Constitutive Expression of Interleukin-8 and its Receptor in Human Myeloid and Lymphoid Leukemia

By Andreas Tobler, Bernhard Moser, Beatrice Dewald, Thomas Geiser, Hugo Studer, Marco Baggiolini, and Martin F. Fey

Interleukin-8 (IL-8), a member of the family of small inducible cytokines, is mainly known for its striking neutrophil-activating properties. Constitutive IL-8 production is negligible in normal leukocytes. We examined expression of IL-8 and its receptor in purified leukemic cells from patients with untreated acute myeloblastic leukemia (AML) and lymphoid leukemias. In the majority of cases (18 of 26 AML, 8 of 15 lymphoid leukemias), the cells constitutively expressed IL-8 mRNA transcripts. In all but 3 of these cases, IL-8 mRNA-expressing cells secreted biologically active IL-8 protein. Immunocytochemical analysis showed intracellular IL-8 (5% to 90% of total cells), demonstrating that the leukemic cells themselves rather than contaminants (monocytes or lymphocytes) were the source of IL-8.

NORMAL HEMATOPOIESIS is governed by an elaborate system of cytokines whose expression is strictly regulated. Most cytokines are produced upon stimulation by various agents, but constitutive expression is negligible in normal tissues. In contrast, myeloid and lymphoid leukemia cells have been shown to produce various cytokines in the absence of stimulation, indicating that constitutive cytokine production may be important in autoregulatory growth stimulation of leukemic cells by autocrine or paracrine circuits.

Interleukin-8 (IL-8) is a potent activator and chemotactic agent of neutrophils. The gene of IL-8 has been cloned and sequenced and has been located on chromosome 4 (q12-21). Fibroblasts, endothelial cells, and monocytes are major producers of IL-8 when stimulated by agents such as IL-1 or tumor necrosis factor α (TNFα), whereas its constitutive expression is negligible. A role for IL-8 in the pathophysiology of inflammatory disorders has been proposed, eg., in rheumatoid arthritis, in which high levels of IL-8 are found in the synovial fluid of affected joints.

Binding studies with IL-8 have indicated that human neutrophils bear two classes of high-affinity receptors corresponding to two proteins of 44 Kd and 70 Kd. Recently, two cDNAs for IL-8 receptors have been isolated, both of which encode polypeptides with seven putative transmembrane domains typical of G-protein–coupled receptors. IL-8 belongs to a family of structurally related proinflammatory cytokines with molecular weights ranging between 7 and 10 Kd that have been termed small (inducible) cytokines. So far, the main activity of these cytokines appears to be the regulation of blood leukocyte functions in inflammatory processes, but little is known about their possible effects in normal and leukemic hematopoiesis. Evidence for a negative and positive regulation of hematopoietic precursor cells by small inducible cytokines has been presented for macrophage-inflammatory protein-1α (MIP-1α), also known as LD78 or stem cell inhibitory factor. MIP-1α was shown to inhibit proliferation of earlier precursors (burst-forming unit-erythroid [BFU-E] and colony-forming unit–granulocyte, erythrocyte, monocyte, megakaryocyte [CFU-GEMM]), and to stimulate clonal growth of committed granulocyte-macrophage precursors (CFU-GM).

Leukemic cells from acute myeloblastic leukemia (AML) and acute lymphoblastic leukemia (ALL) patients were reported to constitutively produce MIP-1α, suggesting that leukemic cells may release small cytokines. We have studied the expression of IL-8 and its receptor in leukemic cells obtained from patients with untreated AML, ALL, and chronic lymphocytic leukemia ( CLL) and in several leukemic cell lines, and have examined whether receptor expression is related to functional activities.

MATERIALS AND METHODS

Cell lines. The following leukemic cell lines were cultured in McCoy’s medium (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Nabi, Miami, FL) in a humidified atmosphere containing 5% CO2 at 37°C: HL-60 (promyelocytic), KG-1 (myeloblastic), HEL and K562 (erythroid/myeloblastic), KCL2 (myeloblastic, Ph+), Raji and Daudi (B-lymphoblastic), Jurkat and MOLT-3 (T-lymphoblastic). In addition, normal human lung fibroblasts (WI38) and human squamous lung carcinoma cells (SK-MES-1) were used.

