that malignancy increased its expression.\textsuperscript{9} Despite the extensive research performed, there is little evidence that either supports or refutes the role of fragile sites in cancer. Our results suggest that the origin of Ph\textsuperscript{c} chromosome is independent of spontaneous instability or fragile site expression, because bands 9q34 or 22q11 were not observed in our series either spontaneously or by induced breakage. The proximity between BCR and ABL genes in specific cell cycle phases may explain the genesis of the translocation.\textsuperscript{10} The increased chromosome instability affecting specific bands could be a systemic manifestation or a consequence of the leukemic process, possibly due to certain unknown clastogenic factors of the neoplastic cells.

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Key words
Chromosome instability, spontaneous breakage, fragile site expression, chronic myeloid leukemia.

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References

Vascular endothelial growth factor isoforms 121 and 165 are expressed on B-chronic lymphocytic leukemia cells

We used flow cytometry to analyze the expression of vascular endothelial growth factor (VEGF) on leukemic cells of 11 B-CLL patients using a monoclonal antibody directed against the 121 and 165 isoforms. All patients tested displayed a positive reaction for VEGF. Interestingly, mean fluorescence intensity (MFI) of cases with a progressive pattern of disease was higher than MFI of patients with stable disease. Cellular VEGF-expression may be involved in disease progression.

Sir,

An increasing body of evidence has been accumulated which suggests a central role for angiogenesis in the pathophysiology of hematopoietic malignancies.\textsuperscript{1-6} Although most information comes from patients with multiple myeloma (MM) it has been recently shown that acute myeloid leukemia (AML) cells express vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis.\textsuperscript{7} Furthermore, elevated levels of basic fibroblastic growth factor (b-FGF) were detected in the urine of patients with acute lymphoblastic leukemia (ALL) and associated with increased bone marrow microvesSEL density.\textsuperscript{8} In B-cell chronic lymphocytic leukemia (CLL), evidence for increased angiogenesis has been demonstrated by Kini et al.\textsuperscript{8} and clinico-prognostic implications of of such a feature have been investigated by our group in a series of CLL patients with early disease.\textsuperscript{9} With respect to the source and mechanisms of production of serum VEGF in CLL much remains unproven. Chen et al.,\textsuperscript{10} on the basis of results obtained in RT-PCR and Slot-blot analysis, showed that B-CLL cells express VEGF m-RNA and identified in VEGF 121 and VEGF 165 the two isoforms produced. We studied 11 B-cell CLL patients using flow cytometry and a monoclonal antibody anti-VEGF whose specificity covered...
either the 165 or 121 isoforms (Clone, 26503; mouse IgG 2b; R & D Systems Inc.). In all instances diagnosis of typical B-cell CLL relied on either cytomorphologic or immunologic analysis (CD5+, CD23+, CD22+/-, FMC7-, CD79b– and dim light chain Sm Ig expression). All experiments were carried out in double staining according to previously reported methods.9

All patients tested displayed a positive reaction for VEGF (Table 1) and the percentage of leukemic cells reactive to VEGF ranged between 37.2% and 97% (median, 62.5%). Interestingly, mean fluorescence intensity (MFI) was higher in patients with a progressive pattern of disease than in patients with stable disease at the time of sampling (median MFI, 138.5 versus 109; p=0.170) (Figure 1).

In lymphoma and in other types of human cancers neoplastic cells produce VEGF, although several accessory cells such as macrophages, peripheral blood T-lymphocytes and platelets also contain appreciable amounts of VEGF.1 Fiedler et al.5 have shown that AM L cells express VEGF as well as VEGF receptors (VEGFR-1 and VEGFR-2). These findings raise the possibility that VEGF may play a role as an autocrine growth factor for AM L cells. Bellamy et al.4 studied different hematopoietic cell lines and found that they all expressed VEGF, whereas only 50% of them expressed b-FGF. In B-cell CLL studies dealing with either cellular or soluble VEGF are limited.8-10 Our results, though based on a small number of patients, lend further support to previous studies suggesting a role of angiogenesis in CLL. Interestingly, the VEGF density changed as a function of disease status, thus supporting a potential involvement of such a protein in the mechanisms of disease progression. Finally, the flow cytometric identification of the isoforms produced raises the possibility of using specific angiogenesis inhibitors as a novel therapeutic strategy for CLL.

