Constitutive Expression and Role in Growth Regulation of Interleukin-1 and Multiple Cytokine Receptors in a Biphenotypic Leukemic Cell Line

By Amos Cohen, Tom Grunberger, Wilma Vanek, Ian D. Dube, Paul J. Doherty, Michelle Letarte, Chaim Roifman, and Melvin H. Freedman

A cell line (B1) was established from the bone marrow of a patient with a relapse of acute leukemia characterized by a 4;11 chromosomal translocation and biphenotypic features of early B and myeloid lineages. Analysis of the growth requirements of this cell line showed density-dependent growth and secretion of an autostimulatory growth factor, suggesting an autocrine mechanism. Several lines of evidence implicate the participation of interleukin-1 (IL-1) in the autocrine growth regulation of B1 cells. These cells constitutively express the messenger RNA (mRNA) for IL-1 and IL-1 receptor and secrete IL-1; recombinant IL-1 stimulated the growth of colonies when cells were seeded at low density, and anti-IL-1 antibodies inhibited the growth of colonies with cells seeded at higher density. B1 cells do not express detectable levels of mRNA for any of the other cytokines tested, and other cytokines failed to support the growth of B1 cells at low density. In addition, B1 cells express multiple cytokine receptor genes, including the receptors for IL-6, IL-7, tumor necrosis factor and γ interferon. Addition of the respective cytokines to the B1 cells resulted in inhibition of the growth of leukemic cells in vitro. The multiplicity of growth inhibitory cytokine receptors on this leukemic cell line might be due to its biphenotypic lineage and may suggest new therapeutic possibilities in controlling leukemic cell proliferation.

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MATERIALS AND METHODS

Establishment of B1 cell line. A fresh bone marrow sample was taken from a 14-year-old patient in first relapse with ALL characterized by a t (4;11) chromosomal translocation. Leukemic cells were isolated from bone marrow by Percoll (Pharmacia, Piscataway, NJ) density gradient centrifugation (1.077 g/mL, 400g, 4°C) and cultured with weekly medium changes in 25-cm² tissue culture flasks (Corning Glass Works, Corning, NY) in α-modified minimum essential medium (αMEM; GIBCO, Grand Island, NY) containing 10% heat-inactivated fetal bovine serum (FBS; Flow, McLean, VA). Medium was changed weekly and cultures were incubated at 37°C in a humidified incubator with 5% CO₂ and 95% ambient air. After 2 months the cells grew as a single cell suspension with a doubling time of 40 to 50 hours. The cell line was negative for mycoplasma and did not carry Epstein-Barr viral sequences.
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The B1 cells were cloned in semisolid methyl cellulose (0.9% Methocel; Dow Chemical Co, Midland, MI). The cloned cell line maintained the immunologic markers of the original leukemic blasts derived from the bone marrow at the time of relapse.

 Colony growth assays. B1 colony growth assays were performed in semisolid medium. B1 cells, at the indicated density, were plated in 25-mm Lux suspension dishes (Nunc, Inc, Naperville, IL) in αMEM medium containing 10% FBS (Flow-Select Fetal Bovine Serum) in methyl cellulose (0.9%, in Methocel, Dow Chemical Co). Duplicate culture dishes were incubated at 37°C with 5% CO₂ in air in a humidified atmosphere. Colonies (≥ 20 cells) were counted after 8 days using an inverted microscope.

Quantitation of messenger RNA (mRNA) of cytokines and cytokine receptors by the polymerase chain reaction (PCR). The quantitation of specific mRNA species by PCR was performed using synthetic DNA as an internal standard, as previously described.¹ The technique involves co-amplification of a target cDNA (produced from the corresponding mRNA by reverse transcription) and of the internal standard. The target cDNA and the internal standard use the same primer sequences but yield PCR products of different sizes that can be separated by gel electrophoresis. In the exponential phase of the amplification, the amount of target cDNA can be quantified by comparison with the amplification of varied amounts of internal standard. The internal standard synthesized contains the primer sequences for multiple cytokines and cytokine receptor genes constructed by the technique of oligonucleotide overlap extension, as previously described.¹ The specific 5’ and 3’ primers used were designed based on published cytokine and cytokine receptor cDNAs sequences⁰⁻¹³ and are listed in Table 1. Specific primers were used for reverse transcription. These primers corresponded to mRNA sequences downstream from each 3’ PCR primer. The use of specific primers (complementary to the corresponding mRNAs rather than oligo[dT]) for the reverse transcription reaction added additional specificity to the PCR and ensured that only the target mRNA was amplified.

