Elevated Intracellular Level of Basic Fibroblast Growth Factor Correlates With Stage of Chronic Lymphocytic Leukemia and Is Associated With Resistance to Fludarabine

By T. Menzel, Z. Rahman, E. Calleja, K. White, E.L. Wilson, R. Wieder, and J. Gabrilove

Chronic lymphocytic leukemia (CLL) is characterized by delayed senescence and slow accumulation of monoclonal, small lymphocytes. Basic fibroblast growth factor (bFGF) is a pleiotropic cytokine that plays a role in hematopoiesis and apoptosis. Elevated bFGF levels have been detected in urine from patients with a variety of neoplastic diseases including various leukaemias; however, the cellular source of the bFGF has not been determined. In this study, the intracellular bFGF level in lymphocytes of 36 patients with B-CLL and 15 normal donors was determined using an enzyme-linked immunosassay. In cells derived from patients with high-risk disease, the median level of intracellular bFGF was 381.5 pg/2 x 10^6 cells, compared with a median of 90.5 pg/2 x 10^6 cells in patients with intermediate disease. In patients with low-risk disease, the median bFGF level was 4.9 pg/2 x 10^6 cells, and in normal controls, it was 6.0 pg/2 x 10^6 cells. The difference in the bFGF levels was significant for the comparison between low- and intermediate-risk (P = .0019), low- and high-risk (P < .0001), and intermediate- and high-risk disease (P = .0001). Immunofluorescent stains of peripheral blood mononuclear cells confirmed CLL lymphocytes as a cellular source of bFGF. To evaluate the potential contribution of elevated intracellular bFGF levels to the phenotype of CLL cells, leukemic cells were cultured in vitro with an apoptotic stimulus (fludarabine). CLL cells with high intracellular levels of bFGF appeared to be more resistant to fludarabine treatment. The addition of bFGF to fludarabine-treated CLL cells resulted in a delay of apoptosis and prolonged survival. These data suggest that bFGF may contribute to the resistance of CLL cells to an apoptotic stimulus.

MATERIALS AND METHODS

Reagents. Fludarabine (9-b-D-arabinosyl-2-fluoroadenine-monophosphate) was obtained from Berlex (Richmond, CA). It was freshly resuspended according to the manufacturer’s guidelines and added to tissue culture media. Fludarabine des-phosphate (F-Ara-A; 9-b-D-arabinosyl-2-fluoroadenine) was obtained from Sigma Chemicals (St Louis, MO). Recombinant human basic fibroblast growth factor (rhbFGF) was a gift from Synergen Inc (Boulder, CO). Stock vials were stored at −20°C. For each experiment, all reagents were diluted in serum-containing medium on the day of use.

Cells. Peripheral blood was drawn into EDTA-containing tubes (Becton Dickinson, Rutherford, NJ). The serum was removed after a brief centrifugation and stored at −20°C, and the cell pellet was resuspended in an equal amount of phosphate-buffered saline (PBS).

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Supported by grants from the Deutsche Forschungsgemeinschaft (Me 1235/1-1), the American Cancer Society (DHP-82), and the National Institutes of Health (DK 48728).

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1056-1063

Blood, Vol 87, No 3 (February 1), 1996: pp 1056-1063
The mononuclear cells were isolated by centrifugation on Ficoll-Hypaque gradients (1.077 g/mL; Pharmacia Fine Chemicals, Piscataway, NJ), washed three times in PBS, and suspended in standard media (RPMI1640 media) containing 10% heat-inactivated fetal calf serum (FCS; GIBCO, Grand Island, NY) supplemented with penicillin (100 U/mL; GIBCO), streptomycin (100 μg/mL; GIBCO), and 3 mg/mL of glutamine (GIBCO). The cell count was determined microscopically using a hemocytometer. The cells were resuspended in either 90% FCS (GIBCO)/10% dimethyl sulfoxide (DMSO; Sigma) and stored under liquid nitrogen until further use or cultured in standard media. An aliquot of 10^6 cells was pelleted in siliconized Eppendorf tubes, supernatant was removed, and cells were stored at −80°C. Cell lysates were prepared by resuspending the cells with 1.0 mL of RPMI1640 containing 0.1% bovine serum albumin (BSA), shockfreezing in liquid nitrogen, and thawing at 37°C, followed by vigorously vortexing the suspension. This procedure was repeated three times. Subsequently, the suspension was centrifuged at 3,000 rpm, and the supernatant was removed and stored at −80°C.

