The Role of Interleukin-8 in Patients with Hematologic Diseases

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Abstract: We measured the plasma Interleukin-8 (IL-8) concentration in 240 patients with hematological diseases to investigate the role of IL-8 in hematologic disease. Significantly higher concentrations of IL-8 were observed in some patients with acute myelogenous leukemia (AML), acute lymphoid leukemia, and chronic myelogenous leukemia than in healthy control subjects. We found no correlation between the plasma IL-8 concentration and the number of white blood cells, neutrophils, or monocytes, and the serum C-reactive protein concentration. In one patient with acute monocytic leukemia (M5), the high plasma concentrations of IL-8 before treatment fell to control levels after chemotherapy along with a decrease in the number of leukemic monocytes. A positive correlation was observed between plasma IL-8 and IL-6, but not between the concentrations of other cytokines. High concentrations of IL-8 were detected in the supernatant of primary cultures of leukemia cells collected from 10 patients with AML. The reverse transcriptase-polymerase chain reaction showed that these cells expressed IL-8 mRNA. Leukemic cells might therefore be the main source of IL-8 in vivo under some circumstances. IL-8 might participate in the fundamental disease process of AML, in addition to its role in inflammation.

Key words: IL-8, hematologic disease, infection, constitutive production

Introduction

Interleukin-8 (IL-8) is an important mediator of inflammation produced by monocytes/macrophages, fibroblasts, and endothelial cells in response to stimulation by interleukin-1 (IL-1), tumor necrosis factor (TNF), or lipopolysaccharide (LPS)1-3. It has attracted attention as a new cytokine with a wide range of biologic activities, such as the facilitation of neutrophilic leukocyte chemotaxis1,2,4), the activation of leukocyte adhesion to the vascular endothelium, the up-regulation of the CD11b/CD18 glycoprotein5-7, and the release of superoxide (O2-)8). Clinically, the role of IL-8 in inflammatory diseases, such as septicemia and various other infections9), adult respiratory distress syndrome10), psoriasis11,12), rheumatoid arthritis13-17), and gout18), has been studied, and a relationship between IL-8 and these diseases has been postulated. Recently, IL-8 production by various cancer cells and acute leukemia cells, especially acute monocytic leukemia (AMoL) cells, has been reported, and the complex metabolic dynamics of IL-8 are gradually being clarified19-22).

To elucidate the relationship between IL-8 and the pathogenesis of various hematologic
diseases, we measured plasma concentrations of IL-8 in the present study. The constitutive production of IL-8 by leukemia cells from patients with AMoL was also studied in a primary culture system.

Materials and Methods

Patients

This study was conducted on 240 patients with hematological diseases who consulted our department over the 2-year period between 1993 and 1995. Seventy-seven patients with acute myelogenous leukemia (AML), 13 with acute lymphoid leukemia (ALL), 36 with multiple myeloma, 33 with malignant lymphoma, 16 with chronic myelogenous leukemia (CML), 8 with chronic lymphoid leukemia, 16 with a myelodysplastic syndrome, and 7 with aplastic anemia were enrolled in the study. None of the patients had achieved complete remission. Inflammatory conditions were sometimes difficult to diagnose with certainty in daily clinical practice (Fig. 1). In addition, 22 healthy volunteers were included in this study as normal controls, for a total of 262 subjects.

Measurement of IL-8 and various other cytokines

Cytokines in the patients' plasma and in the supernatants of leukemia cell cultures were measured by the enzyme-linked immunosorbent assay method using the following kits: IL-8, IL-1β, IL-6, TNF-α, granulocyte macrophage colony-stimulating factor (Toray-Fuji Bionics, Tokyo, Japan), and granulocyte colony-stimulating factor (Amersham, Buckinghamshire, UK).

Primary culture of acute myeloid leukemia cells

Mononuclear cells separated from the bone marrow of patients with AML were suspended in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (Sigma Chemical Co., St. Louis, MO), and incubated for 24 hours at 37°C under 5% CO₂ in air. After adherent cells were removed and the presence of leukemia cells at proportions of 95% or greater was confirmed, primary cultures were started at a concentration of 1 × 10⁶ cells/ml. LPS (Sigma, 10 ng/ml) was added to each culture, and the concentration of IL-8 protein in the supernatant was compared with that without LPS.

