) in patient 6, although our data did not rule out the presence of a breakpoint close to PIM. A breakpoint at 9q34 has recently been described in t(7;9) T-cell acute lymphocytic leukemia (ALL) but it is at least 250 kb 5' of the ABL gene.

Bsp-1 does not react with human mast cells (unpublished observations) and is therefore specific for human basophils. In four of five CML patients, the percentage of Bsp-1+ cells correlated with the percentage of toluidine blue-positive cells and the presence of basophils. However, the pattern of Bsp-1 expression in CML patient 5 and the two AML patients is not observed on normal hemopoietic cells and was not accounted for by the presence of mature basophils. Bsp-1+ cells in these three patients exhibited an immunophenotype and cytochemical features of immature myeloid cells. We suggest that these cells represent precursors of basophils, and this is supported by the fact that 10% to 14% of cells from the basophil precursor cell line KU812 are Bsp-1+.8

![Fig 3. A field inversion gel blot of DNA from patient 6 (1) and a control subject (2). The membrane was first hybridized with a probe for ABL (A), then stripped by washing in 0.4 mol/L NaOH and hybridized with a probe for PIM (B).](image)
LEUKEMIC ORIGIN OF BASOPHILS

T lymphocytes from four of five CML patients did not belong to the leukemic clone. They did not exhibit the Ph' chromosome, and this is in agreement with recent data showing that the bcr gene is not rearranged in the T cells from CML patients.29,33

REFERENCES


ACKNOWLEDGMENT

We thank Dr M.E.J. Beard and staff of the Haematology Department, Christchurch Hospital for cell counts and basophil differentials, Lesley Newton for cytochemical staining, and Dr Anton Berns, Netherlands Cancer Institute, for the PIM probe.
Basophils (Bsp-1+) derive from the leukemic clone in human myeloid leukemias involving the chromosome breakpoint 9q34

MP Bodger, CM Morris, MA Kennedy, JA Bowen, JM Hilton and PH Fitzgerald