Total cellular RNA was prepared according to the methods of Chomczynski and Sacchi.¹⁴ RNA concentrations were measured spectrophotometrically (Beckman Instruments DU-40; Fullerton, CA). The integrity of the RNA was confirmed by electrophoresis under denaturing conditions on a 1% agarose gel.

CDNA was prepared by reverse transcription at 37°C for 60 minutes in a 20-μL reaction mixture containing 2 μg of total cellular RNA, 1 mMol/L dithiothreitol, 0.5 mMol/L dNTP, 0.1 μmol of a specific oligonucleotide primer, and 100 units of recombinant Moloney murine leukemia virus (MMLV) reverse transcriptase (Bethesda Research Laboratories, Rockville, MD). Internal standard DNA (0.1 to 3.0 pg) and cDNA (produced from 100 to 300 ng total cell RNA) were amplified together with 1 unit of Thermus aquaticus DNA polymerase (Taq polymerase; Perkin-Elmer/Cetus, Montreal, Canada) and 50 mMol/L dNTP, 0.1 mMol/L each of the 5’ and 3’ primers (1 X 10⁶ cpm of [γ³²P]-end-labeled primer was added where indicated) in a total volume of 50 μL. The mixture was overlaid with mineral oil and then amplified using a thermal cycler (Epicomp, San Diego, CA) over a number of cycles (indicated in text), denaturing at 95°C for 30 seconds, primer annealing at 54°C for 30 seconds, and extending at 72°C for 1 minute. Oligonucleotides were end-labeled with [γ³²P]-ATP and separated from unincorporated nucleotide with a Sephadex G-50 column. Ten microliters of PCR product were electrophoresed using 8% polyacrylamide gels in Tris borate/EDTA buffer. Gels were stained with ethidium bromide and photographed. When radiolabeled oligonucleotides were incorporated into the amplified product, the gels were dried and autoradiographed at -70°C. For quantitative analysis the appropriate bands were excised from the gel and radioactivity determined by scintillation counting. The amount of radioactivity recovered from the excised internal standard gel bands was plotted against the corresponding internal standard DNA concentration and the amount of cDNA present in each sample calculated from the standard curve.

Cytokines, antibodies, and enzyme-linked immunosorbent assay (ELISA). Human recombinant IL-1α (rhuIL-1α; specific activity 3 x 10⁶ U/mg) was a gift from Hoffmann-La Roche (Nutley, NJ); rhuIL-2 (2 x 10⁶ U/mg protein) was purchased from Genzyme (Boston, MA); rhuIL-3 (6 x 10⁵ U/mg), rhuIL-4 (1.2 x 10⁶ U/mg), rhuIL-5 (1.2 x 10⁶ U/mg), rhuIL-6 (4 x 10⁵ U/mg), rhuIL-7 (3 x 10⁶ U/mg), rhuGM-CSF (1.6 x 10⁶ U/mg), and recombinant human granulocyte-CSF (rhu-G-CSF; 3 x 10⁶ U/mL) were a gift from Genetics Institute (Cambridge, MA); rhuTNF-α (5.6 x 10⁶ U/mL) was a gift from Genentech, Inc (South San Francisco, CA); purified rabbit antihuman IL-1β antibody was purchased from Genzyme;

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Table 1. Sequences of Oligonucleotide Primers Used in the Amplification of Cytokine and Cytokine Receptor cDNAs

Sequences of 5’ and 3’ primers for amplification of published cytokine and cytokine receptor cDNAs were chosen from published cDNA sequences.⁰⁻¹³ The primers were designed to give a melting temperature greater than 58°C, PCR products for the various cytokine cDNAs of 310 to 370 bp, and an internal standard PCR product of 230 to 260 bp as listed in the table above. The PCR reaction was performed on cDNA obtained using specific reverse transcription primers downstream to the 3’ primer sequences listed above.
γ-interferon (γIFN; 1 × 10⁷ U/mg) was a gift from Biogen (Cambridge, MA). Antibodies used in flow cytometry experiments were purchased from Coulter (Hialeah, FL) as rhodamine conjugates for B4-IgG and T3-IgG and fluorescein isothiocyanate (FITC) conjugates for My-9-IgG, My4-IgG, and I3-IgG. An ELISA specific for IL-1β was obtained from Cistron (Pine Brook, NJ). Sensitivity of the assay was shown to be 4 pg/mL.