Leukemic cells. Bone marrow or peripheral blood cells were obtained at diagnosis from 26 AML (including 1 case of chronic myelomonocytic leukemia – M6) and 1 case of acute lymphoblastic leukemia – ALL, and 10 Kd that have been termed small (inducible) cytokines. So far, the main activity of these cytokines appears to be the regulation of blood leukocyte functions in inflammatory processes, but little is known about their possible effects in normal and leukemic hematopoiesis. Evidence for a negative and positive regulation of hematopoietic precursor cells by small inducible cytokines has been presented for macrophage-inflammatory protein-1α (MIP-1α), also known as LD78 or stem cell inhibitory factor. MIP-1α was shown to inhibit proliferation of earlier precursors (burst-forming unit-erythroid [BFU-E] and colony-forming unit–granulocyte, erythrocyte, monocyte, megakaryocyte [CFU-GEMM]), and to stimulate clonal growth of committed granulocyte-macrophage precursors (CFU-GM).

Leukemic cells from acute myeloblastic leukemia (AML) and acute lymphoblastic leukemia (ALL) patients were reported to constitutively produce MIP-1α, suggesting that leukemic cells may release small cytokines. We have studied the expression of IL-8 and its receptor in leukemic cells obtained from patients with untreated AML, ALL, and chronic lymphocytic leukemia (CLL) and in several leukemic cell lines, and have examined whether receptor expression is related to functional activities.
myeloid leukemia in myeloid blast crisis (CML-myBC) and 10 ALL patients. In addition, samples of 3 B-CLL, 1 prolymphocytic leukemia (PLL), and 1 high-grade malignant non-Hodgkin’s lymphoma (NHL) with bone marrow involvement in leukemic phase were examined. In acute leukemia patients, the diagnosis was established according to the French-American-British (FAB) classification and specific esterase staining on cytospin preparations. Mononuclear cells were separated by Ficoll-Hypaque density gradients (1.077 g/L; Nyegaard, Oslo, Norway) and cryopreserved in liquid nitrogen until use. We studied both fresh non-monocyte-depleted samples and samples in which monocytes were depleted by plastic adherence. All cell samples analyzed were composed of ≥95% blast cells as assessed by morphology and specific esterase staining on cytospin preparations.

Culture conditions for leukemic cells. For determination of spontaneous IL-8 protein release, 27 of the 41 samples (16 AML and 11 lymphoid leukemias) were cultured at 10^6 cells/mL in McCoy’s medium supplemented with 10% FBS and penicillin/ streptomycin. Cell viability, as assessed by trypan blue exclusion, was greater than 90% at the end of incubation in all experiments. All reagents used were free of endotoxin, as determined by the limulus-amebocyte assay. After 72 hours of culture, conditioned media plus-amebocyte assay. After 72 hours of culture, conditioned media were analyzed for IL-8 protein release, as described below.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>FAB</th>
<th>IL-8 mRNA</th>
<th>IL-8-R mRNA</th>
<th>IL-8 Protein Release (ng/mL)</th>
<th>IL-8 Protein (intracytoplasmic)</th>
<th>M-CSF mRNA</th>
<th>GM-CSF mRNA</th>
<th>IL-1β mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M1</td>
<td>++</td>
<td>+</td>
<td>1.5</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>M4</td>
<td>+ (+)</td>
<td></td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>M1</td>
<td>–</td>
<td>+</td>
<td>0.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>M2</td>
<td>+ +</td>
<td>+</td>
<td>0.2</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>M4</td>
<td>++ (+)</td>
<td></td>
<td>0.9</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>M3</td>
<td>+</td>
<td>–</td>
<td>0.4</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>M4</td>
<td>++ (+)</td>
<td></td>
<td>58.4</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>8</td>
<td>M5</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>M2</td>
<td>–</td>
<td>–</td>
<td>0.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>10</td>
<td>M0</td>
<td>+ +</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>11</td>
<td>M1</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>12</td>
<td>M1</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>M1</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>14</td>
<td>M2</td>
<td>+ +</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>M0</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>M1</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>M2</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>M1</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>M3</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>M2</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td>M2</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>M3</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>M4</td>
<td>+ +</td>
<td>–</td>
<td>0.9</td>
<td>90</td>
<td>++ (+)</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>24</td>
<td>M1</td>
<td>(+)</td>
<td>–</td>
<td>0.3</td>
<td>80</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>25</td>
<td>M2</td>
<td>+</td>
<td>–</td>
<td>0.4</td>
<td>60</td>
<td>[+] (+)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>26</td>
<td>M6</td>
<td>++</td>
<td>–</td>
<td>1.5</td>
<td>80</td>
<td>(+) – +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>27</td>
<td>M2</td>
<td>++</td>
<td>–</td>
<td>0.7</td>
<td>40</td>
<td>–</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>28</td>
<td>M6</td>
<td>++</td>
<td>–</td>
<td>19.8</td>
<td>80</td>
<td>(+) +</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>29</td>
<td>M2</td>
<td>+ (+)</td>
<td>0.5</td>
<td>40</td>
<td>(+) – +</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Total 26 18/26 10/25 13/16 12/26 6/25 19/26

