Excessive Production of Transforming Growth Factor-β by Bone Marrow Stromal Cells in B-Cell Chronic Lymphocytic Leukemia Inhibits Growth of Hematopoietic Precursors and Interleukin-6 Production

By L. Lagneaux, A. Delforge, C. Dorval, D. Bron, and P. Stryckmans

To explore the pathogenesis of marrow failure in B-cell type chronic lymphocytic leukemia (B-CLL), we have examined the production of interleukin-6 (IL-6), granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage CSF (GM-CSF) by the adherent cell population of bone marrow (BM) derived from B-CLL patients and their capacity to support hematopoietic cell proliferation. Lipo-polysaccharide-stimulated B-CLL stromal cells produced G-CSF and GM-CSF in amounts similar to normal stromal layers, whereas IL-6 production was significantly decreased. Using the blast-colony forming cell assay (B-CFC) and the classical colony-forming unit granulocyte macrophage (CFU-GM) assay, we found that: (1) marrow stromal cells of B-CLL were able to support only 25% of the Bi-CFC growth supported by normal marrow stromal cells; (2) this anomaly was partially corrected by the addition of exogenous IL-6; (3) the colony-stimulating activity (CSA) of the conditioned medium (CM) of B-CLL stromal cells was lower than that of normal CM; (4) that this was the result of the presence of an inhibitor rather than of a growth factor defect; (5) this inhibition could be abrogated by addition of anti-transforming growth factor-β (TGF-β) neutralizing antibody; (6) this antibody corrected the deficient colony supportive activity of the B-CLL stromal cells; (7) TGF-β production by marrow stromal cells was significantly increased in CLL compared with normal; and (8) that this was not caused by the effect of the B-CLL lymphocytes on the stromal cells. It is concluded that this increased TGF-β production in B-CLL is probably responsible for the decreased IL-6 production by stromal cells and for the inhibiting activity on hematopoietic precursors as well. We hypothesize that TGF-β generated at a high level by B-CLL marrow stromal cells could play a major role in the pathophysiology of the BM failure seen in advanced stages of B-CLL.

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cells were counted. Stromal layers without addition of nonadherent cells were treated in the same way and acted as controls for the C02 in a humidified atmosphere and colonies of greater than 20 G-CSF (Quantikine; R&D Systems, Minneapolis, MN). and the medium was replaced by 1 mL of 0.3% agar in a-MEM and volume.''' Before plating, I

Blot- Colony Forming Cell (Bi-CFC) Assay

Bi-CFC were grown using the assay described by Gordon et al.17 Two different normal marrow were tested in parallel for Bi-CFC growth on normal versus B-CLL stromal cells. Mononuclear cells from normal BM were diluted to 1 × 10^9 cells per milliliter in α-MEM supplemented with 15% FCS and depleted of adherent cells by incubation in plastic culture flasks for 2 hours at 37°C in 7.5% CO2 and 100% humidity.

The nonadherent fraction (5 × 10^5 cells/mL) was then layered on top of the normal and the B-CLL stromal layers and incubated for 2 hours at 37°C, 7.5% CO2.

After the 2-hour incubation, the stromal layers were washed three times to remove any cells that had not attached to the stromal cells and the medium was replaced by 1 mL of 0.3% agar in α-MEM and 15% FCS. The plates were incubated for 5 days at 37°C and 7.5% CO2 in a humidified atmosphere and colonies of greater than 20 cells were counted. Stromal layers without addition of nonadherent cells were treated in the same way and acted as controls for the colony origin. Bi-CFC experiments were always performed 3 days or more after feeding of the stromal layers.

Day 7 Colony-Forming Unit Granulocyte Macrophage (CFU-GM) Assay

GM committed progenitor cells (CFU-GM) were grown as described by Pike and Robinson.18 Colony growth was stimulated by human placenta conditioned medium (HPCM) used at 10% of final volume.19 Before plating, 1 × 10^9 marrow cells were added to 1 mL Iscove's modified Dulbecco's medium (IMDM) (GIBCO) supplemented with 20% FCS and 0.3% agar. The cells were incubated for 7 days at 37°C, 7.5% CO2 and in a 100% humidified atmosphere. CFU-GM included colonies (≥ 40 cells) and clusters (5 to 39 cells) that were scored using an inverted microscope.

