In Vivo and In Vitro Suppression of Primary B Lymphopoiesis by Tumor-Derived and Recombinant Granulocyte Colony-Stimulating Factor

By Minako Y. Lee, Karen L. Fevold, Kenneth Dorshkind, Rikiro Fukunaga, Shigekazu Nagata, and Cornelius Rosse

Transplantation of a granulocytosis-inducing murine CE mammary carcinoma into mice suppresses primary B lymphopoiesis in the marrow. The mechanisms of this tumor-induced B-cell suppression were investigated using Whitlock-Witte-type lymphoid cultures. When seeded with normal marrow progenitors, stromal cells of tumor-bearing mice supported the production of B220⁺ cells as well as did either stromal cells derived from control mice or the stromal cell line S17. Cultured over normal stroma, marrow cells of tumor-bearing mice depleted of adherent cells and B220⁺ cells generated B220⁺ cells as effectively as a similar cell population from control mice. However, interleukin-7–responsive progenitors, were completely depleted from the marrow of tumor-bearing mice. When conditioned medium (CM) of cloned CE tumor cells known to produce granulocyte colony-stimulating factor (G-CSF) and macrophage-CSF, or recombinant murine G-CSF was added to the cultures established with S17 cells, B220⁺ cell production was significantly diminished. Anti-serum to murine G-CSF blocked these effects. These in vitro observations were corroborated by the elimination of marrow B220⁺ cells in mice injected with G-CSF. These in vitro and in vivo studies suggest that G-CSF plays an inhibitory role in primary B lymphopoiesis by blocking stromal cell-mediated differentiation of early B-cell progenitors into phenotypically recognizable B220⁺ pre-B cells.

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COLONAL ASSAYS for hematopoietic progenitors, well-characterized cytokines, and long-term bone marrow (BM) culture systems, along with the availability of defined cell lines, have contributed much to our understanding of the factors that maintain and promote the differentiation of most lympho-hematopoietic cell types produced in the BM. Less is known about the mechanisms that limit the response of certain BM cells to physiologic, pathologic, or experimental stimuli that call for escalating cell production in a particular lineage. Reciprocal adjustments in lineage-specific cell production have been observed under clinical and experimental conditions suggesting that there are mechanisms that regulate a balance in multilineage hematopoiesis. Examples include classic studies of reciprocal changes in the erythroid and lymphoid compartments of the BM during hypoxia and polycythemia,¹² similar relationships observed between BM lymphocytes and granulocytes in cyclic neutropenia,² and more recent studies showing suppression of erythropoiesis during a granulocytic response⁴⁻⁵ and suppression of thrombopoiesis during enhanced erythropoiesis.⁶

The murine experimental model we have developed offers opportunities for investigating the mechanisms that could account for the downregulation of one cell lineage while another lineage is being upregulated. We have previously shown that transplantation of a nonmetastasizing murine tumor (CE mammary carcinoma) greatly augments the production of neutrophils,⁷⁻⁸ but suppresses erythropoiesis⁹,¹⁰ and lymphopoiesis¹¹ in the BM of tumor-bearing mice. Kinetic characterization of marrow lymphocytes in these animals suggested that the tumor inhibited primary B-cell production by eliminating progenitor cells that feed into the phenotypically recognizable, pre-B–cell compartment.¹² Our more recent work has established that cloned CE mammary carcinoma cells constitutively produce granulocyte colony-stimulating factor (G-CSF), macrophage-CSF (M-CSF), and possibly other CSFs.¹³ We designed the present study with the following objectives: (1) to test whether suppressed B lymphopoiesis in tumor-bearing mice was a consequence of compromised B-cell progenitor or stromal cell function, and (2) to determine whether the tumor’s suppressive effect on B lymphopoiesis could be attributed to a known or unknown cytokine that it produces.

MATERIALS AND METHODS

Mice and tumor transplantation. Eight- to twelve-week-old male and female (Balb/c × CE) F1 mice,¹⁴ bred in the vivarium of University of Washington, were used for all experiments, unless otherwise noted. A granulocytosis-inducing murine CE mammary carcinoma¹⁴ that has been passaged in mice in our laboratory was used for tumor transplantation.⁹ Mice with a blood neutrophil count exceeding 40,000/µL on days 17 through 21 after tumor cell inoculation were used for the experiments. Age- and sex-matched normal mice served as controls in all experiments.