A blank space means that a value was not determined.
Abbreviations: IL-8-R, IL-8 receptor; –, not detectable; (+) – ++, detectable at various levels.

* Percentage of leukemic cells containing intracytoplasmic IL-8 protein.

Table 1. Expression of IL-8, IL-8 Receptor, and Other Cytokines in AML Cells

IL-8 levels in culture supernatants. IL-8 protein was determined by a double-ligand immunoassay method.33 Samples and recombinant IL-8 standards were incubated in microtiter plate coated with a mouse anti-IL-8 MoAb. After washing, a goat anti-IL-8 pAb conjugated to alkaline phosphatase was added. Finally, enzyme activity was measured with p-nitrophenylphosphate as a substrate.

Detection of intracytoplasmic IL-8. Determination of intracytoplasmic IL-8 was performed as described.34 Briefly, cytosin preparations were incubated with a polyclonal anti-IL-8 antibody, followed by a biotinylated goat anti-rabbit Ig antibody (Dako, Glostrup, Denmark) and ExtrAvidin-Alkaline Phosphatase (Sigma, St Louis, MO). Substrate solution was added (mixture of naphthol AS-MX sodium salt, Fast Red TR salt, and Levamisole, all in veronal acetate buffer). Finally, the slides were counterstained with hematoxylin Mayer. All incubations were performed at room temperature. No background staining or only faint background staining was observed when the primary antibody was omitted or when a control primary antibody (normal rabbit Ig fraction 1:20, Dako) was used.

Cytosolic free calcium [Ca2+]i changes. Cells were loaded with Fura-2 by incubation for 20 minutes at 37°C with 0.1 mmol Fura-2-AM per 10^6 cells in a buffer containing 130 mmol/L NaCl, 4.6 mmol/L KCl, 1 mmol/L CaCl2, 5 mmol/L glucose, and 20 mmol/L HEPES, pH 7.4. The cells were then washed, resuspended in the same buffer, stimulated at 37°C, and fluorescence-related [Ca2+]i changes induced by human synthetic IL-8 were measured.21,33

From www.bloodjournal.org by guest on August 30, 2017. For personal use only.
RNA extraction and Northern blotting. Total cellular RNA was extracted by the acid guanidinium thiocyanate phenol-chloroform method.\textsuperscript{38} RNA samples (10 μg) were size-separated by an agarose-formaldehyde gel (1% wt/vol) and transferred to a nylon membrane (Hybond-N; Amersham, Amersham, UK). Hybridization with randomly-primed\textsuperscript{125I}-labeled probes (1 to 2 × 10\textsuperscript{6} cpm/mL hybridization solution) was performed for 16 to 24 hours at 42°C, as described.\textsuperscript{38} Filters were washed to a final stringency of 0.1% SSC at 65°C, and exposed for 6 to 96 hours at −70°C to XM-films. Filters were washed to a final stringency of 0.1% SSC at 65°C, and exposed for 6 to 96 hours at −70°C to XM-films. Some filters were deliberately overexposed with respect to the positive controls to detect low levels of mRNA.

DNA extraction and Southern blotting. High molecular weight DNA was extracted from 14 AML and 9 ALL cases, and aliquots of 5 μg were digested with EcoRI, HindIII, Kpn I, and BamHI, and Southern blotted by standard procedures. Filters were hybridized with the IL-8 cDNA probe, washed under stringent conditions (0.1× SSC/0.1% sodium dodecyl sulfate [SDS], 55°C to 60°C), and autoradiographed at −70°C.

