Cytokine Gene Expression in B-Cell Chronic Lymphocytic Leukemia: Evidence of Constitutive Interleukin-8 (IL-8) mRNA Expression and Secretion of Biologically Active IL-8 Protein

By Paola Francia di Celle, Anna Carbone, Daniela Marchis, Dan Zhou, Silvano Sozzani, Simona Zupo, Massimo Pini, Alberto Mantovani, and Robin Foa

To extend our knowledge on the cytokines possibly involved in the pathophysiology of B-cell chronic lymphocytic leukemia (B-CLL), the mRNA expression of a panel of 10 cytokines was investigated on purified B-CLL cells using a reverse-transcriptase polymerase chain reaction method. Whereas negative RT-PCR signals were recorded for interleukin-1α (IL-1α), IL-2, IL-3, IL-4, IL-5, IL-7, tumor necrosis factor α (TNFα), and granulocyte-macrophage colony-stimulating factor, we detected the expression of IL-1β, IL-6 and TNFα. Furthermore, the constitutive expression of IL-8 mRNA was observed in all 17 B-CLL samples analyzed. mRNA expression was associated with the capacity of the leukemic cells to release IL-8 both constitutively (4.6 ± 8.1 SD ng/mL) and, to a further extent, after stimulation (14.5 ± 19.4 ng/mL). The circulating levels of IL-8 were also evaluated in 12 untreated B-CLL sera samples and the overall mean level was significantly higher (P < .01) than in normal sera. In addition, supernatants of purified B-CLL cells cultured in the presence of 12-O-tetradecanoylphorbol-13-acetate showed chemotactic activity towards neutrophils; this activity was neutralized in the presence of an anti-IL-8 antiserum. The mRNA for IL-8 was absent in five B-cell preparations from hairy cell leukemia cases and in four B-cell lines. Normal tonsil CD5+ B cells showed a low expression of IL-8 mRNA only in two of the nine preparations tested and the overall quantity of IL-8 released by these cells after 3 days' incubation was significantly lower compared with that released by B-CLL cells (0.4 ± 0.3 and 1.6 ± 0.9 ng/mL under basal and stimulated conditions, respectively). These findings point to an involvement of a member of the proinflammatory chemokine supergene family in human CD5+ B lymphocytes. The different IL-8 behavior observed between B-CLL cells and their normal counterpart is likely to reflect an activation state of the leukemic population.

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Several cytokines have been shown to be expressed at the mRNA level and/or to be produced by B-chronic lymphocytic leukemia (B-CLL) cells, a malignancy of relatively immature B cells. Furthermore, many cytokines have been suggested to play a paracrine or autocrine function in malignant B-cell proliferation.

In the present paper, we show the results of a sensitive reverse-transcriptase polymerase chain reaction (RT-PCR) analysis performed to evaluate the expression of a panel of cytokine mRNAs in unstimulated B-CLL cells. In addition to detecting RT-PCR positivities specific for cytokine mRNAs already described for B-CLL cells, the constitutive presence of interleukin-8 (IL-8) mRNA was documented in all samples analyzed. Furthermore, elevated IL-8 levels may be found in the serum of untreated B-CLL patients. IL-8 may be released by B-CLL cells, and supernatants of purified B-CLL cells contain IL-8–related chemotactic activity for neutrophils.

IL-8/NAP-1 is a member of the proinflammatory supergene family and is known for its potent and specific neutrophil activation and chemotactic properties. It is produced in response to several inflammatory stimuli including microbial products, such as lipopolysaccharide and the cytokines tumor necrosis factor α (TNFα) and IL-1. IL-8 chemotactic activity appears to affect also T lymphocytes and basophils. The natural cellular sources of IL-8 production have been described to be monocyte/macrophages, T cells, large granular lymphocytes, fibroblasts, endothelial cells, mesothelial cells, keratinocytes, neutrophils, hepatocytes, and chondrocytes; there is no evidence of IL-8 production by normal B cells.