BM cell suspensions. BM cells were flushed from femurs with lymphoid BM culture (LBMC) medium, originally described by Whitlock and Witte.¹⁵ LBMC medium contained RPMI 1640 (GIBCO BRL, Grand Island, NY), 5% (vol/vol) heat-inactivated fetal calf serum (FCS; HIFCS, Hyclone lot no. 111879; Hyclone, Logan, UT), 2 mmol/L L-glutamine, and 5 × 10⁻⁵ mol/L 2-mercaptoethanol (2-ME). Viable cells pooled from six femurs of either tumor-bearing or control mice were enumerated by trypan blue exclusion and aliquots of these cells were used to establish an adherent stromal cell layer or to recharge the stromal cells in LBMC.

Establishment of marrow adherent cell layer. Pooled BM cells (6.5 × 10⁶) from either tumor-bearing or normal mice were cultured in 6.5 mL of LBMC medium in 25-cm² tissue culture flasks

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with vented filter caps (Costar, Cambridge, MA) at 37°C in a humidified incubator with 5% CO₂. Eight flasks were established for each experiment, and were fed twice weekly after a published protocol. After 3 weeks of culture, when a confluent stromal layer was established, the hematopoietic cells were eliminated using mycophenolic acid (MPA; Sigma, St. Louis, MO) and were then inoculated with BM cells of normal or CE tumor-bearing mice from which adherent cells have been depleted. Several of the stromal cell cultures were not rechallenged; hematopoiesis was never observed in such cultures for the experimental period.

Preparation of rechallenged cell population. Adherent cells were depleted from BM cell suspensions by passing 5 × 10⁶ to 1 × 10⁸ BM cells over a 10-ml sterile Sephadex G-10 (Pharmacia, Piscataway, NJ) column. Cells expressing B220 antigen were depleted from narrow cell suspensions using petri dishes coated with 10-fold concentrated culture supernatant of 14.8 hybridoma (American Type Culture Collection [ATCC], Rockville, MD). Monoclonal antibody (MoAb) 14.8 recognizes a determinant of the Ly-5.2 (B220) cell-surface molecule expressed by lymphoid cells that include pre-B and B cells. The efficiency of cell depletion was tested by immunofluorescence staining (see below) of nonadherent cells. The cell suspension depleted of both adherent and B220⁺ cells is referred to herein as the depleted rechallenged population (adr⁺, B220⁻).

Coculture of stromal cells and rechallenged cell population. In initial experiments, adherent cell stromal monolayers established from tumor-bearing or normal mouse BM were seeded with 10⁶ of the adherent cell-depleted BM population obtained from normal or tumor-bearing mice. Two, four, and six weeks after the inoculation, the number of viable nonadherent cells generated per flask was determined and the cells were stained to detect B220⁺ cells. In later experiments, a confluent monolayer of the stromal cell line S17 that has been shown to support B lymphopoiesis was established in LBMC medium and seeded with the depleted rechallenged population.

Immunofluorescent staining. B-lineage cells were identified by indirect immunofluorescent labeling. One million cells were incubated with 75 µL of the neat supernatant of 14.8 antibody for 30 minutes on ice, washed one time, and then incubated with the secondary antibody, either phycoerythrin-conjugated affinity-purified goat antirat Ig (Southern Biotechnology Associates, Birmingham, AL) or fluorescein isothiocyanate (FITC)-conjugated affinity-purified goat antirat Ig (Kirkegaard and Perry Laboratories, Inc, Gaithersburg, MD), at saturating concentrations. The samples were then washed, fixed in 1% paraformaldehyde, and stored at 4°C until analysis. Nonspecific staining was assessed using the secondary antibody only.

Flow cytometric analysis. Ten thousand stained cells were analyzed for their forward scatter (cell size), right angle (granularity), and fluorescence intensity by flow cytometry (FACScan; Becton Dickinson, Mountain View, CA) using Consort 30 software (Becton Dickinson). The lymphocyte population was gated using the forward-versus-side scatter and the percentage of B220⁺ cells among this population was determined. The absolute number of B220⁺ lymphocytes per culture flask was then calculated using the number of viable cells per flask. Immunolabeled cells were also examined under a fluorescence microscope to confirm flow cytometric analysis of cells.