RESULTS

Establishment and phenotype of the B1 cell line. The cell line B1 was established from bone marrow obtained from a 14-year-old child in first relapse. The patient’s bone marrow sample at diagnosis and relapse contained over 95% malignant cells characterized by the t(4;11) (q21;q23) chromosomal translocation and biphenotypic expression of lymphoid and myeloid cell markers (often associated with this translocation).13-16

The cell line was established by incubating leukemic cells (10⁷/mL) in α-MEM containing 10% heat-inactivated fetal calf serum (FCS). After 8 weeks, the cells were cloned in semisolid methylcellulose and single colonies were isolated and expanded in liquid culture medium. The cell line established this way resembled the donor’s leukemic cells. The karyotype of the line showed t(4;11) (q21;q23) in all metaphases (Fig 1). In addition, other chromosomal abnormalities, including trisomy 6, der(1)t(1;8) (p36;q13), der(10)t(1;10)(q11;p15), were consistently observed in all metaphases. Cytogenetic analysis showed a profile of periodic acid Schiff (PAS)-positive, acid phosphatase-positive, nonspecific esterase-positive, and Sudan black-negative staining. The leukemic cells lacked T- and B-cell markers (E−, slg−, clg−) and were CD10⁺ and CD20⁺, but had undergone IgH(μ) gene rearrangement. Flow cytometric analysis showed that B1 cells expressed early pre-B-cell markers such as CD19⁺ and HLA-DR⁺ (Fig 2). HLA-DR is coexpressed with My-9 (CD33), a marker of myeloid lineage on 20% of the cells (Fig 2). Other myeloid differentiation markers, such as My-7, Mo-1, and Mo-2, were undetectable on the surface of B1 cells.

These differentiation markers expressed on the B1 cell line are consistent with the early B and myeloid biphenotypic nature of the original bone marrow cells from this patient at relapse, and with previous reports of the association of the 4;11 translocation with biphenotypic leukemia.13-16

Autocrine growth regulation. The B1 cell line exhibited density-dependent growth regulation (Fig 3). In a colony assay, cell growth, as a function of density of the initial cells seeded, was slow below 1 × 10⁴ cells/mL and became linear above this cell density. No growth was observed at or below a cell density of 2.5 × 10⁴ cells/mL. This pattern of density-dependent cell growth is consistent with the secretion of an autocrine factor by B1 cells that stimulates their growth. According to this hypothesis the threshold concentration of the growth factor required to stimulate its own receptor is reached at cell densities above 2.5 × 10⁴ cells/mL.

Support for this hypothesis was obtained from experiments in which the growth of B1 cells, seeded at low density (5 × 10⁴ cells/mL), was stimulated (294% over control cultures) by the supernatant from high-density serum-free cultures of B1 cells. Fractionation of the culture supernatant by Amicon filters showed that the growth-stimulatory

45, XY, der (1)t(1;8)(p36;q13),-4,-6,-9, der (10)t(1;10)(q11;p15), der(11)t(4;11)(q21;q23)
were counted after 7 days.

Methods.

ing 10% heat-inactivated FCS as described in detail in Materials and Methods.

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colonies are the mean values of duplicate cultures ± standard deviation; four other experiments gave similar kinetics. Colonies were counted after 7 days of incubation in semisolid medium containing 10% heat-inactivated FCS as described in detail in Materials and Methods.

Activity resided in the molecular weight range between 10 and 30 Kd.

These data suggest the participation of autocrine factor(s) in the growth of B1 cells. To identify and characterize these growth factors we studied the repertoire of cytokines and their receptors expressed in the B1 leukemic cells.

Expression of cytokine and cytokine receptor genes in the B1 leukemic cell line. A nonquantitative PCR was used as a preliminary screen to determine the presence of cytokine and cytokine receptor mRNAs in the B1 cells (Fig 4). An internal standard DNA was used as a positive control in each reaction (refer to Materials and Methods) (lower bands of ~250 bp in Fig 4). The results of this analysis showed the presence of mRNAs for both IL-1β and IL-1R in B1 leukemic cells (350-bp band in Fig 4A) and raised the possibility that IL-1 may participate in the autocrine growth observed in B1 cells.