The results of this study indicate that, unlike B-CLL, IL-8 mRNA is absent in other B-lymphoid leukemia cells tested, whereas normal CD5+ tonsil B cells rarely show constitutive IL-8 mRNA and the IL-8 released by these cells is significantly lower compared with that of CD5+ neoplastic B-CLL lymphocytes. These findings represent the first indication of a potential immunoregulatory function exerted by CD5+ B cells through IL-8 production.

MATERIALS AND METHODS

Cell and sera samples. Seventeen cases of B-CLL entered this study. The clinical stage and the immunologic features are reported in Table 1. At the time of the study, all patients were untreated or had been off treatment for at least 4 months. Five untreated hairy cell leukemia (HCL) cases, as well as the Daudi, Raji, Nalm-6, and K422 B-cell lines, were also analyzed. Sera samples from 12 of the 15 cases of B-CLL and from 7 normal volunteers were collected and stored at −80°C until titration.

Neoplastic B-cell purification. Enriched B-CLL and HCL cells were obtained after a Lymphoprep (Nycomed AS, Oslo, Norway)
Cytokine Gene Expression in B-CLL

Table 1. Clinical and Immunologic Data of the B-CLL Patients Studied

<table>
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<tr>
<th>Case No.</th>
<th>Clinical Stage</th>
<th>Light-Chain Restriction</th>
<th>CD5/CD19* or CD3+</th>
<th>% Positive Cells</th>
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<td>IV</td>
<td>κ</td>
<td>83</td>
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<td>17</td>
<td>I</td>
<td>λ</td>
<td>80</td>
<td>18</td>
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</table>

* Assessed by FACStar analysis on 10,000 cells. The immunophenotype was evaluated before monocyte and T-cell depletion. After purification of the leukemic cells, all B-CLL samples contained greater than 98% of CD5/CD19 double-positive cells.

Gradient separation of mononuclear cells and depletion of monocytes by adherence to plastic for 60 minutes at 37°C. T lymphocytes were removed by rosette formation with sheep red blood cells (RBCs) (E-rosettes) and repeated Lymphoprep fractionations. After purification, the samples to be tested were stained with selected monoclonal antibodies (MoAbs) and contained more than 98% CD5/CD19 double-positive leukemic cells, less than 1% CD3+ T cells and less than 1% CD14+ monocytic cells.

B cells were sensitized with an anti-CD5 MoAb (1 μg/10^6 cells) (Leu-1; Becton Dickinson, San Jose, CA) and subsequently rosetted with ox RBCs coated with the purified IgG fraction of a goat-antimouse Ig immunosum (Southern Biotechnology Associates, Birmingham, AL). Rosetting CD5+ B cells were separated from nonrosetting CD5- B cells on Lymphoprep density gradients. Purified cells were controlled for viability and analyzed by flow cytometry using a FACStar (Becton Dickinson); they were characterized by B cells (CD5/CD19+ cells) with less than 3% T cells (CD3+ cells) or macrophages (CD14+ cells). Immunofluorescent staining for CD5 was performed after overnight culture to allow the cells to reexpress CD5 that was down-modulated by treatment with anti-CD5 MoAb.