Pre-B colony-forming cell assay (CFU-Pre-B). BM cells were obtained from mice by grinding the femurs. Pre-B colony-forming cells were assayed in agar or in methylcellulose in the presence of interleukin-7 (IL-7). In brief, 10⁶ BM cells were cultured in 1 mL of Medium 199 (Whittaker, Walkerville, MD) enriched with 1% l-glutamine, 1% l-asparagine, 1% sodium pyruvate, 0.5% antibiotic solution (GIBCO), 20% FCS, 5 × 10⁻⁵ mol/L 2-ME, and 0.3% agar or 0.9% methylcellulose. Recombinant murine IL-7 (Genzyme, Cambridge, MA) was used at 12.5 or 50 U/mL. Cultures were incubated at 37°C with 5% CO₂ and day-7 colonies (cell aggregates of more than 40 cells) were counted. Colonies grown in methylcellulose were lifted using a drawn pipet, pooled, and suspended in Hanks’ Balanced Salt Solution (HBSS) containing 2% FCS. These cells were labeled with 14.8 antibody as described above and analyzed by flow cytometry. Colonies grown in agar were stained with specific esterase to distinguish lymphoid colonies from granulocyte colonies.

Conditioned media. A CESJ4 clone, derived from the CE mammary carcinoma; a Bc66 clone, derived from a nongranulocytosis-inducing murine mammary carcinoma; and NIH 3T3 cells (ATCC) were cultured in LBMC medium. The CESJ4 line has been shown to produce G-CSF and M-CSF, whereas the Bc66 line produces M-CSF, but not G-CSF. Supernatants of confluent cultures were stored at −80°C in aliquots and used without refreezing.

Murine G-CSF and antibody production. Recombinant murine G-CSF was produced and purified as previously described. Biologic activity of G-CSF was assessed by proliferation of NFS60 cells. Polyclonal antibody to murine G-CSF was raised in rabbits that were subcutaneously injected with approximately 0.4 mg of the purified recombinant murine G-CSF in Freund's complete adjuvant. After 4 and 6 weeks, two booster injections were given with 0.2 mg of murine G-CSF. Three weeks after the last injection, the serum was collected. The serum neutralized 20 U/mL of murine G-CSF at a dilution of 10,000.

G-CSF injections in vivo. The purified recombinant murine G-CSF with a specific activity of 2 × 10^{10} U/mg was diluted in phosphate-buffered saline (PBS) containing 10% normal mouse serum. Four 12-week-old female C57Black6 mice and two (Balb/c × CE) F1 mice received daily subcutaneous injections of murine G-CSF, 2.5 μg in 0.1 mL solution for 21 days, whereas the equal number of control mice received injections of G-CSF diluent. On day 22, the mice were killed, and their BM was analyzed for B220⁺ cells.

RESULTS

Confirming our previous observations, transplantation of CE mammary carcinoma cells consistently provoked marked neutropenia by 3 weeks posttumor inoculation. The BM of these tumor-bearing mice showed extensive neutrophilic hyperplasia coupled with a depletion of lymphocytes and erythroblasts as previously described. Strontial cell and progenitor cell function. Table 1 summarizes data of experiments that compared the function of stromal cells and progenitor cells obtained from tumor-bearing and control mice. When stromal cell cultures established with tumor-bearing or control mouse BM were inoculated with normal BM cells depleted of adherent cells, no difference in B220⁺ cell production was observed (Table 1, section A). These results suggested that BM stromal cells explanted from tumor-bearing mice can support B-cell production in vitro, and any potential effects of the tumor or its products on the ability of stromal cells to support B-cell development are reversible.

Consistent with our previous observations, B220⁺ cells were essentially absent from the BM of tumor-bearing mice, whereas they were present in normal BM (data not shown). Nevertheless, when BM cells from normal or tumor-bearing mice were depleted of adherent cells and seeded onto confluent marrow stromal cell monolayers and cultured for 4 weeks, comparable numbers of B220⁺ cells (4.9 × 10⁶ cells
for normal and 5.0 x 10^5 cells for tumor-bearing mouse BM) were generated by these two recharge populations. To confirm these findings, the recharge cell population was depleted of both adherent and B220+ cells and inoculated at 10^4 cells/well onto an S17 monolayer in 24-well culture plates. S17 cells provide the in vitro requirements necessary for the differentiation of immature B220+ progenitor cells to B220+ pre-B cells. At 2, 3, and 4 week postrecharge, all nonadherent cells from six replicate wells were harvested and analyzed at each time point.