**Cell culture.** Cells were suspended in standard media. Fludarabine was added on day 1 at a final concentration of 0.3, 3, or 30 μM/L, respectively, to the specified culture. Basic FGF diluted in standard media was added daily to each condition at a final concentration of 10 or 100 ng/mL. The same amount of standard media was added to all other conditions. The incubation was performed for 7 days at 37°C in 5% CO₂. Cell viability was assessed by trypsin blue (GIBCO) exclusion using a hemocytometer. Each sample was evaluated in duplicate. The percentage of viable cells in treated samples (treated with fludarabine with or without bFGF) was divided by the average viability of the untreated (standard media alone) samples (controls) at the same time.

**Measurement of bFGF levels.** Cells lysates and EDTA plasma samples (200 μL per well) were tested for the presence of bFGF by a sandwich enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN) that has a detection limit of 1 pg/mL. Briefly, 200 μL of cell lysate or standard rhbFGF (5 pg/mL to 640 pg/mL) was pipetted into wells precoated with a monoclonal anti-bFGF antibody. After a 2-hour incubation period at room temperature (RT), the wells were washed three times, and a secondary enzyme-bound polyclonal anti-bFGF antibody was added to the wells. After another 2-hour incubation at RT, wells were washed again, and substrate solution was added. The color reaction was stopped after 20 minutes. Absorption at 490 nm was measured immediately in an automated ELISA reader (Emax-Reader; Molecular Devices, Menlo Park, CA). Basic FGF content was calculated using an analytical program (Softmax; Molecular Devices, Sunnyvale, CA).

**Morphologic detection of apoptosis.** All 15 healthy volunteers were included in the study. Of the 36 patients (female, n = 13; male, n = 23), 8 presented with low-risk, 14 with intermediate-risk, and 14 with high-risk disease. The median age was 65.5 years (range, 38 to 85 years); median WBC count at presentation was 55.2 × 10^9/μL, with a range of 2.5 × 10^9/μL to 217 × 10^9/μL (Table 1). Flow cytometry analysis indicated that B-CLL cells coexpressed CD5, CD19, and CD20.

**RESULTS**

A total of 36 patients with documented B-CLL and 15 healthy volunteers were included in the study. Of the 36 patients (female, n = 13; male, n = 23), 8 presented with low-risk, 14 with intermediate-risk, and 14 with high-risk disease. The median age was 65.5 years (range, 38 to 85 years); median WBC count at presentation was 55.2 × 10^9/μL, with a range of 2.5 × 10^9/μL to 217 × 10^9/μL (Table 1). Flow cytometry analysis indicated that B-CLL cells coexpressed CD5, CD19, and CD20.

In cells derived from patients with high-risk disease, the median level of intracellular bFGF (per 2 × 10^9 cells) was 381.5 pg (range, 134.1 to 732.2 pg). Levels were found to be lower in patients with intermediate-risk disease, with a median of 90.51 pg (range, 3.3 to 373.4 pg). In patients with low-risk disease (Rai, low risk) the median bFGF level (per 2 × 10^9 cells) was 4.9 pg (range, 1.5 to 22.6 pg). Lymphocytes from healthy volunteers exhibit a comparable median intracellular bFGF level of 6.0 pg (range, 0.8 to 17 pg) to
that found in CLL cells derived from patients with low-risk disease (Fig 1).

Intracellular bFGF levels in lymphocytes from CLL patients with advanced-stage disease (modified Rai classification, high risk) were significantly higher than bFGF levels detected in cell lysates from normal controls ($P < .0001$) or from patients with low- or intermediate-risk disease ($P < .0001$ and $P = .00119$, respectively). Comparison between normal controls and patients with intermediate-risk disease also revealed a significant difference ($P < .0001$). No correlation between bFGF plasma levels and clinical stage of the disease could be established (data not shown). No correlation between intracellular bFGF levels and factors other than stage of the disease (ie, other chronic or acute diseases, infectious episodes, immunoglobulin infusion, splenectomy, or concurrent medication) could be established. No bias was detectable for any of those parameters in any of the three different risk groups.