**RT-PCR** Total cellular mRNA was extracted according to the method of Chomczynski and Sacchi27 by using the RNazol solution (CinnaBiotech, Houston, TX). cDNAs were prepared by reverse transcription at 42°C for 30 minutes in a 50-μL reaction mixture containing 2 μg of total RNA, 0.5 μg oligo-dT, 1 mmol/L 2'-deoxy-nucleotide-5'-triphosphate, 5 μl 10 × RT-buffer (100 mmol/L TRIS-HCL, pH 8.8, 500 mmol/L KCL and 1% Triton X-100), MgCl2 5 mmol/L, 40 U Rnasin and 25 U Avian Moloney virus-reverse transcriptase. Five microliters of cDNA (corresponding to 0.2 μg of total RNA) were amplified in the presence of 40 mmol/L of each primer, 10 × buffer (0.5 mol/L KCL, 0.4 mol/L TRIS-HCL pH 8.8, 0.025 mol/L MgCl2, 0.002 mol/L each dNTPs and 2 mg/mL bovine serum albumin [BSA]) and 0.5 U of Taq (Thermus aquaticus) polymerase. The mixture was overlaid with mineral oil and then amplified in a Thermal Cycler (Cetus Corp, Emeryville, CA) with PCR cycle conditions individual for the different cytokine mRNAs tested. Fifteen microliters of PCR product were electrophoresed in a 2% agarose gel in TRIS/boric-acid/EDTA buffer. Gels were stained with ethidium bromide and photographed. To better quantitate the observed differences in IL-8 mRNA expression, the cDNAs have been further coamplified for β2-microglobulin (β2-M) and IL-8 mRNAs in the same reaction tube; this approach has been possible because the two systems use the same cycle conditions. The yield of β2-M and IL-8 coamplification results in a 289-bp IL-8 signal with variable intensity together with a 334-bp β2-M fragment constant in all reactions. Lymphokine-specific primer pairs for IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, TNFα, TNFβ, granulocyte-macrophage colony-stimulating factor (GM-CSF), and β2-M were obtained from Clontech (Palo Alto, CA). Specific primers for CD3, CD5, and CD14 mRNA amplification were designed on the basis of published sequences28-30 and used to analyze the possible contamination of T cells and monocytes respectively. All primers are designed to incorporate an exon-exon boundary to distinguish between amplification of mRNA and any contaminating genomic DNA. All reagents for cDNA synthesis and PCR are from Promega Corporation (Madison, WI).

Table 2. RT-PCR Cytokine Gene Expression in B-CLL Samples

<table>
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<tr>
<th>Case No.</th>
<th>IL-1α</th>
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RT-PCR analysis for constitutive presence of cytokine mRNAs in B-CLL-purified neoplastic B cells. All cDNAs tested have been controlled by β2-M mRNA amplification. The case numbers refer to those of Table 1.

Abbreviation: ND, not determined.
Northern blot analysis. Total cellular RNA was isolated from unstimulated purified B-CLL cells by a guanidine isothiocyanate method; U937 cells, unstimulated and stimulated with 12-O-tetradecanoylphorbol-13-acetate (TPA), were used as positive control. Twenty micrograms of each sample were denatured at 60°C for 10 minutes in an electrophoresis buffer (20 mmol/L morpholinopropane sulphonic acid, 15% formaldehyde, 50% formamide, 0.05 mg/mL ethidium bromide), size fractionated by electrophoresing through a 1.5% agarose gel containing 15% formaldehyde, and then transferred to nitrocellulose filters. Filters were dried, baked at 80°C in a vacuum oven for 2 hours, prehybridized for 6 hours at 42°C in 0.1% NaDodSO₄, 1× Denhardt’s solution, 100 μg/μL denatured salmon sperm DNA, 50% formamide and hybridized overnight at 42°C in the same solution containing the 32P random-primed probe. The IL-8 message was detected using a probe a 0.3-kb PstI-EcoRI fragment of monocyte-derived neutrophil chemotactic factor cDNA. Filters were washed twice in 2× SSC and twice in 0.1× SSC with 0.1% NaDodSO₄ at 60°C for 20 minutes, and then exposed for 5 days at −80°C to Kodak X-OMAT XAR-5 films (Eastman Kodak, Rochester, NY).

Culture supernatants. Purified B-CLL and CD5⁺ tonsillar B cells from the same samples used for the PCR studies were tested for their capacity to release IL-8, both spontaneously and after different stimuli. B cells (1.5 × 10⁶), in 1 mL of RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) (Irvine Scientific, Santa Ana, CA), were cultured for 72 hours alone and with 1% phytohemagglutinin (PHA) (Wellcome Research Lab, Beckenham, UK) plus 10⁻³ mol/L TPA (Sigma Chemical Co, St Louis, MO). The supernatants were collected after centrifugation and stored at −80°C until titration.