### Table 1. Comparison of Normal and Tumor-Bearing Mouse BM Cells for the Production of B220+ Cells In Vitro

<table>
<thead>
<tr>
<th>No. of B220+ Cells Generated per Flask (x10^5)</th>
<th>2 wk</th>
<th>4 wk</th>
<th>6 wk</th>
</tr>
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<tbody>
<tr>
<td><strong>(A) Stromal function</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Stromal source</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Normal mouse</td>
<td>1.1 ± 0.2</td>
<td>2.4 ± 0.1</td>
<td>6.6 ± 1.3</td>
</tr>
<tr>
<td>Tumor-bearing mouse</td>
<td>1.2 ± 0.2</td>
<td>2.2 ± 0.4</td>
<td>6.5 ± 2.4</td>
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</tbody>
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<table>
<thead>
<tr>
<th>No. of B220+ Cells Harvested per Well (x10^5)</th>
<th>2 wk</th>
<th>3 wk</th>
<th>4 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(B) Progenitor function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progenitor source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal mouse</td>
<td>1.1 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>5.0 ± 1.6</td>
</tr>
<tr>
<td>Tumor-bearing mouse</td>
<td>1.3 ± 0.4</td>
<td>1.8 ± 1.0</td>
<td>5.8 ± 1.9</td>
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* Means ± SD of cumulative number of B220+ cells generated from eight replicate flasks.
† Means ± SD of B220+ cells harvested from six replicate wells.

Incidence of CFU-pre-B. To further understand the inhibition of B-cell development in tumor-bearing mice, we examined the incidence of IL-7-responsive colony-forming cells in the BM of normal and tumor-bearing mice. IL-7 is a soluble mediator that stimulates the proliferation of B220+ B-cell progenitors to form colonies of pre-B cells in semisolid culture medium. Because of evidence suggesting that B-cell progenitors in vivo may predominate close to the endosteum, BM cells were obtained from normal and day-21 CE tumor-bearing mice by grinding the femur instead of flushing the cells from the medullary cavity. Colony-forming cells were then assayed in methylcellulose or in agar in the presence of two different concentrations of IL-7. As shown in Table 2, tumor-bearing mouse BM contained practically no IL-7-responsive progenitors, whereas normal mouse BM contained numbers of pre-B colony-forming cells comparable with those reported by others. This marked depletion of IL-7-responsive progenitors in the BM of tumor-bearing mice suggested that the tumor blocked the in vivo differentiation of B220+ progenitors into the IL-7-responsive B220+ pre-B cells.

Role of cytokines secreted by the CE tumor. We speculated that the in vivo suppression of lymphopoiesis by the tumor may be caused by soluble factors produced by the tumor. In preliminary experiments, daily injections of serum-free medium conditioned by CE mammary tumor cells, or by the CESJ4 cell line, derived from the tumor, provoked neutrophilic hyperplasia associated with the suppression of lymphoid cells in mouse BM (unpublished observation). We have undertaken in vitro experiments to study humoral factor-mediated suppression of lymphopoiesis, in which the unique roles of the cell inoculum, stromal cells, and growth factors can be more readily determined than in the in vivo system. In 25-cm² flasks, normal BM cells, depleted of adherent and B220+ cells, were seeded onto confluent S17 stromal monolayers and cultured in the presence of media conditioned by CESJ4, Bc66, or NIH3T3 cells, at 10% (vol/vol) each. Two weeks later, the phenotype of the nonadherent cells generated under each experimental condition was analyzed. Results are summarized in Fig 1. Cultures containing CESJ4 medium generated significantly higher numbers of total cells than cultures containing Bc66 medium or NIH3T3 medium, or medium alone. This increase in the total cell production in the flasks supplemented with CESJ4 medium was caused primarily by high numbers of neutrophilic granulocytes. The production of lymphocytes and B220+ cells was significantly (P < .005) suppressed in cultures supplemented with CESJ4 conditioned medium, but not in cultures supplemented with Bc66 or NIH3T3 conditioned medium when compared with medium control. Because CE mammary tumor clones

### Table 2. IL-7-Responsive CFU-Pre-B in the BM of Normal and Tumor-Bearing Mice

<table>
<thead>
<tr>
<th>IL-7</th>
<th>Normal BM</th>
<th>Tumor BM</th>
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<tbody>
<tr>
<td>12.5 U/mL</td>
<td>82.7 ± 45.4</td>
<td>0*</td>
</tr>
<tr>
<td>50.0 U/mL</td>
<td>72.0 ± 29.4</td>
<td>0.3 ± 0.6*</td>
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BM cells (10^5) from normal or tumor-bearing mice were cultured in agar or in methylcellulose in the presence of IL-7 at 12.5 or 50 U/mL. No colony growth was observed in the absence of IL-7. Colony values shown are means ± SD of colonies in stained agar preparations, obtained from five normal and five tumor-bearing mice. More than 96% of cells pooled from colonies grown in methylcellulose in parallel experiments were B220+ when analyzed by flow cytometry.