Preparation of Stromal Cell Conditioned Media (CM)

In all experiments, CM were prepared from 6-week-old stromal layers when the marrow stromal cells had grown to form a confluent adherent layer.

To investigate the production of colony-stimulating factors (CSFs) by B-CLL and normal stromal cells, bacterial LPS (Sigma, St Louis, MO) at 20 μg/mL or IL-1 (Hoffman-La Roche, Nutley, NJ) at 5 ng/mL were added for 24 hours. After this incubation, culture supernatants were collected and stored in aliquots at −80°C until future use.

Measurement of Cytokines in CM

Cytokines were measured using specific immunologic procedures [enzyme-linked immunosorbent assay (ELISA)]; IL-6 (Eurogenetics, Tessonerto, Belgium), granulocyte-macrophage colony-stimulating factor (GM-CSF) (Medgenix, Fleurus, Belgium) and G-CSF (Quantikine; R&D Systems, Minneapolis, MN).

CM were also tested for tumor necrosis factor-α (TNF-α) using the L929 biologic assay.20 One unit of TNF-α was defined as the concentration at which 50% of the L929 cells showed cytopathic effect after 3 days of incubation.

Assay for Colony-Stimulating Activity (CSA)

Stromal cell CM were assayed at 10% for CSA content on the basis of their capacity to induce GM colonies using the CFU-GM assay as described above but without HPCM.

Results were expressed as a percentage of colony number present in the control plates with HPCM.

Assay for Colony-Inhibiting Activity (CIA)

To detect the presence of CFU-GM inhibitors in B-CLL and normal CM, CFU-GM formation was induced by the addition of 10% HPCM in the presence or absence of 10% CM from B-CLL or normal subjects.

Results were expressed as a percentage of control CFU-GM growth in the presence of 10% HPCM.

Transforming Growth Factor-β (TGF-β) Determination

The CCL64 assay was based on the procedure described by Ikeda et al.21 Mv 1Lu mink lung epithelial cells (CCL64; American Type Culture Collection, Rockville, MD) were subcultured in flat bottomed 96-well tissue culture plates (Nunc, Roskilde, Denmark) in 50 μL of IMDM (GIBCO) at 2 × 10^5 cells/well. Standard (R&D Systems) or conditioned media for TGF-β detection were diluted in complete medium and assayed in triplicate (50 μL/well). Cells were incubated at 37°C for 24 hours, then they were pulsed with 1 μCi/well of [3H]thymidine (Amersham, Aylesbury, UK) during the last 4 hours. At the end of the incubation, the cells were harvested onto glass-fiber filters and radioactivity was measured.

Neutralizing Studies in the Presence of Anti-TGF-β Antibody

The inhibiting activity of CM was also assessed in the presence of a chicken antihuman TGF-β neutralizing antibody (R&D Systems) used at a concentration of 1 μg/mL.

Phenotype Analysis of Stromal Cells

For direct immunofluorescence, 6-week-old stromal cells were detached by 0.25% trypsin in phosphate-buffered saline (PBS) (pH 7.4), washed with PBS and incubated at room temperature for 30 minutes with specific monoclonal antibodies (MoAbs).

Double-labeling fluorescence staining was performed using CD5 fluorescein isothiocyanate (FITC) and CD19-phycocerythrin (CD19; PE; Dakopatts, Glastrup, Denmark). The cells were analyzed with an EPICS-Profile II flow cytometer (Coulter, Hialeah, FL). Positive cells were determined by reference to nonspecific staining by control nonrelevant monoclonal antibodies (MoAbs) of the same isotype.

Contamination Experiments With CLL B-Lymphocytes

Peripheral blood mononuclear cells (PBMC) from one B-CLL patient were isolated by Ficoll Hypaque gradient (International Medical Product). Highly purified B cells (no detectable CD3 or CD14 positive cells) were negatively selected by a combination of rosetting with aminoethyl-isothouronium bromide treated sheep red blood cells (SRBC) and treatment with L-leucine ester (Sigma).

Increasing concentrations of highly purified B cells (10^3 to 10^6) were incubated for 1 week on two normal stromal layers. After this incubation period and appropriate stimulation, supernatants were collected and tested for IL-6 and TGF-β production.