Examination of IL-8 mRNA in leukemia cells reverse transcriptase-polymerase chain reaction (RT-PCR)

Total cellular RNA was extracted from acute monocytic leukemia (AMoL) cells with the acid guanidium isothiocyanate phenol-chloroform method. First-strand cDNA was synthesized with avian myeloblastosis virus reverse transcriptase from 5 µg of RNA and was used for the PCR reaction.

Primer sequences were as follows: IL-8, 5'-primer 5'-AACATGACTTCCAAGCTGGC-3' and 3'-primer 5'-ACTGGCATCTTCACTGATTC-3', β-actin, 5'-primer 5'-CTTCTACAATGAGCTGCCTG-3' and 3'-primer 5'-TCATGAGGTAGTCAGTCAGG-3'.

Various amounts of cDNA were amplified with PCR under the following conditions: 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute with 19 cycles for β-actin or with 24 cycles for IL-8. The amplification products were visualized under ultraviolet light after staining of the gel with ethidium bromide and were subsequently transferred onto a nylon membrane (Biodyne B, Pall, East Hills, NY). Specific PCR products were detected by hybridization with synthetic oligonucleotide probes (for IL-8, 5'-TTGAGAGTGGACCACAICTGCAGCAACACAG-3'; for β-actin, 5'GAGAAGATGACCACACAGATCATGTGTTG-
AGACC-3’) labeled with 32P. The relative expression levels of the IL-8 genes were evaluated with a densitometer (Model 300A, Molecular Dynamics, Sunnyvale, CA).

Results
Plasma concentrations of IL-8 in patients with hematologic diseases
The plasma concentrations of IL-8 in healthy normal subjects ranged from 12.3 to 31.3
pg/ml, indicating stable values (Fig. 1). Significant increases were noted in some patients with AML, ALL, and CML (Fig. 1). Twenty-four patients who had no obvious inflammatory conditions or infectious disease were selected from among 77 patients with AML. Of these patients, higher concentrations were noted in patients with acute myelomonocytic leukemia (AMMoL) or AMoL (Fig. 2).

Correlation between laboratory data and the plasma concentrations of IL-8

Other clinical laboratory data in addition to IL-8 were investigated in most patients. No significant correlation was found between the plasma concentrations of IL-8 and other clinical laboratory data, such as white blood cell count, neutrophil count, monocyte count, blast count in peripheral blood, and serum C-reactive protein concentration (data not shown).

Correlation between the plasma concentration of IL-8 and that of other cytokines

Concentrations of other cytokines in addition to IL-8 were measured in the plasma of
Fig. 3. The correlation between IL-8 and IL-6 concentrations in some patients.

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\begin{align*}
n &= 39 \\
y &= 0.49206 + 0.11802x \\
r &= 0.734
\end{align*}
\]

Fig. 4. Clinical course and plasma IL-8 values in a patient with AMoL (case No. 9 in Table 1) on the left, and a patient with ALL on the right. Details are described in the results.
Table 1. Detection of IL-8 and various cytokines in culture supernatants from acute myelogenous leukemia cells.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>FAB</th>
<th>IL-8 (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
<th>IL-1β (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LPS (-)</td>
<td>LPS (+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M1</td>
<td>60</td>
<td>58</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>2</td>
<td>M1</td>
<td>587</td>
<td>748</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>3</td>
<td>M2</td>
<td>487</td>
<td>700</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>4</td>
<td>M2</td>
<td>440</td>
<td>420</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>5</td>
<td>M4</td>
<td>1372</td>
<td>1350</td>
<td>940</td>
<td>1190</td>
</tr>
<tr>
<td>6</td>
<td>M5a</td>
<td>826</td>
<td>813</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>7</td>
<td>M5a</td>
<td>1035</td>
<td>984</td>
<td>38</td>
<td>&lt;10</td>
</tr>
<tr>
<td>8</td>
<td>M5b</td>
<td>822</td>
<td>813</td>
<td>16</td>
<td>66</td>
</tr>
<tr>
<td>9</td>
<td>M5b</td>
<td>227</td>
<td>1052</td>
<td>26</td>
<td>&lt;10</td>
</tr>
<tr>
<td>10</td>
<td>M5b</td>
<td>591</td>
<td>640</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