Immunofluorescence staining was performed to confirm that CLL lymphocytes were the actual cellular source of bFGF. Immunofluorescence stains of cells derived from patients with low-, intermediate-, and high-risk disease are shown in Fig 2. The morphology of the fluorescent cells is consistent with CLL lymphocytes. Strong cytoplasmatic staining can be seen in the CLL cells derived from a patient with high-risk disease (Fig 2D); lesser staining occurs in the cytoplasm of cells obtained from a patient with intermediate-risk disease (Fig 2C). The cytoplasm of cells from a patient with low-risk disease is only faintly stained (Fig 2B). This cytoplasmic fluorescence observed in these patients’ cells was consistent with the results obtained with the ELISA (Fig 1). Peripheral blood lymphocytes from a healthy donor are shown in Fig 2A. Only very weak cytoplasmic staining is seen in these cells, demonstrating that the bFGF content of normal lymphocytes is equivalent to that found in low-risk CLL cells. In addition, it does not differ from rabbit IgG controls (data not shown), suggesting that these cells do not contain a significant amount of bFGF.

We next evaluated the effects of intracellular bFGF on the life span of CLL cells. No correlation between the intracellular bFGF levels and factors other than stage of the disease (ie, other chronic or acute diseases, infectious episodes, immunoglobulin infusion, splenectomy, or concurrent medication) could be established. No bias was detectable for any of those parameters in any of the three different risk groups.

![Fig 1. Distribution of intracellular bFGF content in CLL cells according to the stage of the disease. Basic FGF content of cell lysates was determined by ELISA as described in Materials and Methods. Each symbol (○) represents the bFGF level of CLL cells or normal control cells, respectively. The broken line (---) identifies the median for each of the four groups representing cells derived from patients with low-risk (low, $n = 8$), intermediate-risk (interm, $n = 14$), and high-risk (high, $n = 15$) disease according to the modified Rai classification. Low-density cells from normal donors (normal, $n = 15$) were used as controls.](image)
cellular amount of bFGF and the spontaneous decrease in viability was observed for CLL cells cultured in the presence of media containing 10% FCS (data not shown).

We then examined whether the viability of CLL cells exposed to an apoptotic stimulus could be related to the intracellular bFGF content. For these experiments, B-CLL lymphocytes were cultured in vitro for 7 days in the presence or absence of fludarabine, a chemotherapeutic drug inducing apoptosis in CLL cells. When CLL lymphocytes were exposed to three different concentrations (0.3, 3.0, and 30 μmol/L), cell viability decreased abruptly at 30 μmol/L. At a concentration of 3 μmol/L, fludarabine-induced cell death was as effective, but the decrease in viability was slower. Fludarabine at 0.3 μmol/L did not yield to any significant decrease in cell viability (data not shown). We, therefore, chose a fludarabine concentration of 3 μmol/L for subsequent experiments.

B-CLL cells from 19 patients were exposed to fludarabine alone and evaluated for viability. Trypan blue exclusion analysis revealed a significant difference in the survival of B-CLL cells when the results were compiled according to the intracellular bFGF content (Table 2). After 4 days of culture in the presence of fludarabine (3 μmol/L), 46% of B-CLL cells containing low intracellular bFGF (1.5 to 6 pg/2 × 10⁵ cells) were viable, compared with 47% of the cells with intermediate bFGF content (45 to 134 pg/2 × 10⁵ cells) and 64% of the cells with high intracellular bFGF content (234 to 732 pg/2 × 10⁵ cells; Table 2). On day 7 of culture in the presence of fludarabine (3 μmol/L), the percentages of viable cells were 16%, 24%, and 46% for cells containing low, intermediate, or high intracellular bFGF, respectively. These differences were significant (P = .0063) between cells containing high and low levels of bFGF, and between cells containing intermediate and high levels of bFGF (P = .0114; Table 2). Further statistical evaluation of the association between intracellular bFGF content and resistance to fludarabine suggested arranging the samples into two groups containing either less than 134 pg/2 × 10⁵ cells or more than 234 pg/2 × 10⁵ cells, respectively (Table 3; in the patients’ B-CLL cells evaluated in vitro, no values between 134 pg/2 × 10⁵ cells and 234 pg/2 × 10⁵ cells were measured). On day 4, 47% of the cells in the group with an intracellular bFGF content between 1.5 and 134 pg/2 × 10⁵ cells were viable, compared with 64% of the cells with an intracellular bFGF content between 234 and 732 pg/2 × 10⁵ cells. On day 7, only 19% of B-CLL cells with the lower intracellular bFGF content were still viable, as compared with 46% of the cells with higher intracellular bFGF content. The calculated P values for the differences in survival on day 4 and day 7 were significant (P = .0282 and .0041, respectively).

