RAPID COMMUNICATION

Activation of the Interleukin-3 Gene by Chromosome Translocation in Acute Lymphocytic Leukemia With Eosinophilia

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The t(5;14)(q31;q32) translocation from B-lineage acute lymphocytic leukemia with eosinophilia has been cloned from two leukemia samples. In both cases, this translocation joined the IgH gene and the interleukin-3 (IL-3) gene. In one patient, excess IL-3 mRNA was produced by the leukemic cells. In the second patient, serum IL-3 levels were measured and shown to correlate with disease activity. There was no evidence of excess granulocyte/macrophage colony stimulating factor (GM-CSF) or IL-5 expression. Our data support the formulation that this subtype of leukemia may arise in part because of a chromosome translocation that activates the IL-3 gene, resulting in autocrine and paracrine growth effects.

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Fig 1. Breakpoint sequences for Case 2. The germline IgJh5 region sequence (protein coding region and recombination signal sequences are underlined) is on top, the translocation sequence from Case 2 (PCR primer sequences and putative N region are underlined) is in the middle, and the germline IL-3 sequence, which we derived from a normal IL-3 clone, is on the bottom. + indicates that each sequence has the same nucleotide. The sequence documents the head-to-head joining of the IL-3 and IgH genes. The breakpoint in the IL-3 gene occurred at position -934 (+).

metric method of Mosmann using a VMax microtiter plate reader (Molecular Devices, Menlo Park, CA) set at 570 and 650 nm.16

Cytokine immunoassays. These assays used rat monoclonal anti-cytokine antibodies (10 μg/mL) to coat the wells of a PVC microtiter plate. The capture antibodies used were BVD3-6G8, JES1-39D10, and BVD2-21C11, for the IL-3, IL-5, and GM-CSF assays, respectively. Patient sera were then added (undiluted and diluted 1:2 for IL-3, undiluted for IL-5, and undiluted and diluted 1:5 for GM-CSF). The detecting immunoreagents used were either mouse antiserum to IL-3 or nitroiodophenyl (NIP)-derivatized rat monoclonal antibodies (10 pg/mL) to coat the wells of a PVC microtiter plate. The capture antibodies used were BVD3-6G8, JES1-39D10, and BVD2-21C11, for the IL-3, IL-5, and GM-CSF assays, respectively. Patient sera were then added (undiluted and diluted 1:2 for IL-3, undiluted for IL-5, and undiluted and diluted 1:5 for GM-CSF). The detecting immunoreagents used were either mouse antiserum to IL-3 or nitroiodophenyl (NIP)-derivatized rat monoclonal antibodies JES1-39D10, and BVD2-21C11, specific for IL-3 and GM-CSF, respectively. Bound antibody was subsequently detected with immunoperoxidase conjugates: horseradish peroxidase (HRP)-labeled goat anti-mouse Ig for IL-3, or HRP-labeled rat monoclonal antibodies (JES1-5A2 and BVD2-21C11, specific for IL-3 and GM-CSF). The chromogenic substrate was 3-3'azino-bis-benzthiaziline sulfonate (ABTS; Sigma, St Louis, MO). Unknown values were interpolated from standard curves prepared from dilutions of the recombinant factors using Softmax software available with the VMax microplate reader (Molecular Devices).

RESULTS

Leukemic DNA from Case 2 was studied by Southern blotting. When digested with the HindIII restriction enzyme and hybridized with a human immunoglobulin heavy chain joining region (Jh) probe, a rearranged fragment was detected (data not shown). When digested with two different IL-3 probes, a rearranged 14 kb fragment, comigrating with the rearranged Jh fragment, was identified. When leukemic DNA was digested with HindIII plus EcoRI, a rearranged Jh fragment was detected at 6 kb. The IL-3 probes also identified a comigrating fragment of this size. These experiments indicated that the leukemic sample studied was clonal and that a single fragment contained both Jh and IL-3 sequences, suggesting a translocation had occurred.

To characterize better the joining of the IL-3 gene and the immunoglobulin heavy chain (IgH) gene, the polymerase chain reaction (PCR) was used to clone the translocation. A Jh primer and an IL-3 primer were designed to produce an amplified product in the event of a head-to-head translocation. While control DNA gave no PCR product, Case 2 DNA yielded a PCR-derived fragment of approximately 980 bp, which was cloned and sequenced.