Table 1.

<table>
<thead>
<tr>
<th>Pts</th>
<th>VEGF+/CD19+</th>
<th>VEGF (MFI)</th>
<th>Disease-status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>143</td>
<td>PD after FLUDA</td>
</tr>
<tr>
<td>2</td>
<td>72.1</td>
<td>129</td>
<td>PD after FLUDA</td>
</tr>
<tr>
<td>3</td>
<td>86.4</td>
<td>108</td>
<td>PD after CLB</td>
</tr>
<tr>
<td>4</td>
<td>60.2</td>
<td>99</td>
<td>SD (Stage A)</td>
</tr>
<tr>
<td>5</td>
<td>56.1</td>
<td>105</td>
<td>SD (Stage A)</td>
</tr>
<tr>
<td>6</td>
<td>51</td>
<td>128</td>
<td>SD (Stage A)</td>
</tr>
<tr>
<td>7</td>
<td>48</td>
<td>144</td>
<td>PD after CLB</td>
</tr>
<tr>
<td>8</td>
<td>97.7</td>
<td>134</td>
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<tr>
<td>9</td>
<td>97.3</td>
<td>148</td>
<td>SD (Stage A)</td>
</tr>
<tr>
<td>10</td>
<td>70.5</td>
<td>156</td>
<td>PD after CLB</td>
</tr>
<tr>
<td>11</td>
<td>37.2</td>
<td>109</td>
<td>SD (Stage A)</td>
</tr>
</tbody>
</table>

MFI = mean fluorescence intensity; PD = progressive disease; SD = stable disease.

Figure 1. VEGF-FTIC/ CD19-PE double staining: A) negative control; B) patient with stable disease; C) patient with progressive disease.

Key words
Chronic lymphocytic leukemia, VEGF-expression, disease-progression.

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References
Expansion of CD3-CD56+ cytotoxic cells from patients with chronic lymphocytic leukemia: in vitro efficacy

Cytokine-induced killer cells were expanded from 12 patients with chronic lymphatic leukemia. In these cultures, T-cells increased significantly from less than 10% to 56.3±29.4% after 14 days. Similarly, the percentage of cells expressing the natural killer-cell marker CD56 increased significantly to 31.8±26.3%.

Sir,

Cytokine-induced killer (CIK) cell cultures were generated from 12 patients with chronic lymphatic leukemia (CLL) and assayed for their expression of various cell surface markers by flow cytometry. On day 0 all patients had at least 90% CD19 positive lymphocytes in their blood. After two weeks of culture CD19 positive cells had decreased significantly to 33.3±30.5% with the range being 1.5 and 78.6% (p=0.02; Figure 1).

In contrast, fewer than 10% of the lymphocytes were CD3+ on day 0 of culture. Expression of CD3 increased to 56.3±29.4% after two weeks of culture (p=0.03). Similarly, CD8 positive cells increased to 53.8±31.4% after two weeks (p=0.08). The percentage of CD56 positive cells increased significantly to 11.0±11.1% after one week of culture and to 31.8±26.3% after two weeks (p=0.01; Figure 1). CD56 positive cells co-expressed CD3. Next, we tested the cytotoxic activity of CIK cells using a 51Cr release assay. Fourteen-day old CIK cells were tested using autologous or allogeneic leukemia cells as targets. In the autologous setting CIK cells were unable to lyse leukemia cells. However, CIK cell lysis could be increased by addition of anti-CD3 monoclonal antibody. Addition of anti-CD32 antibody did not abolish this effect. In contrast, addition of anti-CD19 antibody did not produce an increase in cytotoxicity. In the allogeneic setting CIK cells showed a weak cytotoxic effect on leukemia cells. Again, this effect could be increased by addition of anti-CD3 antibody. This effect was not abolished by addition of anti-CD32 (Figure 2).

CLL cells are resistant to T-lymphocytes.