DNA probes. The following purified inserts were used as human cDNA probes: granulocyte-macrophage colony-stimulating factor (GM-CSF) (0.8 kb, EcoRI, BamHI) from pCSF-1\textsuperscript{39}, macrophage-CSF (M-CSF) (1.6 kb, XhoI-EcoRI) from pCSF-1\textsuperscript{29}, IL-1β cDNA (0.9 kb, Pst I) from pA-3\textsuperscript{41}, and IL-8 (0.85 kb, BamHI) from p(NAP)/T3\textsuperscript{42}. A 700-bp fragment (BamHI) of an isolated cDNA clone corresponding to one\textsuperscript{34} of the two published cDNAs was used as a probe for IL-8 receptor expression.\textsuperscript{43}

RESULTS

IL-8 expression in human acute myeloid leukemia cells. Northern blot analysis of total RNA from purified blast cells from 26 AML patients was performed with an IL-8 cDNA probe that detects a 1.8-kb mRNA transcript. About two-thirds of the samples (18 of 26) expressed low to high levels of IL-8 mRNA (Table 1 and Fig 1). No aberrant transcripts were detected. There was no apparent correlation between the FAB type and expression of IL-8 mRNA. TNFα-stimulated WI38 fibroblasts that had previously been shown to accumulate high levels of IL-8 mRNA served as positive controls and Jurkat cells served as negative controls.\textsuperscript{38}

The release of IL-8 protein into the culture medium and the presence of intracytoplasmic IL-8 were also studied. The supernatants of the leukemic samples were collected after 3 days of culture and analyzed for IL-8 release (Table 1). The quantity of IL-8 varied considerably and ranged from 0.1 ng/mL (case no. 3) to 58.4 ng/mL (case no. 9). With one exception (case no. 14), IL-8 mRNA production was always associated with IL-8 protein release. Using the same double-ligand immunoassay, we have previously observed that TNFα-stimulated human WI38 fibroblasts secrete about 120 ng/mL IL-8 during 2 days of culture.\textsuperscript{38} To show that the IL-8 produced by the AML cells was biologically active, we tested the supernatants from cases no. 9 and 44 for β-glucuronidase release by cytochalasin-B-pretreated neutrophils.\textsuperscript{44} A good agreement was found between the activity of the two AML supernatants to induce the release of β-glucuronidase from neutrophils and their levels of IL-8 as determined by enzyme-linked immunosorbent assay (ELISA). The β-glucuronidase activity was neutralized by anti–IL-8 antibodies by 90% or more.

To establish whether the leukemic cells themselves, rather than contaminating cells such as monocytes, were the source of IL-8 and to determine the fraction of individual IL-8-producing neoplastic cells, we performed immunocytochemical analyses of cytofilm preparations from 8 AML cell samples before and after culturing of the cells (Table 1). In 7 of these 8 samples, the percentage was high and ranged from 40% to 90%. Values were not affected by culturing the cells for 3 days, indicating that IL-8 production was constitutive and not a result of stimulation by components in the culture medium. Furthermore, the expression patterns we observed in non-monocyte-depleted samples, including cases with and without constitutive IL-8 expression (Table 1, cases no. 1, 2, 4, 9, 10, 11, 15, 16, 17, 26, 27, 28, and 30), did not differ markedly from those seen in monocyte-depleted cells.

The presence of intracytoplasmic IL-8 was always associated with IL-8 mRNA production and IL-8 protein release. Case no. 21, in which no intracytoplasmic IL-8 was detected, was also negative for IL-8 mRNA expression and IL-8 protein release. IL-8 containing leukemic blasts from a patient with AML (M2, FAB, case no. 44) and a patient with
Intracytoplasmatic IL-8 protein in individual AML and ALL cells, as determined by immunocytochemistry (original magnification X 1,300). Cytospins were prepared and further processed with a polyclonal IL-8 antibody as described. (A) Leukemic cells from case no. 44 (AML M2, FAB). (B) Leukemic cells from a patient (case no. 40) with erythroleukemia (AML M6, FAB). (C) Leukemic cells from a patient (case no. 29) with T-ALL. (D) Leukemic cells from a patient (case no. 34) with B-ALL.

