Impaired Expression of Cell Surface Receptors for B Cell Growth Factor by Chronic Lymphocytic Leukemia B Cells

By Robert T. Perri

Normal human B cell proliferation is controlled by various immunoregulatory signals including the T cell-derived lymphokine B cell growth factor (BCGF). The role of BCGF in the regulation of malignant B cell proliferation is unclear. Therefore, we studied the proliferative response of purified chronic lymphocytic leukemia (CLL) B cells to BCGF. For all CLL patients studied, CLL B cells showed a decreased proliferative response as compared with control B cells for BCGF-induced B cell proliferation (patient 291 ± 69 cpm vs control 3,942 ± 622, mean ± SEM). This impaired proliferative response appeared to be intrinsic to CLL B cells since it was not corrected by incubation with increasing concentrations of BCGF. Attainment of normal B cell responsiveness to BCGF requires the processing of an initial activation signal which results in the expression of cell surface receptors for BCGF. Increasing concentrations of the B cell activation signal (the Fab', fragment of goat anti-human μ chain) did not improve CLL B cell responsiveness to BCGF. Three-day activated CLL B cells compared with activated control B cells demonstrated a marked impairment in their ability to absorb the BCGF activity present in the BCGF preparation (BCGF activity absorbed out, patient 12.8% vs control 53%). Pretreatment of CLL B cells with neuraminidase failed to improve either the proliferative response to BCGF or the expression of cell surface receptors for BCGF by the CLL B cells. This study suggests that the impaired responsiveness to BCGF by CLL B cells is the result of impaired expression of cell surface receptors for BCGF when CLL B cells are exposed to activation signals.

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and interleukin 2 (IL-2). This BCGF preparation supports both the short-term proliferation of activated human B cells and the proliferation of human B cells in long-term culture. This BCGF preparation does not support the proliferation of T cells. It is primarily enriched for the human B cell stimulating factor (BSF) that appears to correspond to BSF-1. Both SPA 1% (vol/vol) and goat F(ab′)2 anti-human IgM chain 15 μg/mL gave comparable results when used as the initial activation signals in this B cell proliferation assay. The cultures were pulsed with 1H-thymidine (1 μCi per well) (6.7 Ci/mmol/L, New England Nuclear, Boston) over the last 16 to 20 hours of incubation, harvested by a MASHII cell harvester in H2O, and counted in a Packard scintillation spectrometer.

Absorption of BCGF activity by activated B cells. Control or CLL B cells were prepared as described above. These control or CLL B cell-enriched populations were cultured for three days in the presence of SPA 1% (vol/vol) or varying concentrations of goat F(ab′)2 anti-human IgM chain. After this three-day incubation, 1 x 106 mL of viable control or CLL B cells were incubated for two hours at 4°C in an equal volume mix with the BCGF preparation. After this incubation, the preparation was tested for residual BCGF activity in the standard BCGF proliferation assay with freshly isolated control B cells as the target cells. Both SPA 1% (vol/vol) and goat F(ab′)2 anti-human IgM chain 15 μg/mL gave comparable results when used as the initial activation signals. Postabsorption values are those seen with the BCGF preparation before exposure to the three-day activated control or CLL B cells. Postabsorption values are those seen after exposure to the activated control or CLL B cells. PHA-stimulated lymphoblasts failed to absorb BCGF activity for the BCGF preparation. Percentage of activity adsorbed is calculated by dividing postabsorption cpm by preabsorption cpm then subtracting this from one, and multiplying that value by 100.

Neuraminidase treatment of B cells. Control or CLL B cells were isolated as described above. As previously reported, 1 x 106 control or CLL B cells were incubated with neuraminidase (Clostridium perfringens 50 U/mL, Sigma) for 30 minutes at 37°C with constant shaking. The cells were then washed five to seven times and resuspended in RPMI 1640 with 10% FCS prior to their use in the proliferation assays described above.

RESULTS

The responsiveness of CLL B cells to the proliferation signal of BCGF was studied. The proliferative response of CLL B cells to BCGF was markedly impaired (Fig 1). In the presence of both an activation signal and BCGF, control B cells had a stimulation index of 16.7. In contrast, under similar culture conditions, CLL B cells had a stimulation index of only 2.3. All of the CLL B cells we have studied have demonstrated an impaired proliferative response to BCGF.