Radioimmunoassay for IL-8. IL-8 was measured in the cell culture supernatants and in sera using a radioimmunoassay according to the manufacturer’s instructions (Advanced Magnetics Inc, Cambridge, MA). Briefly, 200 μL of each sample to be tested and 100 μL of anti-IL-8 serum prepared in rabbit were mixed to a final volume of 600 μL in phosphate-buffered saline containing 0.1% BSA and 0.1% sodium azide at pH 7.0. After incubation for 16 hours at room temperature, 100 μL of 125I-IL-8 were added to each sample and incubated for 4 hours at room temperature. Five-hundred microliters of magnetic goat-antirabbit IgG was added to each sample; after further incubation for 20 minutes, the samples were pelleted by centrifugation (15 minutes at 1,000g) and washed twice in washing buffer (0.01 mol/L TRIS, 1.0 mol/L sodium chloride, 0.002 mol/L EDTA with 1.0% BSA and 0.1% sodium azide at pH 7.4). The tubes were counted in a gamma counter for 60 seconds. The standard curve for IL-8 was established to be between 10 pg/0.1 mL and 2,500 pg/0.1 mL. IL-8 concentrations were determined by interpolation of the cpm values in the standard curve. All samples were tested in duplicate.

Neutrophil migration assay. Neutrophil migration was evaluated using a microchamber technique, as previously described. Briefly, 25 ± 1 μL of 10⁻⁸ mol/L N-Formylmethionyl-leucyl-phenylalanine (FMLP; Sigma) or the cell supernatant to be tested were seeded in the lower compartment of the chemotaxis chamber and 50 μL of polymorphonuclear leukocytes (PMN) (1.5 × 10⁶/mL) were seeded in the upper compartment. The two compartments were separated by a 5-μμm pore-size polycrylpolylpyroridone-free polycarbonate filter. Chambers were incubated at 37°C in air with 5% CO₂ for 60 minutes. At the end of the incubation, filters were washed twice in washing buffer (0.01 mol/L TRIS, 1.0 mol/L sodium chloride, 0.002 mol/L EDTA with 1.0% BSA and 0.1% sodium azide at pH 7.4). The tubes were counted in a gamma counter for 60 seconds. The standard curve for IL-8 was established to be between 10 pg/0.1 mL and 2,500 pg/0.1 mL. IL-8 concentrations were determined by interpolation of the cpm values in the standard curve. All samples were tested in duplicate.

Neutralization with an anti-IL-8 antiserum. Rabbits were injected subcutaneously with recombinant human IL-8 in complete Freund’s adjuvant. After four injections (every 2 weeks) the antiserum was titrated by an enzyme-linked immunoabsorbent assay. The antiserum was capable of blocking completely the chemotactic activity of 5 ng/mL of IL-8 at the dilution of 1:100. For neutralization experiments, human natural IL-8 purified to homogeneity (a kind gift of Dr J. Van Damme, Rega Institute, University of Leuven, Belgium) was used.