Examples of IL-8 containing T-ALL and B-ALL cells are shown in Fig 2C and D. As observed for AML cells, culturing of lymphoid leukemia cells for 3 days did not change the fraction of positive cells.

Northern blots were rehybridized with M-CSF, GM-CSF, and IL-1β cDNAs probes to determine a possible association of IL-8 mRNA expression with that of other cytokines (Fig 3). Five of 14 samples expressed IL-1β mRNA. Low M-CSF mRNA levels were observed in 3 ALL samples (cases no. 20, 36, and 43), but all CLL samples were negative. No GM-CSF mRNA transcripts were detected in any of the samples. Four ALL samples and one B-CLL sample coexpressed IL-1β mRNA and IL-8 mRNA.

IL-8 mRNA production in leukemic cell lines. IL-8 mRNA expression was also examined in several myeloid and lymphoid leukemia cell lines at various stages of differentiation (Fig 4). IL-8 mRNA accumulation was observed in the bipotent (erythroid/myeloblastic) HEL and K562 cells, the myeloblastic KG-1 cells, the promyelocytic HL-60 cells, and in the KCL22 cell line derived from a CML in myeloid blast crisis. These results agree well with those obtained in fresh leukemia samples, suggesting that myeloid
In our study, we used Northern blot analysis of total cell RNA to detect IL-8 mRNA transcripts. HL-60, KG-1, and Jurkat leukemia cell lines did not express detectable IL-8 mRNA. In contrast, fresh lymphoid leukemia cells at early stages of differentiation are able to produce IL-8 mRNA. In contrast to fresh lymphoid leukemia samples, B-lymphoblastic (Raji, Daudi) and T-lymphoblastic (MOLT-3, Jurkat) leukemia cell lines did not express IL-8 mRNA. In contrast to fresh lymphoid leukemia samples, B-lymphoblastic (Raji, Daudi) and T-lymphoblastic (MOLT-3, Jurkat) leukemia cell lines did not express IL-8 mRNA. In contrast to fresh lymphoid leukemia samples, B-lymphoblastic (Raji, Daudi) and T-lymphoblastic (MOLT-3, Jurkat) leukemia cell lines did not express IL-8 mRNA. In contrast to fresh lymphoid leukemia samples, B-lymphoblastic (Raji, Daudi) and T-lymphoblastic (MOLT-3, Jurkat) leukemia cell lines did not express IL-8 mRNA.

**Fig 3.** Steady-state mRNA levels of IL-8 and other cytokines in fresh lymphoid leukemia cells. Northern blot analysis of total cellular RNA. The blot was hybridized sequentially with cDNA probes for IL-8, M-CSF, GM-CSF, and IL-1β. Numbers on top of the lanes indicate case numbers (Table 2). The same controls were included as in Fig 1. The small bottom panel shows the ethidium bromide-stained Northern gel with 28s and 18s rRNA, demonstrating equivalent RNA loading per lane. Transcript sizes are indicated in kilobases.

**Southern blot analyses.** Southern blot analyses using an IL-8 cDNA probe were performed in IL-8-expressing and nonexpressing lymphoid and myeloid leukemia samples (9 and 14 cases, respectively) after DNA digestion with restriction endonucleases (EcoRI, HindIII, Kpn I, and BamHI) based on a cDNA restriction map. No IL-8 gene rearrangements or evidence of gene amplification were seen in any of these cases (data not shown).

**DISCUSSION**

We report constitutive production of IL-8 mRNA and IL-8 protein in acute myeloblastic and lymphoblastic leukemia cells. Expression was assessed by Northern hybridization, immunoassay of IL-8 released in culture, and cytchemistry of cell-associated IL-8.

The possibility that contaminating normal cells (monocytes and lymphocytes) could be the source of IL-8 expression appears unlikely for a number of reasons. We and others have shown that resting blood and bone marrow monocytes produce little if any IL-8 when cultured in the absence of stimuli. In the cytopsin samples used for immunocytochemistry, less than 5% of the cells could be identified as monocytes, whereas the fraction of IL-8-positive AML cells was 40% to 90%. The fact that the fraction of IL-8-positive cells did not change appreciably with culturing indicates that IL-8 expression was constitutive rather than dependent on in vitro induction. Also, we did note a fair number of cases with constitutive cytokine expression that had not been depleted of monocytes. Like monocytes, T lymphocytes produce IL-8 only after stimulation with mitogens, albeit in considerably lower amounts than monocytes. Similarly, IL-8 mRNA production was not observed in resting and lipopolysaccharide-treated B lymphocytes.