The mRNAs for the receptors for IL-6, IL-7, γIFN, and TNF were expressed in B1 cells but no mRNA was detected for the corresponding cytokines (Fig 4B). Furthermore, no mRNA for any of the cytokines IL-2, IL-3, IL-4, IL-5, and GM-CSF or their corresponding receptors was detectable in B1 cells (data not shown). The PCR products of the preliminary screening were detected by ethidium bromide staining under amplification conditions that ensure detection of mRNA species exceeding greater than 0.002% of total cell RNA.

To determine whether the expression of IL-1β by B1 cells is associated with the production of IL-1β protein, we have measured the secretion of IL-1β by B1 cultures using specific ELISA. B1 cells (4 × 10⁷ cells/mL) found to secrete 24.5 pg/mL IL-1β into the medium over 24 hours of incubation. This value is a minimal estimate for IL-1β by B1 cells because some of the cytokine secreted by these cells may be bound and internalized by the corresponding receptors present on B1 cells.

The role of IL-1 in autocrine growth of the B1 leukemic cell line. To determine whether IL-1 was involved in autocrine growth control of B1 cells, we first analyzed the effect of IL-1α on the growth of B1 cells at various cell densities (Fig 5). IL-1α and IL-1β can be used interchangeably for this purpose since both bind to the same receptor. IL-1 markedly stimulated B1 cell growth at low cell densities (15-fold stimulation at 2.5 × 10⁶ cells/mL) while its effect progressively decreased at higher cell densities and was completely ineffective at the highest cell density tested (5 × 10⁷ cells/mL). These results are consistent with a role for IL-1 in the positive autocrine growth control observed in B1 cells. In addition, we tested the effect of anti–IL-1β antibody on the growth of B1 cells (Fig 6). Addition of rabbit anti–IL-1β antibodies significantly inhibited (47%) the growth of B1 cells relative to unstimulated controls. Normal rabbit serum did not affect the growth of B1 cells (data not shown). Taken together, these data suggest that IL-1 participates in an autocrine cycle promoting the growth of B1 cells.

Inhibition of B1 growth by IL-6, IL-7, TNFα, and γIFN. Because B1 cells express the genes of four other cytokine receptors, IL-6, IL-7, TNF, and γIFN, we analyzed the effect of exogenous cytokines on B1 cell growth. Figure 6 illustrates that IL-6, IL-7, TNF, and γIFN are potent inhibitors of B1 cell growth. Furthermore, the growth stimulatory effect of IL-1 can be overcome by the addition of these cytokines (data not shown). The corticosteroid
dexamethasone also inhibited the growth of B1 cells (Fig 6). Other cytokines, the receptors for which were undetectable on B1 cells, such as IL-2 and IL-4, failed to affect B1 cell growth (Fig 7). The hematopoietic growth factors IL-3, G-CSF, and GM-CSF did not affect the growth of B1 cells (Fig 7). In general, there was excellent agreement between our ability to detect the expression of a particular cytokine receptor gene, as shown by the PCR assay, and our ability to detect biologic effects of the cytokine on B1 cells (either growth stimulatory [IL-1] or inhibitory [IL-6, IL-7, TNF, and γIFN]).

Regulation of IL-1, IL-1R, IL-6, and IL-6R gene expression in B1 leukemic cells. The expression of IL-1, IL-1R, IL-6, and IL-6R genes in normal lymphoid and myeloid cells is regulated by IL-1, IL-6, and corticosteroids. For instance, IL-1 is not normally expressed by lymphoid and myeloid cells unless induced by exogenous inducers such as IL-1 or IL-6. However, in B1 cells IL-1 is constitutively expressed (Fig 4A), suggesting a deviation from the normal control of gene expression. To test this possibility, we studied the regulation of IL-1, IL-1R, IL-6, and IL-6R mRNA levels following treatment of B1 cells with IL-1, IL-6, and dexamethasone. A representative autoradiogram...
role of cytokines in leukemic cell growth

![Graph showing cell growth (in % of control) for different cytokines: IL-1, IL-2, IL-3, IL-4, IL-5, G-CSF, GM-CSF.]