RESULTS

Expression of cytokine mRNAs in B-CLL samples. The RT-PCR data on the constitutive cytokine gene expression
in 10 B-CLL are summarized in Table 2. The $\beta_2$-M gene was used as positive control of cDNA transcription. A significant constitutive mRNA expression for the IL-1$\beta$ gene was found in 7 B-CLL samples analyzed, for IL-6 in 9, and for TNF$\alpha$ in 5. Expression of the IL-8 gene was found in all 10 B-CLL samples tested. IL-1$\alpha$, IL-2, IL-3, IL-4, IL-5, IL-7, TNF$\beta$, and GM-CSF mRNA PCR amplification analyses were always negative. Figure 1 shows the PCR signals for IL-1$\beta$, IL-6, IL-8 and TNF$\alpha$ mRNAs amplification in these 10 B-CLL cDNAs, compared with the $\beta_2$-M PCR bands that are present in all samples and represent control reactions. In Fig 1, one can note the differences in intensity of the amplified PCR signals between cases, especially for IL-6 and TNF$\alpha$, but no correlation was found between IL-1$\beta$, IL-6, IL-8 and TNF$\alpha$ amplification of the same cDNA. The different intensities of IL-8 fragments observed in B-CLL cases have been emphasized by the coamplification of $\beta_2$-M and IL-8 mRNAs in the same RT-PCR reaction. In Fig 2, the variable intensities of the 289-bp IL-8 specific bands are coupled with the homogeneous 334-bp $\beta_2$-M fragments present in all lanes. To exclude that the cytokine PCR signals could be caused by the presence of residual contaminant T cells or monocytes, in all cases, an RT-PCR for the presence of CD3 and CD14 mRNAs was performed. In agreement with the phenotypic data, CD3- and CD14-specific fragments were present only in T cells and monocytes, respectively (very faint CD3 and CD14 signals are present in lanes 4 and 5), whereas variable quantities of CD5 mRNA were found in B-CLL, CD5$^+$ tonsil B cells, and in normal T cells. The $\beta_2$-M messages are shown as cDNA control reactions. (*) Contamination-free reactions.
with the phenotypic data, all PCR reactions for CD3 and CD14 messages were negative (Fig 3) compared with the strong signal observed with peripheral blood T- (CD3⁺) and monocyte (CD14⁺) cell preparations used as positive controls; B-CLL samples were all positive for CD5 mRNA.

On the basis of these findings, the mRNA for IL-8 was evaluated in seven further purified B-CLL samples (no. 11 through 17, Table 1); as expected, a strong IL-8 PCR signal was found in all of them (data not shown). The presence of IL-8 mRNA was also confirmed by Northern blot hybridization in three representative cases (Fig 4, lanes 4, 5, and 6).

No IL-8 mRNA expression was, instead, detected by RT-PCR in the four B-cell lines tested (Daudi, Raji, Nalm-6, and K422), and in the purified pathologic B cells from five HCL samples (data not shown).

Expression of IL-8 mRNA in CD5⁺ tonsil B cells. In view of the results obtained in B-CLL samples, the RT-PCR coamplification of IL-8 and β₂-M mRNAs was extended to normal CD5⁺ B lymphocytes purified from tonsils; these cells can be considered the normal cellular counterpart of B-CLL cells. Two of the nine preparations studied (Fig 5, lanes A and E) showed specific IL-8 and β₂-M fragments, but the remaining seven displayed only the β₂-M 334-bp bands (Fig 5). Cytofluorimetric analysis was performed to detect the purity of CD5⁺ tonsil cells (see the representative profile in Fig 6) and again we assessed for the presence of contaminating T cells and monocytes by RT-PCR detection for CD3 and CD14 mRNAs, respectively. The two CD5⁺ tonsil B-cell preparations that were positive for IL-8 mRNA (Fig 5, lanes A and E) showed very faint, almost invisible, CD3 and CD14 mRNA-specific signals (Fig 3, lanes 4 and 5).

Release of IL-8 by B-CLL cells and serum levels. To determine whether the IL-8 mRNA detected in B-CLL cells is translated into a secreted protein, we assayed the culture supernatants of 11 purified B-CLL samples under basal and stimulated conditions using a radioimmunoassay. The data concerning IL-8 release from B-CLL cells are summarized in Table 3. In all cases tested but one (no. 7), a constitutive IL-8 secretion, above the limit of sensitivity of the method (0.064 ng/mL), was observed. The overall mean IL-8 release was 4.6 ± 8.1 SD ng/mL. Under basal conditions, the release of IL-8 was particularly evident in cases no. 2, 9, 11, and 14. The amounts of IL-8 detected in the supernatants of stimulated B-CLL cells, increased in all cases studied (mean 14.5 ± 19.4 ng/mL). The possibility that the IL-8 released could originate from contaminating T cells or monocytes is most unlikely, because the culture supernatants were collected from controlled cellular populations (the same used for the RT-PCR studies) containing more than 98% of neoplastic B-CLL cells (see Materials and Methods).