The majority of AML samples (18 of 26) and lymphoid leukemia cell samples (8 of 15) accumulated IL-8 mRNA. There was no correlation with the FAB type, although particularly high expression was found in acute myelomonocytic leukemias. Constitutive IL-8 mRNA expression was also present in the KG-1, HEL, and K562 myeloid cell lines.
Table 2. Expression of IL-8, IL-8 Receptor, and Other Cytokines in Lymphoid Leukemia Cells

<table>
<thead>
<tr>
<th>Case No.</th>
<th>mRNA</th>
<th>Protein Release (ng/mL)</th>
<th>Protein M-CSF (intracytoplasmic)*</th>
<th>M-CSF mRNA</th>
<th>GM-CSF mRNA</th>
<th>IL-1 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>B-ALL</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>19</td>
<td>B-ALL</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>20</td>
<td>B-ALL</td>
<td>+</td>
<td>0.4</td>
<td>10</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>B-ALL</td>
<td>+</td>
<td>0.2</td>
<td>30</td>
<td>--</td>
<td>++</td>
</tr>
<tr>
<td>29</td>
<td>T-ALL</td>
<td>(+)</td>
<td>0.02</td>
<td>20</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>34</td>
<td>B-ALL</td>
<td>(+)</td>
<td>0</td>
<td>5</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>36</td>
<td>B-ALL</td>
<td>++</td>
<td>0.05</td>
<td>30</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>43</td>
<td>ALL</td>
<td>++</td>
<td>3.9</td>
<td>10</td>
<td>(+)</td>
<td>++</td>
</tr>
<tr>
<td>45</td>
<td>B-ALL</td>
<td>--</td>
<td>0</td>
<td>0</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>48</td>
<td>B-ALL</td>
<td>--</td>
<td>0.40</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>18</td>
<td>B-CLL</td>
<td>(+)</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>(+)</td>
</tr>
<tr>
<td>22</td>
<td>B-CLL</td>
<td>--</td>
<td>0.40</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>42</td>
<td>B-CLL</td>
<td>--</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>25</td>
<td>PLL</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>31</td>
<td>NHL</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Total 15 8/15 0/9 6/11 3/14 0/14 5/14

A blank space means that a value was not determined.

Abbreviations: IL-8-R, IL-8 receptor; --, not detectable; (+) – ++, detectable at various levels.

* Percentage of leukemic cells containing intracytoplasmic IL-8 protein.

Demonstrating that spontaneous IL-8 expression occurs in (leukemic) myeloid cells at early stages of differentiation.

Different studies have demonstrated spontaneous expression of hematopoietic and inflammatory cytokines by myeloid and lymphoid leukemias, such as IL-1β,6,7,10 IL-6,4,8 TNFα,4,9 GM-CSF,1,4 granulocyte-CSF (G-CSF),2,3 and M-CSF2.5 These studies showed a considerable heterogeneity in spontaneous expression and coexpression of several cytokines in AML. For example, spontaneous IL-1β production was seen in one study in 14 of 44 AML patients (31%)4 and in another in 10 of 17 patients (58%).6 Among the AML samples examined here, few expressed GM-CSF mRNA, approximately half expressed M-CSF mRNA, and the majority (73%) expressed IL-1β mRNA. In both AML and lymphoid leukemia samples we observed frequent coexpression of IL-8 and IL-1β mRNA. Because IL-1β is a strong inducer of IL-8 expression in monocytes,17 This frequent coexpression of IL-1β and IL-8 raises the possibility that, at least in some leukemic cells, IL-1β may enhance IL-8 expression in an autocrine or paracrine manner. The incidence of coexpression of IL-8 and M-CSF was lower in both types of leukemia, and very few AML patients coexpressed IL-8 and GM-CSF.