Statistical Analysis

Comparison of G-CSF, GM-CSF, TGF-β, and IL-6 production by stromal cells from normal and B-CLL patients was performed.
using the nonparametric Mann-Whitney U-test. Growth of normal Bl-CFC on normal or B-CLL stromal cells was compared using the paired Student’s t-test. CSA activity and presence of inhibitor in supernatant from stromal cells of normal or B-CLL patients were compared using the Student’s t-test.

RESULTS

Production of GM-CSF, G-CSF, and IL-6 by the Stromal Layers

As shown in Table 1, low levels of CSFs are produced by stromal layers but the production can be greatly enhanced by LPS or IL-1. Moreover, the mean IL-6 production by LPS or IL-1-stimulated stromal cells was, respectively, 123 ± 47 (mean ± SEM) or 87 ± 20 ng/mL for eight controls compared with 36 ± 5 or 40 ± 7 ng/mL for the five B-CLL. These differences between B-CLL and controls were statistically significant (respectively, P < .004 and P < .04). On the other hand, no significant differences were seen for LPS or IL-1-stimulated GM or G-CSF production when comparing normal or B-CLL stromal layers.

Bl-CFC Growth on Normal Versus B-CLL Stromal Layers

The stromal cells were tested for growth-supporting activity of Bl-CFC by adding normal BM mononuclear cells of two normal subjects in parallel on stromal layers of four normal or B-CLL stromal cells. These experiments were done in the presence or absence of 100 ng/mL of IL-6. Table 2 shows a significantly decreased growth of normal Bl-CFC on B-CLL stromal layers: 25% of normal growth (P < .011). Addition of exogenous IL-6 increased this value to 53% of normal Bl-CFC growth.

Production of CSA by BM Stromal Cells

Stroma that had not been stimulated by LPS or IL-1 did not produce biologically active CSA as measured by the ability to stimulate human BM cells to produce CFU-GM. Stimulation by LPS or IL-1 resulted in biologically detectable CSA.

Table 1. Endogenous, LPS, and IL-1-Stimulated Production of GM-CSF, G-CSF, and IL-6 by Adherent Marrow Stromal Layers From Normal Subjects or B-CLL Patients

<table>
<thead>
<tr>
<th>CSA</th>
<th>Inducer</th>
<th>N (n = 8)</th>
<th>CLL (n = 5)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>None</td>
<td>23 ± 5</td>
<td>37 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>(pg/mL)</td>
<td>LPS</td>
<td>119 ± 13</td>
<td>132 ± 31</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>IL-1</td>
<td>100 ± 12</td>
<td>114 ± 33</td>
<td>NS</td>
</tr>
<tr>
<td>G-CSF</td>
<td>None</td>
<td>90 ± 3</td>
<td>90 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>(pg/mL)</td>
<td>LPS</td>
<td>5,500 ± 130</td>
<td>3,820 ± 600</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>IL-1</td>
<td>ND</td>
<td>5,260 ± 160</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6</td>
<td>None</td>
<td>2.45 ± 0.54</td>
<td>1.7 ± 0.16</td>
<td>NS</td>
</tr>
<tr>
<td>(ng/mL)</td>
<td>LPS</td>
<td>123 ± 47</td>
<td>36 ± 5</td>
<td>.004</td>
</tr>
<tr>
<td></td>
<td>IL-1</td>
<td>87 ± 20</td>
<td>40 ± 7</td>
<td>.04</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.
Abbreviations: NS, not significant; ND, not determined.
* Mann-Whitney U-test.

Fig 1. CSA in supernatant of stromal layers. Data were expressed as percentage (mean ± SEM, N = 6) of total number of colonies obtained on day 7 in the presence of HPCM. ( ) Unstimulated stromal layer; ( ) LPS-stimulated stromal layer; ( ), IL-1-stimulated stromal layer.