We next tested whether the addition of exogenous bFGF at different concentrations (1, 10, 100 ng/mL) to B-CLL.
cells could increase their resistance to fludarabine. Adding bFGF to the fludarabine-treated CLL cells resulted in an increased survival that was most distinct at a bFGF concentration of 100 ng/mL. As shown in Table 4, the survival of fludarabine-treated CLL cells containing low intracellular bFGF (1.5 to 6 pg/2 × 10^6 cells) was significantly increased by the addition of exogenous bFGF. The addition of exogenous bFGF resulted in a 10% increase in viable cells on day 1. On day 2, 68% of the cells treated with fludarabine alone were viable, whereas cells treated with fludarabine plus bFGF displayed 81% viability (Table 4). After 7 days of culture in the presence of fludarabine, 16% of the cells were viable, compared with 47% of the cells treated with fludarabine plus bFGF. These differences in survival were significant. Furthermore, the addition of exogenous bFGF to B-CLL cells containing low intracellular bFGF rendered them as resistant to fludarabine as cells with high intracellular content.

B-CLL cells with intermediate bFGF content (45 to 134 pg/2 × 10^6 cells) treated with fludarabine alone yielded a 50% decrease in viability observed on day 4. When exogenous bFGF was added, this 50% decrease in survival was delayed and was observed, instead, on day 7. This difference in survival of these B-CLL cells was also significant (Table 4).

Interestingly, the addition of 100 ng/mL of exogenous bFGF to B-CLL cells with high intracellular bFGF content (234 to 732 pg/2 × 10^6 cells) also resulted in a significant increase in cell survival after fludarabine treatment (Table 4).

No effect on survival was observed in either instance when bFGF was added to LLC cells under standard serum conditions (data not shown).

Since the assessment of viability by trypan blue exclusion does not provide a direct correlation with apoptosis, the morphology of cells treated with fludarabine alone or fludarabine plus bFGF was examined microscopically. Treatment with fludarabine alone for 24 hours resulted in changes consistent with apoptosis, such as nuclear condensation, micronuclei formation, and the appearance of apoptotic bodies (data not shown). These changes were more prominent after 48 hours of exposure, confirming the apoptotic effects of fludarabine that have been previously described. In cells treated with fludarabine plus bFGF, morphologic changes consistent with

### Table 2. Delay of Fludarabine-Induced Cell Death Is Associated With Intracellular bFGF Content

<table>
<thead>
<tr>
<th>Day</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>87 ± 2</td>
<td>86 ± 6</td>
<td>88 ± 7</td>
<td>47</td>
</tr>
<tr>
<td>2</td>
<td>68 ± 13</td>
<td>73 ± 17</td>
<td>82 ± 11</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>46 ± 13</td>
<td>47 ± 16</td>
<td>64 ± 15</td>
<td>0.002</td>
</tr>
<tr>
<td>7</td>
<td>16 ± 15*</td>
<td>24 ± 8*</td>
<td>46 ± 15*</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Values indicate % viability of B-CLL cells in standard medium treated with fludarabine (3 μmol/L) alone, according to intracellular bFGF content (per 2 × 10^6 cells): low, 1.5 to 6 pg (n = 4); all patients with low-risk disease according to the modified Rai classification (intermediate); 45 to 117 pg (n = 7; six patients with intermediate-risk disease, one patient with high-risk disease according to the modified Rai classification); high, 234 to 732 pg (n = 8; six patients with high-risk disease, two patients with intermediate-risk disease according to the modified Rai classification). Each patient sample was evaluated in duplicate. Numbers represent the mean and standard deviation of the group of patients for each respective bFGF content.

* P < .006 for the comparison between low and high, by Student's t-test.

† P = .01 for the comparison between intermediate and high, by Student's t-test.