Bone marrow leukemia cells were cultured in 24-well plates at a concentration of 10^4 cells/ml per well with and without LPS (10 ng/ml). The supernatants were removed after 48 hours of incubation. Cytokine concentrations were measured using ELISA kits, as described in the text. FAB, French-American-British classification system.

some of the 240 patients. A positive correlation was found between the concentrations of IL-8 and IL-6 (γ=0.734) but not with the concentrations of other cytokines (Fig. 3).

Clinical course of patients with leukemia and high plasma IL-8 concentrations

Changes in the concentrations of IL-8 and other cytokines during the clinical course of two patients were investigated (Fig. 4). In one patient with AMoL (M5b), the plasma IL-8 was high when the leukemic monocyte count was elevated before chemotherapy was started. The IL-8 concentration fell as the leukocyte, neutrophil, and monocyte counts decreased in response to treatment (Table 1, case 9). No infectious complications were noted during the observation period. In a patient with ALL (LT), the plasma IL-8 concentration increased markedly during an episode of severe pneumonia when severe leukopenia had developed after maintenance chemotherapy.

Production of IL-8 and other cytokines and expression of IL-8 mRNA by leukemia cells primary culture

Leukemia cells separated from 10 patients with AML were thought to be competent to be investigated, and primary culture was established. IL-8 was produced in each culture supernatant of the leukemia cells (Concentration, 60 to 1372 pg/ml). TNF-α, IL-1β or IL-6 were also produced in addition to IL-8 in four cases (cases 5, 7, 8, and 9; Table 1). The IL-8 concentration in the culture supernatant of leukemia cells increased in response to LPS in four cases (case 2, 3, 9, and 10) but not in the other six cases. IL-8 mRNA was expressed by RT-PCR within 12 hours of the beginning of culture in cells isolated from patient 9 with an increase in the ratio of IL-8:β-actin mRNA during stimulation with LPS (Fig. 5).

Discussion

In the present study, plasma concentrations of IL-8 were measured in 240 patients with the severe hematologic diseases encountered in daily clinical practice. Significant increases
in the plasma concentration of IL-8 were noted in some patients with AML, ALL, and CML.

Previous studies of plasma IL-8 have been conducted, mainly with reference to infectious diseases. No studies, therefore, have been performed on the significance of IL-8 in hematologic diseases. In this study, the plasma concentration of IL-8 was measured at random in patients with hematologic diseases. In most patients, no significant correlation was noted between the plasma concentration of IL-8 and the white blood cell count, leukemia cell count, neutrophil count, monocyte count, or serum CRP concentration. In a time-course study of a patient with leukemia, the plasma concentration of IL-8 was elevated during an episode of pneumonia (Fig. 4).

The intensity of inflammation reflects the concentrations of the proinflammatory mediators IL-1 and TNF at the site of inflammation. In cells subjected to such inflammatory stimuli, IL-8 is synthesized as a chemical mediator of inflammation\(^1\)\(^-\)\(^3\). Ostermann et al.\(^9\) have studied the dynamic changes in IL-8, IL-6, and TNF-\(\alpha\) in serum during septic shock in leukopenic patients after intensive chemotherapy. Concentrations of these cytokines reached their peaks within 48 hours of the onset of sepsis and were not correlated with the peripheral leukocyte count\(^9\). Infection after chemotherapy for acute leukemia is frequently encountered in clinical practice, especially in patients with severe leukopenia. Significantly

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**Fig. 5.** IL-8 mRNA expression demonstrated by RT-PCR in a primary culture of leukemia cells from a patient with acute monocytic leukemia (case No. 9 in Table 1). Methods are described in the text.