Table 2. Growth of Normal Marrow Bl-CFC on Normal Versus B-CLL Stromal Cells

<table>
<thead>
<tr>
<th>Type of Stroma</th>
<th>N</th>
<th>Mean Number Bl-CFC</th>
<th>% of Normal Mean Growth</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4</td>
<td>445 ± 81</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Normal + IL-6</td>
<td>3</td>
<td>368 ± 75</td>
<td>83</td>
<td>.011</td>
</tr>
<tr>
<td>B-CLL</td>
<td>4</td>
<td>111 ± 53</td>
<td>25</td>
<td>NS</td>
</tr>
<tr>
<td>B-CLL + IL-6</td>
<td>4</td>
<td>238 ± 95</td>
<td>53</td>
<td>NS</td>
</tr>
</tbody>
</table>

These experiments were done in the presence or absence of 100 ng/mL of IL-6.
*Paired Student’s t-test.

normal-cell CM but that the stimulatory effect of B-CLL-conditioned media was significantly less than CM from normal controls (32% ± 6% v 99% ± 9%, P < .0001).

Assay for the Presence of CFU-GM Inhibitors in CM

The presence of possible CFU-GM inhibitors in conditioned media was tested by adding 10% of normal or B-CLL stromal-cell CM to HPCM-stimulated CFU-GM cultures. Results presented in Fig 2 show that only B-CLL CM contained colony inhibitors that significantly reduced HPCM-stimulating activity (44 ± 4 CFU-GM v 83 CFU-GM, P < .0001, n = 5).

Production of TNF-α by the Stromal Layers

To investigate the nature of the inhibitory activity present in B-CLL CM, we have measured the production of TNF-α by B-CLL stromal cells.

Our results show a low TNF-α production that is similar
for B-CLL and normal stromal layers (3 ± 2 U/mL). Thus, these data indicate that the inhibitory activity of B-CLL CM could not be attributed to TNF-α.

**Production of TGF-β by Stromal Cells**

Normal and B-CLL CM were tested in the CCL64 assay. Fig 3 shows that bioactive TGF-β was found in all CM. In normal CM, TGF-β concentration did not change significantly after LPS stimulation (respectively, 8 ± 4 ng/mL vs 15 ± 4 ng/mL). TGF-β in unstimulated supernatants from B-CLL stromal cells was slightly increased in comparison with normal stromal layers (respectively, 15 ± 3 ng/mL vs 8 ± 4 ng/mL). However, in B-CLL stroma, LPS induced a threefold to fourfold increase of TGF-β production (53 ± 10 ng/mL) (P < .03). These results show that B-CLL stromal cells can produce significantly more TGF-β than normal stromal cells.

**Neutralization Experiments With Anti-TGF-β**

**On CFU-GM.** To identify inhibitors responsible for the colony inhibition detected in B-CLL stromal cells, neutralization experiments were performed. Each conditioned medium was incubated with 1 μg/mL of a chicken antihuman TGF-β neutralizing antibody and then retested for its inhibiting activity on CFU-GM growth. Results presented in Fig 4 show that incubation with anti-TGF-β neutralizing antibody had no effect on the growth of CFU-GM stimulated by HPCM additioned with normal CM; however, this antibody neutralized colony-inhibiting activity present in B-CLL CM (80% ± 8% vs 22% ± 4% of control CFU-GM growth).

**On Bl-CFC.** Because we have shown (1) that TGF-β is produced in excess by B-CLL stromal cells and (2) that anti-TGF-β can suppress the inhibition exerted by CM of B-CLL stromal cells on the proliferation of CFU-GM, we questioned whether the proliferation of normal Bl-CFC on B-CLL stromal layers could also be modified after incubation with an anti-TGF-β neutralizing antibody. The results of two experiments are presented in Fig 5. First, it is shown that anti-TGF-β and IL-6 have no effect on Bl-CFC growth on normal stromal cells. Secondly, on B-CLL stromal layers, addition of IL-6 improved Bl-CFC growth to 73% ± 14% versus 38% ± 4% of normal growth, whereas anti-
TGF-β induced an even more significant increase in Bl-CFC growth (138% of normal growth).

On IL-6 production. The anti–TGF-β antibody (results not shown) also totally normalized the production of IL-6 by B-CLL stromal cells in three experiments.

Detection of CLL B Lymphocytes in Stromal Layers

Using flow cytometry, we found less than 1% B lymphocytes in B-CLL stromal layers tested after 6 weeks of culture. Moreover, identical percentages (close to the background) were obtained for two normal stromal layers. These results and microscopic examination of stromal cells confirmed that only very few B lymphocytes were present in stromal layers after 6 weeks of culture.