Unlike hematopoietic growth factors and inflammatory cytokines, IL-8 binds to two distinct G-protein-linked receptors in human neutrophils.20,21 Specific IL-8 binding was previously reported in myeloid leukemia, but not in T- and B-lymphoblastic cell lines.22 Using an IL-8 receptor cDNA probe, we found that 10 of 25 AML samples accumulated IL-8 receptor mRNA, whereas all lymphoid leukemia cells

Fig 4. IL-8 mRNA transcripts in human leukemic cell lines. Northern blot analysis of total cellular RNA hybridized with IL-8 cDNA. Lane 1, MOLT-3; lane 2, Raji; lane 3, HEL; lane 4, Daudi; lane 5, Jurkat; lane 6, KG-1; lane 7, K562; lane 8, KC122; lane 9, HL-60, untreated; lane 10, HL-60 cells induced for 4 days with 1.25% dimethylsulfoxide for 4 days; lane 11, TNFα-induced WI38 fibroblasts; lane 12, HL-60 cells induced for 18 hours with phorbol ester. The small bottom panel shows the ethidium bromide-stained Northern gel with 28s and 18s rRNA, demonstrating equivalent RNA loading per lane. Transcript sizes are indicated in kilobases.

---
EXPRESSION OF IL-8 AND ITS RECEPTOR IN LEUKEMIA

Fig 5. Steady-state mRNA levels of IL-8 and its receptor in fresh AML cells. Northern blot analysis of total cellular RNA. The blot was hybridized sequentially with cDNA probes for IL-8 and IL-8 receptor (IL-8-R). Numbers on top of the lanes indicate case numbers (Table 1). The following controls were included: Jurkat cells (a); HL-60 cells, untreated (b), TNF-α-stimulated W138 fibroblasts (c), and SK-MES-1 lung carcinoma cells (d). The small bottom panel shows the ethidium bromide-stained Northern gel with 28s and 18s rRNA, demonstrating equivalent RNA loading per lane. Transcripts are indicated in kilobases.

were negative. With one exception (CML in myeloid blast crisis), IL-8 receptor mRNA expression was always associated with that of its ligand.

In contrast to neutrophils, no biologic effects of IL-8 have been described on immature myeloid cells. We now show that IL-8 induced cytosolic free calcium changes in myeloid leukemic cells expressing the IL-8 receptor. This indicates that IL-8 activates the same signaling pathway in myeloid leukemic cells and neutrophils. Therefore, it is conceivable that one of the two IL-8 receptors, presumably IL-8 receptor-1, is involved in functions that are unrelated to the recruitment of inflammatory cells, and that constitutively released IL-8 could act in myeloid leukemic cells in an autocrine manner. Most recently, it was shown that IL-8 as well as several other CXC and CC chemotactic cytokines suppress colony formation of immature subsets of normal myeloid progenitor cells stimulated by GM-CSF plus stem cell factor.59

Our study shows that AML and lymphoid leukemias frequently express IL-8 constitutively, and that the expressed IL-8 receptor is functional. At this point, our findings suggest that neutrophil activation may not be the only biologic raison d'être of IL-8, and that a search for a broader spectrum of IL-8 activity in hematopoiesis may be warranted.

ACKNOWLEDGMENT

We thank G. Niklaus, M. Oestreicher, and J. Zbären for excellent technical assistance.

REFERENCES


12. Matsushima K, Oppenheim JJ: Interleukin 8 and MCAF: Novel inflammatory cytokines inducible by IL 1 and TNF. Cyto-
kine 1:2, 1989


15. Strieter RM, Kunkel SL, Showell HJ, Marks RM: Endothelial cell gene expression of a neutrophil chemotactic factor by TNF, IL-1 and LPS. Science 243:1467, 1989


31. Yamamura YT, Hattori T, Obaru K, Sakai K, Asou N, Ta-


34. Studer H, Gerber H, Zbären P, Peter HJ: Histomorphologi-
al and immunohistochemical evidence that human nodular goiter grow by episodic replication of multiple clusters of thyroid follicular cells. J Clin Endocrinol Metab 75:1151, 1992


Human GM-CSF: Molecular cloning of the complementary DNA and purification of the natural and recombinant proteins. Science 228:810, 1985
Constitutive expression of interleukin-8 and its receptor in human myeloid and lymphoid leukemia

A Tobler, B Moser, B Dewald, T Geiser, H Studer, M Baggiolini and MF Fey