* P < .001 when compared with normal BM.
SUPPRESSION OF LYMPHOCYTOPOIESIS BY G-CSF

Fig 1. Effect of tumor-cell-conditioned media on lymphocyte production from normal BM cells cultured over S17 stromal cells. Established S17 stromal cell monolayers in flasks were inoculated with the depleted recharge population obtained from normal mouse BM, and cultured in the presence of CESJ4, (●), 12, Bc66 (○), 10, or NIH3T3 (□, 4) cell-conditioned media, 10% (vol/vol) each, for 2 weeks. Nonadherent cells, pooled from three replicate flasks per each medium, were analyzed for lymphocyte and B220+ cell production. Values represent means and SD (vertical bars) of data obtained from the number of experiments shown in parentheses. *P < .005 when compared with medium controls (●, 11).

produce G-CSF and M-CSF, and Bc66 tumor produces M-CSF, but not G-CSF, we eliminated M-CSF as a likely suppressor of B-cell production in vitro. On the other hand, G-CSF or other factors unique to the CE mammary tumor clones could be responsible for the in vivo and in vitro suppression of lymphopoiesis.

The effect of murine G-CSF on B-cell production in vitro. These findings prompted us to examine the effect of recombinant murine G-CSF on B-cell production. In a preliminary experiment, confluent S17 stromal cell monolayers in flasks were inoculated with the depleted recharge population obtained from normal mouse BM at 1.5 × 10³ cells/flask. Culture media were supplemented with recombinant murine G-CSF to achieve a range of 0 to 4.8 ng/mL final concentrations of G-CSF throughout the culture period. Two weeks after inoculation, nonadherent cells were analyzed for B220 expression. Recombinant murine G-CSF suppressed B220+ cell production in a dose-related manner (Fig 2). Using an effective dose (2.4 ng/mL) of G-CSF, the production of total cells, lymphocytes, and B220+ cells was compared. Recombinant G-CSF stimulated the production of total cells that were predominantly granulocytes, whereas the production of lymphocytes and B220+ cells was significantly reduced (Fig 3). These findings indicate that the in vitro suppressive effects of CE mammary carcinoma on lymphopoiesis can be assigned to G-CSF.

To confirm that this B-cell suppression could be attributed solely to the G-CSF produced by the tumor, we examined whether specific antiserum to murine G-CSF could eliminate the inhibitory effect of CESJ4 medium in vitro. Rabbit polyclonal antibody to murine G-CSF was used at a 10,000-fold dilution to neutralize the activity of 20 U/mL of G-CSF (equivalent to 2.2 ng/mL of G-CSF). S17 stromal cell cultures were established in 25 cm² flasks and inoculated with 10⁶ cells of the depleted recharge population obtained from normal mouse BM. Cells were cultured in lymphoid medium supplemented with CESJ4 conditioned medium (10% vol/vol) or recombinant murine G-CSF (2.4 ng/mL) in the presence of G-CSF antiserum at the neutralization titer. These supplemented media were used at the time of inoculation and at the twice weekly feedings. Two

Fig 2. A dose-related effect of recombinant murine G-CSF (rmG-CSF) on B220+ cell production. Confluent S17 cell monolayers in culture flasks were inoculated with the depleted recharge population obtained from normal mice at 1.5 × 10³ cells/flask. Cells were cultured in media supplemented with rmG-CSF to achieve 0 to 4.8 ng/mL final concentration of G-CSF or G-CSF diluent (PBS containing 0.002% Tween 20) for 2 weeks. Nonadherent cells were analyzed for B220 expression. Each data point represents the number of B220+ cells, generated from a pool of three flasks.
weeks after seeding, the nonadherent cells were analyzed. The results obtained from two repeated experiments are shown in Table 3. G-CSF again reduced the number of B220+ cells generated per flask to less than 30% of the medium control. This G-CSF-induced inhibition of B220+ cell production was specifically reversed by G-CSF antiserum but not by normal rabbit serum. In culture flasks containing CESJ4 conditioned medium, the number of B220+ cells was reduced to 52% of the medium control. G-CSF antiserum completely reversed this reduction. These studies confirmed that G-CSF present in CESJ4 medium is responsible for the suppression of B lymphopoiesis in these cultures. Therefore, the inhibitory action of CESJ4 medium on B-cell production can be attributed to G-CSF produced by the tumor cells.