Contamination of Normal Stromal Layers With CLL B Lymphocytes

To confirm that CLL B lymphocytes were not responsible for altered cytokine production detected in B-CLL stromal layers, we performed contamination experiments. As shown in Fig 6, normal BM stromal layers contaminated by CLL B lymphocytes (10^3 to 10^6) secreted similar amounts of IL-6 and TGF-β as uncontaminated stromal cells. These observations suggest that CLL B lymphocytes do not play a direct role in the increased cytokine production by B-CLL stromal cells.

DISCUSSION

The present results show that it is possible in B-CLL, as in normal controls, to prepare confluent stromal layers from marrow cells. However, in CLL an increase (by a factor of 10) in the number of marrow adherent cells plated may be required. A confluent layer of stromal cells indistinguishable from that of normal controls and containing rare lymphocytes can then be obtained within 4 to 8 weeks.

A previous observation made on CLL peripheral blood mononuclear cells (PBMC) had indicated a decreased production of IL-6 by PBMC of B-CLL patients compared with that of normal subjects. This observation suggested a decreased IL-6 production by blood monocytes but could not rule out the involvement of T and/or B lymphocytes. Therefore, it was hypothesized that if blood monocytes were implicated, marrow stromal cells of CLL patients could also behave abnormally and that this could have some implication on proliferation and maturation of myeloid as well as lymphoid cells growing in the marrow of CLL patients. Therefore, our first step after obtaining CLL stromal cells was to examine the production of IL-6 by the marrow stromal cells after exposing them to LPS or IL-1. For the sake of comparison with normal controls, the production of G- and GM-CSF in addition to that of IL-6 were also measured in the stromal supernatants. These measurements clearly showed a significant decrease of IL-6 production by B-CLL stromal cells (P < .04 to P < .004), whereas G-CSF and GM-CSF were produced normally. This last observation emphasizes that normal and CLL stromal layers were not only morphologically comparable but also functionally.

B-CLL stromal layers were then compared with normal stromal cells for their capacity to support the growth of normal Bl-CFC. These experiments showed that the CLL stromal cells had only 25% of the Bl-CFC proliferation supporting activity of normal stromal cells. Based on our previous results, these experiments were performed in the presence and in the absence of 100 ng/mL recombinant human (rh)IL-6. Addition of IL-6 did not improve the growth of Bl-CFC on normal stromal layers whereas it doubled that seen on CLL stromal layers. However, IL-6 was not able to restore growth to normal. This suggested that the decreased IL-6 production was only a partial or a secondary cause of the decreased Bl-CFC growth.
The supernatant of stromal cells from normal and B-CLL marrow stimulated by LPS were then compared for their growth-promoting activity in the CFU-GM culture system (CSA). The results were expressed in percentage of growth obtained by HPCM. This experiment showed that the supernatant of normal stromal cells had 99% ± 9% activity, thus the same as the HPCM, whereas the CLL derived ones exhibited only 32% ± 6% of this activity. Thus, these results indicate that the anomaly, whether it is caused by lack of a stimulant or excess of an inhibitor, decreases similarly Bl-CFC and CFU-GM.

The next step was designed to distinguish, in the CFU-GM system, between lack of stimulation or excessive inhibition as the cause of the anomalies observed in CLL. Therefore, HPCM used as stimulant was mixed with only 10% CM generated by either normal stromal cells or CLL stromal cells. This experiment showed clearly an excessive inhibition generated by the CLL stromal cells.

Three potential inhibitors were then envisaged: (1) prostaglandins, (2) TNF-α, and (3) TGF-β. These three substances are known for their inhibiting activity on hematopoietic precursors. Previous studies have indicated that recombinant TNF-α can inhibit the in vitro colony growth of normal granulocyte/macrophage progenitors (CFU-GM). However, it appears unlikely that TNF could play an important role by itself in our experiments for the following reasons: (1) B lymphocytes of B-CLL that are able to produce TNF constitutively are not seen in our stromal cell layer of CLL marrows; (2) in the present study TNF production by stromal cells was very low (2 to 3 U/mL), whereas levels on the order of 5,000 U/mL seem to be required for maximal CFU-GM suppression; (3) the very low production of TNF, was similar for CLL and normal stromal layers (3 ± 2 U/mL); and (4) TNF has been shown to induce the production by monocyte-macrophage cells of IL-6 that is significantly decreased in our studies.