Fig. 7. Effects of IL-1 and other cytokines on B1 cell growth. B1 cells were seeded at a density of $5 \times 10^4$ cells/mL in the presence or absence of the indicated recombinant cytokines. Colony number in the control cultures was 46 colonies/dish. Cytokine concentrations used were IL-1α, 10 U/mL; IL-1β, 10 U/mL; IL-3, 10 U/mL; IL-4, 10 U/mL; IL-5, 10 U/mL; and G-CSF, 10 U/mL. Demonstrating the amplification of IL-1 measured by quantitative PCR is presented in Fig. 8. A summary of the experiments performed on IL-1, IL-1R, and IL-6R gene expression is presented in Fig. 9.

The level of IL-1 mRNA remained unchanged in the presence of exogenously added IL-1 or IL-6 but the combination of these two cytokines increased IL-1 expression (90% over control levels; Figs 8 and 9A). Dexamethasone, on the other hand, markedly decreased IL-1 mRNA expression (Figs 8 and 9A), as observed in normal lymphoid cells. These results indicate that, although the IL-1 gene is constitutively expressed in the B1 leukemic cells, it still maintains some of the regulatory control elements of normal hematopoietic cells.

Unstimulated B1 cells constitutively expressed high levels of IL-18 mRNA amounting to 0.15% of total cell RNA (150 ng IL-1 mRNA per mg total cell RNA) (Fig 9A). The mRNA for IL-1R is present in B1 cells at a much lower level (8 ng/mg total cell RNA) and is not affected by any combination of IL-1 or IL-6 (Fig 9B). As reported for normal peripheral blood B cells, IL-1R mRNA is markedly induced by dexamethasone in B1 cells (sixfold stimulation over control levels). The levels of IL-6R mRNA in unstimulated B1 cells are unaffected by IL-1 and IL-6 but are decreased in the presence of dexamethasone (Fig 9C).

**DISCUSSION**

We describe here the establishment of a leukemic cell line with a 4;11 chromosomal translocation exhibiting both pre-B and myeloid features. The growth of this cell line is controlled by an autocrine loop involving IL-1 and its receptor and is inhibited by at least four different cytokines. The establishment of this cell line allowed us to analyze the growth regulation of biphenotypic leukemic cells in vitro.

The 4;11 translocation often occurs in leukemic cells with an early B-cell precursor phenotype together with myelomonocytic or T-cell features. In this respect, our work confirms the previous descriptions of leukemic cells with this phenotype, including a report of the establishment of a cell line with a similar phenotype. The B1 cell line has characteristics of early precursor B cells, such as the expression of CD19 antigen found early in normal B-cell ontogeny and restricted to B-lineage cells and the absence of the CD20 antigen, which appears somewhat later in

**Fig. 8. Effects of IL-1, IL-6, and dexamethasone on the expression of IL-1 mRNA in B1 cells. B1 cells were incubated in MEM medium containing 10% FCS for 4 hours in the presence of the indicated cytokines or dexamethasone ($1 \times 10^{-7}$ mol/L). Total cell RNA was extracted as described in Materials and Methods, and reversed transcribed with specific primers for IL-1β and amplified using the PCR in the presence of the indicated concentrations of the synthetic internal DNA standard as described in the legend to Fig 4. In these experiments the 5’ primer was end-labeled using polynucleotide kinase and γ32P-ATP for quantitative PCR analysis as described by Wang et al. Amplification proceeded for 26 cycles in triplicate samples containing equal amounts of reverse transcribed cDNA (equivalent to 150 ng of total cell RNA) and variable amounts of internal standard DNA as indicated. Ten microliters of the PCR products were electrophoresed on a polyacrylamide gel that was dried and autoradiographed after 2 hours of exposure at 70°C. Lower bands (–250 kb) represent the PCR products of the internal standard and larger bands (–350 kb) represent PCR products of cDNAs of IL-1β.**

**Internal standard (pg)**

- 300
- 250

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Fig 9. Effects of IL-1, IL-6, and dexamethasone on the expression of IL-1β, IL-1R, and IL-6R mRNAs. B1 cells were incubated in the presence of either IL-1β (10 U/mL), IL-6 (10 U/mL), or dexamethasone (1 × 10^{-7} mol/L). RNA was extracted and reverse transcribed (0.15 μgRNA/reaction) and the cDNA amplified by quantitative PCR as described in the legend to Fig 7 with specific primers for IL-1 (A), IL-1R (B), and IL-6R (C). The products of the individual PCR reactions were electrophoresed, the gel dried, and autoradiographed. The radioactive bands corresponding to the internal DNA standard, and the amplified cDNA were excised from the dried gel and counted. The amount of cytokine cDNA present in the original sample was calculated from the coamplified internal standard as described by Wang et al. The amount of mRNA was calculated by assuming a 30% yield of the reverse transcription reaction.