### Table 3. Fludarabine-Induced Cell Death Is Delayed in Cells With High Intracellular bFGF Content

<table>
<thead>
<tr>
<th>Day</th>
<th>&lt;120 (n = 11)*</th>
<th>&gt;230 (n = 8)†</th>
<th>PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86 ± 5</td>
<td>86 ± 7</td>
<td>.7074</td>
</tr>
<tr>
<td>2</td>
<td>71 ± 15</td>
<td>82 ± 11</td>
<td>.1114</td>
</tr>
<tr>
<td>4</td>
<td>47 ± 14</td>
<td>64 ± 15</td>
<td>.0282</td>
</tr>
<tr>
<td>7</td>
<td>19 ± 16</td>
<td>46 ± 15</td>
<td>.0041</td>
</tr>
</tbody>
</table>

Values indicate percent viability of B-CLL cells in standard medium treated with fludarabine (3 μmol/L) alone. Each patient sample was evaluated in duplicate. Numbers represent the mean and standard deviation of the group of patients for each respective bFGF content.

* Intracellular bFGF levels ranging from 1.5 to 117 pg/2 × 10^6 cells (four patients with low-risk disease, six patients with intermediate-risk disease, and one patient with high-risk disease according to the modified Rai classification).

† Intracellular bFGF levels ranging from 234 to 732 pg/2 × 10^6 cells (two patients with intermediate-risk disease and six patients with high-risk disease according to the modified Rai classification).

Student's t-test was used to determine the P values.

### Table 4. Addition of Exogenous bFGF Results in a Significant Delay of Fludarabine-Induced Cell Death

<table>
<thead>
<tr>
<th>Intracellular bFGF Content and Day</th>
<th>Fludarabine</th>
<th>Fludarabine + bFGF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>87 ± 2</td>
<td>97 ± 3</td>
<td>.0266</td>
</tr>
<tr>
<td>2</td>
<td>68 ± 13</td>
<td>81 ± 13</td>
<td>.0002</td>
</tr>
<tr>
<td>4</td>
<td>46 ± 13</td>
<td>61 ± 18</td>
<td>.0398</td>
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<tr>
<td>7</td>
<td>16 ± 15</td>
<td>47 ± 16</td>
<td>.0049</td>
</tr>
<tr>
<td>Intermediate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>86 ± 6</td>
<td>98 ± 4</td>
<td>.0017</td>
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<tr>
<td>2</td>
<td>73 ± 17</td>
<td>88 ± 8</td>
<td>.0213</td>
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<tr>
<td>4</td>
<td>47 ± 16</td>
<td>67 ± 29</td>
<td>.0313</td>
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<tr>
<td>7</td>
<td>24 ± 18</td>
<td>55 ± 20</td>
<td>.0098</td>
</tr>
<tr>
<td>High</td>
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<td></td>
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<tr>
<td>1</td>
<td>88 ± 7</td>
<td>95 ± 5</td>
<td>.0078</td>
</tr>
<tr>
<td>2</td>
<td>82 ± 11</td>
<td>96 ± 11</td>
<td>.0022</td>
</tr>
<tr>
<td>4</td>
<td>64 ± 15</td>
<td>84 ± 15</td>
<td>.0084</td>
</tr>
<tr>
<td>7</td>
<td>46 ± 15</td>
<td>68 ± 11</td>
<td>.0071</td>
</tr>
</tbody>
</table>

Viability of B-CLL cells in standard medium treated with fludarabine (3 μmol/L) alone or plus bFGF (100 ng/mL). Each patient sample was evaluated in duplicate. Numbers represent the mean and standard deviation of the group of patients for each respective bFGF content. See notes to Table 2 for definitions of intracellular bFGF content.

* The paired t-test was used to determine the P values.
apoptosis were observed only in a small fraction of the CLL lymphocytes (data not shown). To quantify the changes observed, we examined cells treated with or without bFGF for changes in response to an apoptotic stimulus with a recently developed flow cytometric method. Apoptosis-associated DNA strand breaks are detected using an in situ TdT assay. The intensity of labeling of apoptotic cells with biotinylated dUTP correlates with the number of DNA strand breaks per cell. Treatment of B-CLL cells from patients with low-risk disease (n = 2) and intermediate-risk disease (n = 2) with F-Ara-A at 3 μmol/L resulted in a significant increase in the percentage of apoptotic cells after 12 and 36 hours (Fig 3). This increase could be effectively reduced by adding bFGF to the culture. In a representative experiment using CLL cells with low intracellular bFGF, 12 hours of incubation with fludarabine resulted in an increase of the apoptotic cell fraction to 27%. When the cells were treated for 12 hours with fludarabine plus 100 ng/mL of bFGF, the fraction of apoptotic cells was reduced to 18.7%. After 36 hours of incubation with fludarabine, 70% of cells were apoptotic; treatment with fludarabine plus bFGF for 36 hours resulted in only 22.5% apoptotic cells (Fig 3).