Therefore, TGF-β appears to be a more likely candidate explaining the results of the present study for the following reasons: (1) TGF-β is produced by monocytes, one of the marrow stromal cells in our culture system; (2) TGF-β is a well-known inhibitor of IL-6 production by LPS-stimulated monocytes, which is consistent along with the observed decrease in IL-6 production by stromal B-CLL cell supernatants we have observed; (3) TGF-β is the most potent endogenous suppressor of lymphocyte proliferation and function, the inhibition it produces on the production of Igs by normal human B lymphocytes is quite compatible with the hypogammaglobulinemia often seen in CLL patients; (4) in other studies, TGF-β has been shown to be an important physiologic negative regulator of early hematopoietic cells; and (5) finally, the results of the present experiments showing that B-CLL stromal cells produce increased amount of TGF-β are quite convincing for the negative regulatory role of TGF-β in B-CLL.

Because TGF-β is shown to be produced by CLL as well as by normal marrow stroma, one wonders how anti-TGF-β antibodies do stimulate only the Bl-CFC growing on CLL stroma and not on normal stroma. This suggests that, at the concentrations generated by normal stromal cells, TGF-β exerts only very little inhibition. An explanation for this could be that in the normal conditions, the stimulatory arm of the control of hematopoiesis (made of all the growth factors positively affecting hematopoiesis) is overriding the inhibitory arm so massively that the lower level of TGF-β characterizing the basic conditions of hematopoiesis is not really affecting the equilibrium between the two opposite regulatory influences. On the contrary, when TGF-β is produced in considerably higher amounts, as is the case in CLL, its inhibitory activity only starts to affect hematopoiesis and as a corollary, its neutralization by antibodies is producing upregulation of hematopoiesis. This explanation is reinforced by our observation that G- and GM-CSF productions are not different in normal and CLL stromal layers.

However, it should be remembered that whereas TGF-β has been shown to inhibit early progenitors such as high proliferative potential colony-forming cell (HPP-CFC), CFU-blast, burst-forming unit-erythroid (BFU-E), and even day 14 CFU-GM, on the other hand, day 7 human CFU-GM have been reported to be potentiated by TGF-β. Thus, these features are different from the present study in which both Bl-CFC and day 7 CFU-GM were found to be inhibited in approximately the same proportions. Therefore, we tested in our system the effect of recombinant human TGF-β on both Bl-CFC and on day 7 CFU-GM and found that both were similarly inhibited.

Because decreased IL-6 production and increased TGF-β production have been seen in CLL B lymphocytes, a doubt could subsist about the role of these leukemic lymphocytes on perturbated cytokine production by stromal cells. However, failure to show by flow cytofluorometry significant contamination of our stromal cell preparation by CLL B lymphocytes and the negativity of the contamination experiments with CLL B lymphocytes suggests that these lymphocytes are not involved directly in the cytokine perturbation observed. For these reasons, we consider that overproduction of TGF-β in CLL is to be attributed to BM environment.

It has been reported recently that PBMC and highly enriched B cells from B-CLL patients express mRNA for TGF-β and release significant amounts of TGF-β in vitro. Moreover, Israel et al. reported that TGF-β, in contrast to its inhibitory action on DNA synthesis of normal B cells in vitro, has little or no effect on lymphocytes from B-CLL patients. The refractoriness of B-CLL to TGF-β suggests that TGF-β might contribute to a growth advantage of leukemic B cells over normal hematopoietic precursors.

Finally, our study supports the hypothesis that increased TGF-β production could play a crucial pathogenic role in some manifestations of this disease, especially BM failure that is constant in advanced stages of CLL.

Further investigations will be necessary to establish the precise role of each stromal cell type in the increased TGF-β production and whether a correlation exists between this overproduction and the stage of the disease.

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Excessive production of transforming growth factor-beta by bone marrow stromal cells in B-cell chronic lymphocytic leukemia inhibits growth of hematopoietic precursors and interleukin-6 production

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