B-cell development. B1 cells also express nonspecific esterase activity characteristic of cells of myeloid lineage.

The profiles of cytokines and cytokine receptors expressed by B1 cells show both similarities and significant differences to those reported for AML cells. Autocrine stimulation of growth by IL-1β has been reported for the majority of AML cases that constitutively express both IL-1β and its receptor as shown here for B1 cells (Figs 4, 8, and 9). In addition, TNF and γIFN in some cases inhibited AML cell growth, as observed for B1 cells (Fig 6). On the other hand, GM-CSF was expressed in the majority of AML samples in combination with either IL-6 or TNF, while neither one of these cytokines is expressed by B1 cells. Little information is available on the cytokine production by ALL cells. However, the growth of ALL cells is stimulated by IL-3 and by IL-7. In contrast, IL-7 inhibits the growth (Fig 6) of B1 cells while IL-3 has no effect on B1 cell growth (Fig 7) indicating that the growth requirements of these cells are quite different from other ALL or AML cells.

Of all the cytokines tested, only IL-1β is constitutively expressed in B1 cells and is capable of promoting B1 cell proliferation, suggesting that IL-1 is directly responsible for the autocrine regulation of B1 cell growth. In contrast, normal hematopoietic cells do not express IL-1 constitutively but rather produce IL-1 in response to inducers. Taken together, these observations suggest that the constitutive expression of IL-1 and the establishment of IL-1 autocrine growth may be involved in the growth of these leukemic cells. The growth of this leukemic cell line is consistent with the growth characteristics of the initial leukemic cells at relapse. Thus, culture supernatant from the freshly isolated leukemic cells exhibited autocrine growth characteristics, contained hematopoietic growth factor activity, and responded to growth stimulation by IL-1 (data not shown).

Other growth factor receptors commonly associated with leukemic cells such as GM-CSF and IL-6 (AML), IL-3, and IL-7 (ALL) failed to stimulate B1 cell growth. GM-CSF receptors were not expressed on B1 cells (data not shown) whereas binding of IL-6, IL-7, and TNF to their corresponding receptors, present on B1 cells, inhibited rather than stimulated B1 cell growth (Fig 6). The unusual expression on B1 cells of the receptors for four growth-inhibitory cytokines is quite different from the cytokine expression and responses of conventional AML or ALL cells and may be a characteristic feature of the leukemic subtype with a 4;11 translocation. Determination of the cytokine profile of other cases with 4;11 translocations will be required to confirm this hypothesis.

The role of these growth inhibitory interactions in the normal differentiation of hematopoietic cells is still unclear. However, it has been proposed that leukemic cells with a 4;11 chromosomal translocation often originate from an early hematopoietic precursor with biphenotypic differentiation potential. It has been proposed that such multipotential hematopoietic precursor cells will express multiple cytokine receptors and may be capable of differentiating into the various lineages in response to specific growth factors. Indeed, differentiation along the monocytic lineage by activation of protein kinase C has been reported in a biphenotypic cell line with a 4;11 translocation. The possibility that certain combinations of the cytokine recep-
tors expressed on B1 cells may induce the differentiation of B1 cells along the myeloid or lymphoid lineages is open for future investigation. On the other hand, the expression of cytokine receptors on the B1 cells suggests potential treatment strategies for patients with this subtype of leukemia.

It is widely accepted that IL-1 is not expressed constitutively in normal hematopoietic cells but is inducible by a variety of factors affecting cells of myeloid or lymphoid lineages. In contrast, the B1 leukemic cells express IL-1 constitutively and IL-1 gene expression could not be induced by either IL-1 or IL-6 alone (Fig 8). These data suggest that the genetic elements controlling IL-1 gene expression may have undergone structural changes during the leukemogenesis process as previously described for other cytokines involved in autocrine growth control.49

ACKNOWLEDGMENT

We thank Ursula Cymerman and Ellen Thompson for excellent technical assistance.

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