The effect of murine G-CSF on stromal cell function. In a single experiment we examined whether G-CSF suppressed B lymphopoiesis by interfering with the supportive role provided by the stroma. Monolayers of S17 cells were treated with G-CSF for 1 week, washed, and then seeded with the depleted recharge population. On days 4, 7, 10, and 15 after reseeding, the incidence of B220+ cells in these cultures was determined. No significant difference was observed between stromal cells with and without G-CSF pretreatment, whereas B220+ cell production was markedly suppressed in the continued presence of G-CSF in culture (data not shown). These results suggest that G-CSF pretreatment does not change the ability of S17 cells to support B-cell differentiation. If the suppression of lymphopoiesis by G-CSF is mediated by the stroma, the continued presence of G-CSF is a requirement to compromise the role of S17 cells in lymphopoiesis.

The effect of recombinant murine G-CSF on lymphocytes in vivo. Finally, we evaluated the suppression of lymphopoiesis by recombinant murine G-CSF in vivo. Recombinant murine G-CSF provoked marked neutrophilia (28,400 ± 3,400/μL) but did not cause significant changes in blood lymphocyte counts (6,100 ± 540/μL for G-CSF-treated mice, 5,800 ± 700/μL for control mice). The in vitro effect of G-CSF on lymphopoiesis was most striking in the BM. Whereas the number of granulocytic cells per femur doubled in G-CSF-treated animals, the number of erythroblasts and lymphocytes was profoundly reduced. The number of BM B220+ cells decreased from 6.2 ± 1.4 × 10⁶ to 0.7 ± 0.4 × 10⁶ (P < .005) per femur in G-CSF-treated mice. These in vivo studies corroborate the above in vitro experiments, and confirm the significant role G-CSF can play in the suppression of primary B lymphopoiesis in the BM.

DISCUSSION

In this report, we have investigated the mechanisms that can account for the suppression of lymphopoiesis in a well-characterized experimental model of tumor-induced granulocytosis. We have documented that (1) BM stromal cells explanted from tumor-bearing mice are capable of supporting B lymphopoiesis in vitro; (2) progenitors capable of differentiating into B cells on normal stromal monolayers persist in the BM of tumor-bearing animals; and (3) these cells, however, are blocked from giving rise to IL-7-responsive B-cell colony-forming cells. We have provided evidence that G-CSF is responsible for the in vitro and in vivo suppression of B lymphopoiesis at an early stage before the expression of B220.

However, the precise mechanism of action of G-CSF on B lymphopoiesis remains to be determined. We may consider at least two possibilities. Firstly, G-CSF may interfere with stroma-progenitor cell interactions that are essential for the differentiation of primitive B-cell progenitors into IL-7-responsive B-cell precursors. In our experiments with CE tumor-bearing mice, IL-7-responsive progenitors were nearly completely eliminated from the BM. IL-7 has been shown to exert most of its proliferative effects on cells before cμ expression at the time of D-J rearrangements.9 Because

<table>
<thead>
<tr>
<th>Culture Component</th>
<th>B220+ Cell Production (% of control)</th>
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<tbody>
<tr>
<td>LBMC alone</td>
<td>100</td>
</tr>
<tr>
<td>G-CSF (2.4 ng/mL)</td>
<td>28.9 ± 8.9</td>
</tr>
<tr>
<td>G-CSF plus anti-G-CSF</td>
<td>99.7 ± 7.9*</td>
</tr>
<tr>
<td>G-CSF plus NRS</td>
<td>36.0 ± 9.3</td>
</tr>
<tr>
<td>CESJ4 (10% vol/vol)</td>
<td>52.2 ± 15.6</td>
</tr>
<tr>
<td>CESJ4 plus anti-G-CSF</td>
<td>113.5 ± 10.71*</td>
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</tbody>
</table>

S17 stromal monolayers in culture flasks were inoculated with 1.5 × 10⁶ cells of the depleted recharge population from normal mouse BM. Cells were cultured in LBMC medium alone (control), or LBMC medium supplemented with murine G-CSF or with CESJ4 conditioned medium, in the presence of anti–G-CSF serum at a neutralizing titer, or normal rabbit serum (NRS) for 2 weeks. Nonadherent cells were analyzed for B220+ cells and the suppression of cell production was expressed as a percentage of control. Data are means ± SD, derived from six culture flasks for each experimental condition.