DISCUSSION

This is the first study to (1) demonstrate elevated intracellular bFGF levels in lymphocytes of patients with intermediate- or high-risk CLL, (2) reveal a correlation between the clinical stage of CLL and the level of intracellular expression of bFGF, and (3) show that bFGF may be a contributing factor to the resistance of B-CLL cells to an apoptotic stimulus.

Basic FGF has been postulated to play a role in malignancy. Elevated levels of bFGF have been previously reported in cells derived from renal cell carcinoma and malignant melanoma, and a correlation between increased levels and impaired prognosis has been established. Other investigators have shown increased urinary excretion of bFGF in a variety of neoplastic diseases, including leukemias; however, the source of the bFGF was not established. In this study, lymphocytes from patients with CLL were identified as a source of bFGF. In addition, the intracellular bFGF level correlated with the stage of the disease, suggesting that in the course of the disease, the accumulation of intracellular bFGF provides an advantage for malignant cells. As the size of a leukemic cell population is regulated not only by proliferation but also by the rate of cell death, a delay in apoptosis caused by elevated bFGF levels may create a survival advantage.

Recently, evidence has emerged that bFGF may be able to interfere with apoptosis. Apoptosis is a selective process of physiologic cell depletion. It is characterized by intranucleosomal DNA cleavage, chromatin condensation, and a reduction in cell size. Cell lines that are consistently overexpressing bFGF after stable transfection with the gene for bFGF show a marked delay in apoptosis after serum starvation. Adding bFGF to the culture media immediately after inducing apoptosis by radiation in bovine endothelial cells resulted in a significant increase in survival at different

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levels of radiation. In addition, Fuks et al demonstrated a protective effect of bFGF in mice receiving total lung radiation. When bFGF was given intravenously before and shortly after radiation, development of radiation-induced pneumonitis was impaired, and samples of irradiated tissue exhibited a lower content of apoptotic cells.

In this study, we were able to demonstrate that higher intracellular bFGF content was associated with an increased resistance to fludarabine-induced apoptosis in vitro. Furthermore, the addition of exogenous bFGF to fludarabine-treated B-CLL cells with lower intracellular bFGF levels yielded delayed apoptosis and increased cell viability similar to that observed for CLL cells with higher intracellular bFGF content. CLL cells with a lower intracellular bFGF content treated with exogenous bFGF cells mimic the phenotype observed in cells containing higher intracellular bFGF content. Moreover, the addition of exogenous bFGF to fludarabine-treated cells with high intracellular bFGF content further increased the survival of these cells, suggesting that the mechanism by which bFGF exerts its effect may be mediated at least in part from the extracellular compartment. These initial observations are promising and will require a larger clinical study to further validate their importance.

Inhibition of apoptosis appears to play an important role in the clonal expansion, tumor progression, and resistance to cytotoxic therapy in CLL. Alterations in specific gene products involved in the regulation of apoptosis have previously been reported in CLL, including deletions of the retinoblastoma gene (Rb), increased expression of bcl-2, and mutations in p53. Several cytokines have also been shown to protect B-CLL cells from undergoing programmed cell death. Interferon-α has been shown to increase bcl-2 expression in B-CLL cells, resulting in a delay of apoptosis in vivo and in vitro. In addition, interferon-γ and interleukin-4 have been shown to inhibit apoptosis of CLL cells and promote survival of cells in vitro. More recently, increased expression of cyclin D2 has been found in CLL, suggesting that this may contribute to the delayed senescence characteristic of this disease. These reports suggest that the potential effects of bFGF on delaying apoptosis and prolonging the survival of B-CLL cells may be indirect, via the induction of one or more of these cytokines or inhibition of specific genes that promote apoptosis. This remains to be determined.

Basic FGF may function as an independent factor or work in concert with one or more of the cytokines or genetic alterations reported to be operative in CLL. Therefore, the expression and biologic activity of bFGF needs to be studied in the context of the expression of other cytokines and gene products involved in the regulation and pathogenesis of this disease. This is presently an area of active investigation in our laboratory.

ACKNOWLEDGMENT

We are especially grateful to Alison Gencarelli for assistance with the collection of B-CLL samples, to Thomas Delohery for excellent performance of the fluorescence-activated cell sorter readings and helpful discussion of the data, and to the Department of Human Genetics for their expert technical assistance with the photomicroscope.

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