Twelve sera collected from the B-CLL cases of Table 1
were also analyzed for IL-8 protein levels; the overall mean value of IL-8 was 0.8 ± 0.7 ng/mL, significantly higher (P < .01) than in 7 normal sera tested (0.2 ± 0.1 ng/mL) (Table 4).

Release of IL-8 by CD5+ tonsil B cells. In four of the nine CD5+ tonsil B-cell preparations (A, C, E, F) sufficient material was obtained to set up cell cultures and to test IL-8 secretion after 72 hours of incubation (Table 5). In all cases, low amounts of IL-8 release were recorded under basal conditions (0.4 ± 0.3 ng/mL), whereas an enhancement of IL-8 secretion (1.6 ± 0.9 ng/mL) was observed after PHA and TPA stimulation. The IL-8 levels released by CD5+ tonsil B cells were significantly lower (P < .1 and P < .05 under basal and stimulated conditions, respectively) compared with those of B-CLL cultures.

Analysis of IL-8 bioactivity. To evaluate whether the IL-8 secreted was biologically active, culture supernatants of purified B-CLL cells were tested for neutrophil chemotactic activity in a Boyden chamber system. As shown in Table 6, the supernatants of the three preactivated samples tested (nos. 5, 6, and 9) were capable of inducing a strong polymorphonuclear chemotactic migration; a moderate chemotactic activity was also recorded with the unstimulated B-CLL supernatants of two samples. The degree of chemotactic activity correlated with the dilution of the supernatant employed (Table 6).

Inhibition with anti-IL-8. The neutralizing action of an anti-IL-8 antiserum on the neutrophil chemotactic activity detected in B-CLL supernatants was investigated. As reported in Table 7, a total inhibition of chemotactic activity was shown in two preactivated samples (nos. 6 and 9), whereas a 70% inhibition was seen in the third case analyzed (no. 5).

DISCUSSION

To better define the spectrum of cytokines constitutively expressed by B-CLL cells, we assessed a panel of cytokine mRNAs in a series of B-CLL-enriched B-cell preparations by using an RT-PCR method. In agreement with the results published so far, in most samples tested, we found constitutive expression of the IL-1β, TNFα, and IL-6 genes.13–15 On the contrary,46 no constitutive GM-CSF and IL-7 mRNA expression was detected. For GM-CSF, the apparent discrepancy is most likely caused by the fact that we assessed mRNA expression under basal conditions and not after a preincubation period, whereas for IL-7 this is probably a consequence of the very large amounts of cellular RNA analyzed and of the high number of PCR cycles used in the study recently published.49 Whereas negative PCR reactions were also recorded for IL-1α, IL-2, IL-3, IL-4, IL-5, and TNFβ, a marked constitutive expression of IL-8 mRNA was observed in all B-CLL samples analyzed. This was further supported by the demonstration that purified B-CLL cells are capable of releasing constitutively and, to a further extent, after stimulation IL-8, and that elevated IL-8 levels can be found in the sera of B-CLL patients.

Several reports have suggested a possible involvement of IL-8 in different pathologic conditions; high IL-8 levels have been detected in the synovial fluid and in the sera of rheumatoid arthritis patients,51,54 suggesting a role for IL-8 in the recruitment of neutrophils in the inflamed synovia. Moreover, an increased expression of IL-8 mRNA by alveolar macrophages and an elevation in the level of IL-8 protein in the bronchoalveolar lavage fluids, related to the level of neutrophilia and the clinical severity of the disease, have been found in patients with idiopathic pulmonary fibrosis.55
With regard to cancer, it has been shown that melanoma cells stimulated with TNFα or IL-1α express IL-8 mRNA and secrete a biologically active IL-8 protein, and that IL-8 can induce a haptotactic migration of melanoma cells; these data suggest a role for IL-8 in the growth regulation of melanoma. In a recent report, constitutive IL-8 mRNA has been documented by Northern blot analysis in 8 of 15 of melanoma. In a recent report, constitutive IL-8 mRNA lymphoid leukemia (6/10 acute lymphoblastic leukemias and secrete a biologically active IL-8 protein, and that IL-8 may be an autocrine factor for B-CLL, we have been unable to detect IL-8 mRNA expression in the other neoplastic B cells analyzed (ie, HCL, another B-cell chronic lymphoproliferative disorder, as well as in the B-cell lines Daudi, Raji, Nalm-6, and K422), suggesting a possible primary role of IL-8 within the CD5⁺ compartment.