We next attempted to determine if CLL B cells were capable of responding to increased concentrations of BCGF (Fig 2). Control B cells responded to the proliferation signal of BCGF in a dose-dependent manner. In contrast, at all concentrations of BCGF studied, CLL B cells consistently exhibited a marked impairment in their proliferative response to BCGF.

We next sought to determine whether the impaired proliferative response of CLL B cells to BCGF was the result of impaired processing of the initial activation signal. Previous studies have demonstrated that the nature of the B cell activation signal provided by anti-μ is dependent upon the concentration of anti-μ chosen. Initially, we studied the effect of increasing concentrations of anti-μ on the responsiveness of CLL B cells to the proliferation signal of BCGF (Fig 3). As shown, increased concentrations of anti-μ as the initial activation signal did not improve the responsiveness of CLL B cells to the proliferation signal of BCGF.

Impaired processing of an initial activation signal by CLL B cells would be expected to result in decreased or abnormal expression of cell surface receptors for BCGF by the CLL B cells. Because these studies suggested that CLL B cells have an impaired activation response, we sought to determine if this impaired activation response by CLL B cells results in a defective expression of cell surface receptors for BCGF. In previous studies, the presence of receptors for BCGF on activated control B cells has been demonstrated by their ability to absorb out selectively the BCGF activity present in a BCGF preparation. The ability of activated control and CLL B cells to absorb out the BCGF activity present in the BCGF preparation was studied (Table 1). As shown, activated control B cells were capable of absorbing out significant BCGF activity from the BCGF preparation. In contrast, activated CLL B cells demonstrated a marked impairment in their ability to absorb out BCGF activity from the BCGF preparation. These results suggest that CLL B cells have an impaired expression of cell surface receptors for BCGF. This impaired expression of BCGF receptors by CLL B cells may be the result of an impaired processing by CLL B cells of the initial activation signal.

A previous study demonstrated abnormal glycosylation of CLL T cell membrane components. In an effort to determine if the impaired expression of BCGF receptors in response to activation signals by CLL B cells was the result

![Fig 1](https://example.com/fig1.png) Comparison of (A) control and (B) CLL B cell proliferative responses to BCGF. Cultures were prepared as described in the Materials and Methods section. Data represent the mean ± SEM.

![Fig 2](https://example.com/fig2.png) Proliferative response of control and CLL B cells to varying concentrations of BCGF in the presence of anti-μ (15 μg/mL). Cultures were prepared as described in the Materials and Methods section. Data represent the mean ± SEM.
of abnormal glycosylation of CLL B cell membrane components, we examined whether neuraminidase pretreatment of CLL B cells resulted in improved expression by CLL B cells of cell surface receptors for BCGF after an initial activation signal. CLL B cells were pretreated with neuraminidase before incubation with SPA 1% (vol/vol) for three days. The ability of neuraminidase-pretreated, three-day-activated CLL B cells to absorb out BCGF activity from the BCGF preparation was examined (Table 2). Neuraminidase pretreatment of CLL B cells did not result in enhanced expression of CLL B cell surface receptors for BCGF. Both neuraminidase-pretreated and nontreated CLL B cells had a similarly impaired capability to absorb out BCGF activity.

To confirm the failure of neuraminidase pretreatment to enhance CLL B cell BCGF receptor expression, we next studied the effect of neuraminidase pretreatment of CLL B cells on CLL B cell responsiveness to the proliferative signal of BCGF. No improvement in the proliferative response of CLL B cells to BCGF was seen when CLL B cells were pretreated with neuraminidase. Neither increased concentrations of neuraminidase nor longer preincubation times with neuraminidase resulted in any improvement in the absorption of BCGF by the CLL B cells. Alterations in the intensity of the initial activation signals did not improve the proliferative response to BCGF by CLL B cells. Alterations in the intensity of the initial activation signals did not improve either the proliferative response to BCGF or the expression of cell surface receptors for BCGF by CLL B cells.

Previous studies have identified intrinsic B cell defects in

Table 1. Defective Absorption of BCGF by CLL B Cells

<table>
<thead>
<tr>
<th>BCGF (vol/vol)</th>
<th>Preabsorption Activity Absorbed (%)</th>
<th>Postabsorption by CLL B Cells</th>
<th>Postabsorption by CLL B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>5,351</td>
<td>2,438</td>
<td>54.4</td>
</tr>
<tr>
<td>20</td>
<td>7,272</td>
<td>3,633</td>
<td>53.0</td>
</tr>
<tr>
<td>10</td>
<td>5,351</td>
<td>—</td>
<td>16.0</td>
</tr>
<tr>
<td>20</td>
<td>7,272</td>
<td>—</td>
<td>12.8</td>
</tr>
</tbody>
</table>

BCGF, B cell growth factor; CLL, chronic lymphocytic leukemia.