(A) M: \(\phi\times 174\) Hae III digest, 1: 2-hour culture without stimulation; 2: 2-hour of LPS stimulation; 3: 4-hour culture without stimulation; 4: 4-hour of LPS stimulation; 5: 12-hour culture without stimulation; 6: 12-hour of LPS stimulation.

(B) The relative level of IL-8 mRNA expression when \(\beta\)-actin is given a value of 1. IL-8 mRNA expression was seen even without stimulation. LPS augmented the expression of IL-8 mRNA.
elevated concentrations of IL-8 in the plasma of patients with leukemia supports the concept of participation of IL-8 in infection. The high plasma concentrations of IL-8, despite a decrease in normal monocytes because of intense bone marrow suppression, also suggests that IL-8 is produced by other cells, such as endothelial cells, tissue macrophages, and fibroblasts. In the present study, we found no correlation between plasma concentrations of IL-1β and TNF-α and those of IL-8. Nevertheless, a positive correlation was found between the plasma concentrations of IL-8 and IL-6, a finding that confirms the relationship between IL-6, a cytokine that induces production of acute-phase inflammatory proteins, and IL-8. These results suggest that other processes for the production of IL-8 and IL-6 could exist in addition to inflammation.

In recent years, attention has been focused on the constitutive production of IL-8 by human tumor cells themselves, and the relationship of IL-8 production to the disease process has been discussed. Suzuki et al. isolated and purified a neutrophil chemotactic peptide with a structure similar to that of IL-8 from the nonstimulated culture supernatant of a lung giant cell carcinoma cell line and suggested the existence of specific reaction between neutrophils and cancer cells with reference to antitumor immune activity. Expression of IL-8 mRNA and production of IL-8 protein have been reported in myelogenous leukemia cell lines, such as HL-60 and U937. Tobler et al. have studied IL-8 mRNA expression, the IL-8 receptor, and IL-8 production by leukemia cells from patients with AML and ALL. Production of IL-8 was demonstrated in cells from most of these patients. According to the authors, leukemic cells themselves, rather than monocytes and lymphocytes, are the source of IL-8. Vinante et al. have also reported the production of IL-8 by leukemia cells themselves in primary culture. Our culture experiment of monocytic leukemia cells demonstrated augmented expression of IL-8 mRNA after 4 hours. The concentration of IL-8 protein in the culture supernatant rose an average of 16 hours later. The peak IL-8 concentration, sometimes reaching levels of 1000 pg/ml or higher, appeared 48 to 60 hours later. LPS stimulation facilitated IL-8 production in four cases (cases 2, 3, 9, and 10) but not in the other six cases. The plasma IL-8 concentration rose to high levels, along with an increase in leukemic monocytes (Fig. 4a). Constitutive production of IL-8 by leukemia cells themselves thus appears to be the main route of IL-8 production in vivo in patients with AMoL. In our study of primary leukemia cell cultures, TNF-α and IL-1β were also induced, in addition to IL-8, in some patients. Production of TNF-α and IL-1β by leukemia cells has been reported on several occasions. The up-regulation of IL-8 mRNA and the production of IL-8 protein by proinflammatory mediators (TNF-α, IL-1β, or IL-6) produced by leukemia cells themselves remains a possibility.

While many details remain unknown concerning the role of IL-8 in hematologic diseases, the results of the present study suggest several possibilities. In addition to its main role as a mediator at the site of inflammation or infection, IL-8 might be abundantly produced by leukemia cells, in response to infection or to the proliferation and differentiation of leukemia cells. In addition, IL-8 might be produced by cells such as monocytes, macrophages, fibroblasts, and endothelial cells. IL-8 production by leukemia cells not directly involved in inflammation has been demonstrated in vivo in studies of the proliferation and differentiation of homogeneous leukemia cell lines such as HL-60 and U937; similar results were obtained by us (data not shown).

The results of the present study suggest that IL-8 participates in the basic aspects of the
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...disease process of leukemia, in addition to the known physiologic functions of IL-8 during inflammation. Further studies are needed to clarify the role of IL-8 in acute leukemia.

References


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