The connection between the described IL-8 chemotactic activity and the pathogenesis of B-CLL is still unclear. An aspect that will require further investigation is the possible action of the IL-8 secreted by B-CLL cells on other potential target cells and on the neoplastic clone itself. In this context, cell populations other than neutrophils—eg, peripheral blood mononuclear cells, T lymphocytes, and an Epstein-Barr virus-positive B-cell line—appear capable of expressing the IL-8 receptor. With regard to the possibility that IL-8 may be an autocrine factor for B-CLL, we have been unable to detect a proliferative action of exogenous IL-8 on B-CLL cells (data not shown) and, to date, lymphoid leukemic cells have been shown not to express the IL-8 receptor mRNA. The possibility that IL-8 may, in addition to its well-described chemotactic function, play a regulatory role on the B-cell compartment gains further support by the recent observation of an inhibitory action exerted by IL-8 on the IL-4-induced IgE synthesis by human B cells.

In our analysis, we detected IL-8 bioactivity in a chemotactic assay for neutrophils in the supernatants of purified B-CLL cells and could show that this activity is specifically inhibited in the presence of an anti–IL-8 antisem. The analysis extended to purified CD5⁺ tonsillar B cells—which are considered the normal cellular counterpart of B-CLL cells—showed the presence of IL-8 mRNA in only two of the nine samples tested. The IL-8 released by unstimulated and stimulated CD5⁺ tonsillar B cells was significantly lower compared with that of B-CLL cells; the different IL-8 behavior between B-CLL cells and the normal counterpart may, in agreement with previous data, reflect an activation state of the leukemic clone. This finding, together with the constitutive IL-8 gene mRNA expression and detection of a functional IL-8 protein release, represents the first demonstration of an IL-8–related chemoattractant activity produced by human CD5⁺ B cells. The amounts of IL-8 released by B-CLL cells are similar to those secreted by monocyte/macrophages, which are the predominant source of IL-8. Furthermore, we were unable to detect IL-8 mRNA expression in the other neoplastic B cells analyzed (ie, HCL, another B-cell chronic lymphoproliferative disorder, as well as in the B-cell lines Daudi, Raji, Nalm-6, and K422), suggesting a possible primary role of IL-8 within the CD5⁺ compartment.
Furthermore, a role of RBCs in binding IL-8 and in limiting IL-8 stimulation of leukocytes has been documented, and the more or less severe anemia associated with B-CLL may contribute to the increased circulating levels of IL-8 observed in this disease. Indeed, a reduction of inflammatory response induced by high levels of intravascular IL-8 has been described in rabbits; these data contrast with the proinflammatory action described for extravascular IL-8. Although hypogammaglobulinemia is the main predisposing factor to the frequent infective episodes that complicate the clinical course of patients with B-CLL, it is conceivable that the high quantities of IL-8 released by B-CLL cells may play a further contributory role.

Taken together, the data presented in this paper allow a more comprehensive overview of the scenario of cytokine gene expression in B-CLL. A better insight in the repertoire of cytokine gene expression and production in the different lymphoproliferative disorders will help to understand the processes that govern the proliferation and growth of the neoplastic clone, and to better correlate the different and often apparently contradicting actions.

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Cytokine gene expression in B-cell chronic lymphocytic leukemia: evidence of constitutive interleukin-8 (IL-8) mRNA expression and secretion of biologically active IL-8 protein

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