*Incorporation of ³H-thymidine refers to the proliferative response of 5 x 10⁸ control B cells to staphylococcal Protein A (SPA) 1% (vol/vol) in the presence of the BCGF preparation, which was added both before and after absorption by SPA-stimulated control and CLL B cells. Cultures were incubated for three days with the addition of ³H-thymidine (1 µCi per well) for the last 16 hours. For the absorption of the BCGF preparation, control and CLL B cells were exposed to SPA 1% (vol/vol) for three days. After this interval, control and CLL B cells (10 x 10⁶ viable cells) were incubated for two hours at 4 °C with the BCGF preparation. After incubation, the preparation was tested for residual BCGF activity. Preabsorption values are those seen with the BCGF preparation before exposure to the SPA-stimulated B cells. Postabsorption refers to those values seen after exposure to the activated B cells. Activated PHA-stimulated T lymphoblasts failed to absorb the BCGF activity. Neuraminidase treatment of control B cells did not alter their absorption of BCGF.

†BCGF (vol/vol) refers to the final concentration of the BCGF preparation added to the proliferation assay.

‡Percentage of activity absorbed is calculated by dividing postabsorption cpm by preabsorption cpm, then subtracting this from one, and multiplying that value by 100.

Table 2. Effect of Neuraminidase Treatment of CLL B Cells on Absorption of BCGF by CLL B Cells

<table>
<thead>
<tr>
<th>BCGF (vol/vol)</th>
<th>Preabsorption by CLL B Cells</th>
<th>Postabsorption by Treated CLL B Cells</th>
<th>Activity Absorbed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9,495</td>
<td>7,042</td>
<td>25.8</td>
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<tr>
<td>20</td>
<td>13,611</td>
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<td>9,495</td>
<td>—</td>
<td>21.3</td>
</tr>
<tr>
<td>20</td>
<td>13,611</td>
<td>—</td>
<td>3.2</td>
</tr>
</tbody>
</table>

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*Incorporation of ³H-thymidine refers to the proliferative response of 5 x 10⁸ control B cells to staphylococcal Protein A (SPA) 1% (vol/vol) in the presence of the BCGF preparation, which was added both before and after absorption by SPA-stimulated neuraminidase-pretreated and nontreated CLL B cells. Cultures were incubated for three days with the addition of ³H-thymidine (1 µCi per well) for the last 16 hours. For the absorption of the BCGF preparation, CLL B cells were exposed to SPA 1% (vol/vol) for three days. Neuraminidase-treated and nontreated CLL B cells (10 x 10⁶ viable cells) were incubated then for two hours at 4 °C with the BCGF preparation. After incubation, the preparation was tested for residual BCGF activity. Preabsorption values are those seen with the BCGF preparation before exposure to the SPA-activated cells. Postabsorption refers to those values seen after exposure to the activated cells. Activated PHA-stimulated T lymphoblasts failed to absorb the BCGF activity. Neuraminidase treatment of control B cells did not alter their absorption of BCGF.

†BCGF (vol/vol) refers to the final concentration of the BCGF preparation added to the proliferation assay.

‡Percentage of activity absorbed is calculated by dividing postabsorption cpm by preabsorption cpm, then subtracting this from one, and multiplying that value by 100.
CLL B cells. CLL B cells have a markedly impaired proliferative response to mitogenic signals such as PWM. The addition of control T cells or monocytes did not improve the proliferative response of CLL B cells to PWM. Decreased CLL B cell colony growth in vitro B cell clonogenic assays has been reported. Membrane abnormalities of CLL B cells have also been reported. CLL B cells incubated with anti-immunoglobulin reagents have abnormal dynamics of surface membrane immunoglobulin redistribution (capping). In addition, impaired motility of CLL B cells has been described.