* P < .01 when compared with cultures without antiserum.
† P < .025 when compared with cultures without antiserum.
SUPPRESSION OF LYMPHOCYTOPOIESIS BY G-CSF

2067

stromal cell contact is considered essential for the differentiation of these early B-cell progenitors. G-CSF might have interfered with the stromal cell-mediated differentiation of immature progenitors into IL-7-responsive progenitors in vivo. Exposing stromal cells to G-CSF for a short time in vitro did not impair the B-cell–supporting ability of stromal cells once G-CSF was removed from the culture medium, indicating that G-CSF must be present to impair stromal cell function. Alternatively, the presence of G-CSF in the BM milieu may affect the progenitor differentiation. Several studies have suggested the involvement of granulopoietic agents in the suppression of primary lymphopoesis. The early B-cell differentiation signal of S17 cells has been recently attributed to insulin-like growth factor I (IGF-I) that, like the kit ligand, appears to act synergistically with IL-7. It may be interesting to examine the effect of G-CSF on IGF-1 expression by stromal cells.

Secondly, G-CSF may affect lymphopoesis by promoting the granulocytic differentiation of a bipotent or multipotent stem cell. According to Suda et al., the addition of both IL-7 and G-CSF to normal BM cells in methyl cellulose cultures did not affect the formation of respective colonies that were generated by either IL-7 or G-CSF alone, suggesting that G-CSF and IL-7 act on two distinct populations of committed progenitors. The murine G-CSF receptor has been detected only on BM cells other than leukemic cells and binding of IGF-1–G-CSF has been restricted to granulocytic and monocytic cells. However, primitive progenitors may be too few to rule out the binding of G-CSF to them by autoradiography. It has been shown that primitive progenitors previously considered as myeloid also possess B-lymphoid potential. Therefore, G-CSF may have direct effect on such lymphohematopoietic progenitors. Alternatively, G-CSF may stimulate neutrophil production to the extent that overcrowding of the BM would prohibit lymphoid progenitors from gaining access to stromal elements necessary for their development. Although this latter possibility cannot be ruled out in vivo, it cannot explain the suppressive effects of G-CSF on lymphopoiesis in vitro.

The association of suppressed lymphopoiesis with neutrophil hyperplasia of the BM may be more prominent or more frequently noted in mice than in other species. In addition to our report and that by Dorskind, reduction of BM lymphocytes in G-CSF-treated mice has been noted by Molineux et al. and Metcalf et al. In studies of G-CSF injections using hamsters, dogs, or primates, BM granulocytic hyperplasia has been well described, but the status of BM lymphocytes in these animals is not clear. G-CSF is effective in relatively low doses in correcting neutropenia in humans, therefore, lymphocyte suppression may not be obvious in short-term, low dose G-CSF treatment. However, in a human case of marked granulocytosis induced by a CSF-producing carcinoma, Sato et al. reported that more than 85% of the BM population was neutrophilic granulocytes, 9% erythroid, and 5% other cell types; thus, reduction in a relative number of lymphocytes may also occur in extensive granulocytic BM in humans. The BM of patients with cyclic neutropenia exhibits a reciprocal cycling of granulocytes and lymphocytes, suggesting that the observed inverse relationship of lymphocytes and granulocytes may be relevant to nonmurine species as well.

These in vivo and in vitro studies reported here provide an important piece of evidence for the pleiotropic effects of a hematopoietic growth factor. Our experiments document without ambiguity that a growth factor that is clearly stimulatory for one cell lineage inhibits another. These studies suggest that, although recombinant hematopoietic growth factors have come into therapeutic use, it is important to clarify their effects on cell lineages other than their primary target.

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In vivo and in vitro suppression of primary B lymphocytopoiesis by tumor-derived and recombinant granulocyte colony-stimulating factor

MY Lee, KL Fevold, K Dorshkind, R Fukunaga, S Nagata and C Rosse