The DNA sequence of the translocation clone from Case 2 confirmed the joining of the Jh region with the promotor of the IL-3 gene in a head-to-head configuration (Fig 1). Sequence analysis indicated that the breakpoint on chromosome 14 was just upstream of the Jh5 coding region. The breakpoint on chromosome 5 occurred 934 bp upstream of the putative site of transcription initiation of the IL-3 gene. We also determined that a putative N sequence of 17 bp was inserted between the chromosome 5 and chromosome 14 sequences during the translocation event.17,18 Figure 2 shows the relationship of chromosome 5 breakpoints to the IL-3 gene. This figure shows the two cloned breakpoints (arrows) in relation to the normal IL-3 gene. One breakpoint occurred at position -602 and the other at -934 (arrows). In both circumstances, the translocations resulted in a head-to-head joining of the IgH gene and the IL-3 gene, leaving the mRNA and protein coding regions of the IL-3 gene intact. Boxes denote the five IL-3 exons; restriction enzymes are (B) BamHI, (P) PstI, (H) HpaI, (E) EcoRI, and (X) XhoI.
Fig 3. Documentation of IL-3 mRNA over-expression. A Northern blot was prepared and hybridized with a probe for IL-3. Lane 1 contained RNA from unstimulated peripheral blood lymphocytes (PBL) as a negative control. Lane 2 contained RNA from PBL stimulated for 4 hours with concanavalan A (ConA), and lane 3 contained RNA from PBL stimulated with ConA for 48 hours. As in the positive control lanes (2 and 3), a 1 kb band was identified in the leukemic sample from Case 1 (lane 4, lower arrow), suggesting aberrant expression of the IL-3 gene. In addition, the leukemic sample showed over-expression of an unspliced 2.9 kb IL-3 transcript (lane 4, upper arrow). We documented that this represented an unspliced precursor of the mature 1 kb transcript by showing that this band hybridized to a probe from intron 2 of the IL-3 gene. A similar band was detectable in normal mitogen-stimulated cells. Lane 5 through 10 represent RNA from six samples of B-lineage acute lymphocytic leukemia analyzed by Northern blot because too few cells were available for study. The locations of the two cloned breakpoints in relation to the IL-3 gene. The two chromosome 5 breakpoints were separated by less than 500 bp.

The genomic structure in Cases 1 and 2 suggested that a normal IL-3 gene product was over-expressed as a result of the altered promoter structure. This would predict that the IL-3 gene on the translocated chromosome was capable of making IL-3 protein. This prediction was tested by expressing a genomic fragment from the translocated allele of Case 1 containing all five IL-3 exons under the control of the SV40 promoter/enhancer in the Cos7 cell line. Cell supernatants were studied in a proliferation assay using the factor dependent erythroleukemic cell line, TF-1. The supernatants derived from transfections using the vector plus insert supported TF-1 proliferation, while supernatants from transfections using the vector alone were negative in this assay (data not shown). Furthermore, the biologic activity could be blocked by an antibody to human IL-3 (BVD3-6G8). This result showed that the translocated allele retained the ability to make IL-3 mRNA and protein.

The level of expression of IL-3 mRNA in leukemic cells from Case 1 was assessed. Northern blotting showed that the mature IL-3 mRNA (approximately 1 kb) and a 2.9 kb unspliced IL-3 mRNA were excessively produced by the leukemia (Fig 3). The 2.9 kb form of the mRNA is also present at low levels in normal peripheral blood T lymphocytes after mitogen activation (Fig 3). Several B-lineage acute leukemia samples without the t(5;14) translocation had undetectable levels of IL-3 mRNA in these experiments. In addition, although genes for GM-CSF and IL-5 map close to the IL-3 gene and might have been deregulated by the translocation, no IL-5 or GM-CSF mRNA could be detected in the leukemic sample (data not shown).19,20

Three serum samples from Case 2 were assayed by immunoassay for levels of IL-3, GM-CSF, and IL-5 (Table 1). Serum IL-3 could be detected and correlated with the clinical course. When the patient’s leukemic cell burden was highest, the IL-3 level was highest. No serum GM-CSF or IL-5 could be detected.