The current model of normal B cell proliferation proposes that normal human B cell responsiveness to the proliferation signal of BCGF requires the expression of cell surface receptors for BCGF. The processing of an initial activation signal by normal human B cells results in the expression of cell surface receptors for BCGF. The presence on activated lymphocytes of receptors for specific growth factors (ie, IL-2 or BCGF) has been identified by their ability to absorb out selectively the specific growth factor activity from preparations containing the specific growth factor. Other investigators have used a BCGF preparation similar to the preparation used in this study and have demonstrated that three-day, anti-μ-activated control human B cells can absorb out 55% of BCGF activity from this BCGF preparation. In the current study, three-day SPA-activated control B cells absorbed out a nearly identical 54.4% of BCGF activity from the BCGF preparation. In contrast, CLL B cells consistently demonstrated a markedly impaired ability to absorb out BCGF activity from the BCGF preparation. These results suggest that CLL B cells have a defective expression of cell surface receptors for BCGF.

Cryptic or hidden expression of cell surface antigenic determinants by CLL T cells have been reported. Some CLL T cells are E rosette positive but fail to react with OKT3, a monoclonal antibody specific for mature human T cells. A cryptic or hidden antigenic expression of OKT3 by these CLL T cells was suggested by the finding that after neuraminidase treatment these CLL E rosette-positive, OKT3-negative T cells became reactive with OKT3. The cryptic or hidden antigenic expression of OKT3 in these CLL T cells appears to be related to an as-yet-undefined membrane abnormality of CLL T cells that results from abnormal glycosylation of cell membrane components. The absence of any improvement in either CLL B cell proliferative response to BCGF or the expression of CLL B cell surface receptors for BCGF suggests that the abnormalities of CLL B cells reported in this study are not the result of cryptic BCGF receptor expression secondary to abnormal glycosylation of cell membrane components.

Other studies have suggested that CLL B cell dysfunction is a result of impaired differentiation. The expression of surface membrane immunoglobulin by CLL B cells is markedly less than expressed by more differentiated B cells. It has been suggested that only normal B cells that express surface IgG are responsive to PWM. Because most CLL B cell surface immunoglobulin is IgM, it has been theorized that CLL B cells may fail to respond to PWM because they lack the binding sites for PWM.

Murine studies have suggested that the influence of proliferation signals such as BCGF on B cells is affected by the stage of development of the target B cell. Anti-immunoglobulin stimulation of BCL1 murine malignant B cells did not result in increased proliferation whereas the BCL1 B cells had assumed a more mature phenotype before exposure to the anti-immunoglobulin. Anti-immunoglobulin stimulation could not induce proliferation of normal immature murine B cells.

The stage of human B cell development and differentiation during which normal B cells are responsive to BCGF is not yet known. A recent study has demonstrated that B cells responsive to BCGF may be selected by the presence on the B cell of the B2 surface antigen. B2 is a 140-kd glycoprotein that expresses distinct binding sites for C3d and for EBV. B2 first appears on the cell surface after the cytoplasmic µ-chain–positive pre-B cell stage and is lost when surface IgD is no longer detectable. B2-positive B cells were responsive to the proliferation signals of anti-µ and anti-µ plus PHA-stimulated leukocyte-conditioned media (PHA-LCM). If CLL is a disorder characterized by a clonal expansion of immature B cells, such immature B cells may not have developed a responsiveness to the proliferation signal of BCGF.

A recent study has demonstrated some proliferative responsiveness of CLL B cells to "T cell factors." However, induction of CLL B cell proliferation required activation by anti-immunoglobulin at 200 µg/mL. In that study, data on the effectiveness of more conventional concentrations of anti-immunoglobulin as an initial activation signal were not presented. However, even with a 15-fold increase in the concentration of anti-µ, the stimulation index was only 3.8, comparable to results in this study in which the stimulation index was 3.1 when anti-µ at 15 µg/mL was used as an activation signal. That occasional CLL B cells may be capable of some degree of responsiveness to BCGF is suggested by the reported ability of BCGF to support the growth of some leukemic B cells in vitro for periods of up to 60 days.

This study does not resolve the role of BCGF in regulating CLL B cell proliferation. It does show that the impaired responsiveness to BCGF by CLL B cells is the result of impaired expression of receptors for BCGF when CLL B cells are exposed to activation signals. The apparent arrest of CLL B cells at an immature stage of B cell development may have impact upon the responsiveness of the malignant B cell to the proliferation signal of BCGF. Because some CLL B cells may be induced to further differentiation by agents such as phorbol esters, we have begun to investigate whether in vitro-induced differentiation of CLL B cells results in improved CLL B cell responsiveness to BCGF. Impaired responsiveness to immunoregulatory lymphokines such as BCGF may contribute to the significant hypogammaglobulinemia observed in CLL patients.

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