Since the IL-3 immunoassay measured only immunoreactive factor, we confirmed that biologically active IL-3 was present by using the TF-1 bioassay. This bioassay can be rendered monospecific using appropriate neutralizing monoclonal antibodies specific for IL-3, IL-5, or GM-CSF. We observed that sera from 1-16-84 and 3-14-84 contained TF-1 stimulating activity that could be blocked with anti-IL-3 MoAb (BVD3-6G8), but not with MoAbs to IL-5 (JES1-39D10) or GM-CSF (BVD2-23B6) (Fig 4; GM-CSF data not shown). The amount of neutralizable bioactivity in these two samples correlated very well with the difference in IL-3 levels obtained by immunoassay for these samples. Furthermore, the failure to block TF-1 proliferating activity with either anti–IL-5 or anti–GM-CSF was consistent with the inability to measure these factors by immunoassay and the corresponding growth factor levels quantified by immunoassay. The patient received chemotherapy between 11/6/84 and 3/14/84 to lower his leukemic burden.2 No serum samples were available for a similar analysis of Case 1.

Table 1. Peripheral Blood Counts and Growth Factor Levels at Different Times in Case 2

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>11/15/83</th>
<th>1/16/84</th>
<th>3/14/84</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>81,800</td>
<td>116,500</td>
<td>12,300</td>
</tr>
<tr>
<td>Lymphoblasts</td>
<td>0</td>
<td>33,785</td>
<td>0</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>46,626</td>
<td>73,080</td>
<td>615</td>
</tr>
<tr>
<td>Serum growth factor levels (pg/mL)</td>
<td>&lt;444</td>
<td>7,995</td>
<td>1,051</td>
</tr>
<tr>
<td>IL-3</td>
<td>&lt;15</td>
<td>&lt;15</td>
<td>&lt;15</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

Peripheral blood counts from Case 2 at three different time points with the corresponding growth factor levels quantified by immunoassay. The patient received chemotherapy between 11/16/84 and 3/14/84 to lower his leukemic burden.2 No serum samples were available for a similar analysis of Case 1.

Abbreviation: WBC, white blood cells.
Reciprocal Dilution

Fig 4. Bioassay of serum IL-3. Leukemic patient sera were tested for bioactive IL-3 and IL-5 in the TF-1 proliferation assay. The reciprocal of the dilution is indicated on the horizontal axis and the optical density indicating the amount of proliferation is indicated on the vertical axis. Serum from all three time points was assayed simultaneously. The assay was rendered monospecific by using a 1 μg/mL final concentration of monoclonal rat anti-IL-3, BVD3-6G8 (□), or anti-IL-5, JES1-39D10 (▲); □ indicates no MoAb. On 1/16/84 and 3/14/84, inhibition of proliferation was evident in the presence of anti-IL-3 antibody, documenting serum levels of IL-3 on those days. Serum IL-5 was not detected in this assay, as anti-IL-5 did not alter TF-1 proliferation.

indicated that these other myeloid growth factors were not detectably circulating in the serum of this patient.

DISCUSSION

In this report, we have extended our analysis of acute lymphocytic leukemia and eosinophilia associated with the t(5;14) translocation. In both cases we have studied, we have documented the joining of the IL-3 gene from chromosome 5 to the IgH gene from chromosome 14. The breakpoints on chromosome 5 are within 500 bp of each other, suggesting that additional breakpoints will be clustered in a small region of the IL-3 promoter. The PCR assay we have developed will be useful in the screening of additional clinical samples for this abnormality.

The finding of a disrupted IL-3 promoter associated with an otherwise normal IL-3 gene implied that this translocation might lead to the over-expression of a normal IL-3 gene product. In this work, we have documented that this is true. In addition, neither GM-CSF nor IL-5 are over-expressed by the leukemic cells. Furthermore, in one patient, serum IL-3 could be measured and correlated with disease activity. To our knowledge, this is the first measurement of human IL-3 in serum and its association with a disease process. The measurement of serum IL-3 in this and other clinical settings may now be indicated.

The finding of the IL-3 gene adjacent to a cancer-associated translocation breakpoint suggests that its activation is important for oncogenesis. It is our thesis that an autocrine loop for IL-3 is important for the evolution of this leukemia.26 The excessive IL-3 production that we have documented would be one feature of such an autocrine loop. The final proof of our thesis must await additional data. In particular, from the study of additional clinical samples, it will be necessary to document that the IL-3 receptor is present on the leukemic cells and that anti–IL-3 antibody decreases proliferation of the leukemia in vitro.

An important aspect of this work is the suggestion of a therapeutic approach for this disease. If an autocrine loop for IL-3 can be documented in this disease, attempts to lower circulating IL-3 levels or block the interaction of IL-3 with its receptor may prove useful. Because it is also possible that the eosinophilia in these patients is mediated by the paracrine effects of leukemia-derived IL-3, similar interventions may improve this aspect of the disease. Antibodies or engineered ligands to accomplish these goals may soon be available.

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