Effects of B Cell Stimulatory Factor-1/Interleukin 4 on Hematopoietic Progenitor Cells

By Christian Peschel, William E. Paul, Junichi Ohara, and Ira Green

B cell stimulatory factor-1 (BSF-1)/interleukin 4 (IL 4) is a T cell product originally characterized on the basis of its actions on B lymphocytes. Recently it has been reported that BSF-1 activates T cell and mast cell lines. We now provide evidence that BSF-1, purified to homogeneity, also has a broad spectrum of activity on hematopoietic progenitor cells (HPC). However, like its action on B cells, proliferative effects were only observed when BSF-1 was combined with an additional factor. Thus BSF-1, in combination with recombinant G-CSF, enhances the proliferation of granulocyte-macrophage progenitor cells (CFU-GM). BSF-1 increases the proliferation of CFU-e in the presence of recombinant erythropoietin (rEPO). Furthermore, BSF-1 induces, together with rEPO, colony formation by primitive erythroid (BFU-e) and multipotent (CFU-mix) progenitor cells comparable to that observed with rEPO and interleukin 3 (IL 3). BSF-1 is also active as a megakaryocyte colony-stimulating factor; in combination with recombinant interleukin 1, rEPO or the supernatant of the T cell hybridoma FS7-20.6.18, BSF-1 induces megakaryocyte colony formation (CFU-Mk). The same factors that synergize with BSF-1 also enhance CFU-Mk proliferation induced by IL 3. Although the precise mechanism of action of BSF-1 on HPC is not yet known, we propose that BSF-1 represents an activation factor for HPC and prepares the progenitor cells to respond to specific growth or differentiation factors.

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ethanol, and adjusted to pH of 7.2. After enrichment of the mononuclear cell fraction (MNC) by centrifugation over Ficoll-Hypaque (FH, density 1.077 g/mL, Pharmacia, Piscataway, NJ), interface cells were depleted of adherent cells by two consecutive adherence procedures in tissue culture dishes (100-mm diameter, Falcon, MA) at a cell density of 1 x 10^6 MNC/mL. Nonadherent cells obtained after the second incubation period of 90 minutes were incubated with a cocktail of appropriate dilutions of Thy1.2, Lyt-1, and Lyt-2 antibodies to remove T cells (Beckton Dickinson, Sunnyvale, CA) for 30 minutes on ice; after centrifugation cells were suspended in RPMI 1640 containing 10% fetal calf serum (FCS) and 20 U/mL IL-3 (Collaborative Research, Bedford, MA). Nonadherent T cell-depleted bone marrow cells were used in all experiments. In certain experiments a monoclonal antibody (MAR 3, Ortho, Raritan, NJ) was used in all experiments. In certain experiments a monoclonal antibody (MAR 3, Ortho, Raritan, NJ) was used in all experiments.

Growth factors. BSF-1 was purified from supernatant fluids of 4x-phorbole-12,13-dimethylacetate (PMA) activated EL-4 cells by a combination of affinity chromatography and high-performance liquid chromatography (HPLC). Briefly, 4L of EL-4 Sn was passed over an affinity column of 10 mL Sepharose-4B conjugated with 20 mg γ-globulin from a monoclonal anti-BSF-1 (11B11) ascites at a flow rate of 4 mL/min. After washing the column thoroughly with 2 L glass-distilled water, BSF-1 was eluted with 0.1% trifluoroacetic acid (TFA). The eluate was loaded onto a C18-reversed phase HPLC column (Supleco, Inc, Bellefonte, PA) with a linear gradient of 35% to 55% acetonitrile in 0.1% TFA over 60 minutes at a flow rate of 0.5 mL/min. The detailed procedure will be described elsewhere (J. Ohara et al, manuscript in preparation). The resultant BSF-1 migrated as a single molecular species of mol wt 20,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

The N-terminal amino acid sequence of this material is identical to that inferred from the nucleotide sequences of cDNA clones of BSF-1. A U/mL of BSF-1 is the concentration required for half maximal stimulation of H-thymidine uptake by purified splenic B cells (10^6 cells/mL) stimulated with goat antiserum IgM antibody (5 to 10 μg/mL).

Highly purified IL 3 was kindly provided by J. N. Ihle, Frederick, MD. Recombinant human erythropoietin (rEPO) purified by sequential chromatography was purchased from Amgen (Thousand Oaks, CA). Recombinant human interleukin 1α (IL 1) was generously provided by Hoffman LaRoche (Nutley, NJ), and recombinant human G-CSF was kindly provided by Amgen. The supernatant of the T cell hybridoma FST-20.6.18 that has recently been described to contain megakaryocyte potentiating activity was kindly supplied by P. Marrack (National Jewish Hospital, Denver, CO).

Hematopoietic progenitor assays. Colony-forming assays were performed in a modified agar culture system as previously described. Nonadherent T cell-depleted bone marrow cells were suspended in culture medium containing 10% heat-inactivated FCS and 0.3% Bacto-Agar held at 37°C (Difco, Detroit). Aliquots of 0.25 mL of this mixture containing 2 x 10^6 or 4 x 10^6 viable cells were pipetted into Nunclon 4-well multidishes (Nunc, Denmark). After solidification at room temperature, a liquid overlayer of 0.25 mL culture medium containing 10% fetal calf serum (FCS) and the growth factors to be studied was added. The growth factors tested in the different progenitor cell assays are shown in Table 1. All stimulating factors suspended in the liquid overlayer were applied in double concentration to achieve final dilutions in the whole culture system as described in Table 1 and Results. After incubation in 6% CO2 in fully humidified atmosphere for 2.5 to seven days, the whole agar cultures were fixed with 2.5% glutaraldehyde, washed in distilled water for four hours, mounted on microscopic slides, and dried on a slide warmer. May-Grünwald-Giemsa, benzidine, and acetylcholinesterase stainings were performed on the dried agar sheets as described; the slides were permanently mounted with Permount (Fisher, Fairlawn, NJ); and numbers of colonies or clusters were scored under a light microscope.

A summary of the progenitor cell assays, definition of colony types, and factors used, as detailed below, are also given in Table 1.

**CFU-GM assay.** 2 x 10^6 cells/well were incubated with G-CSF 10 to 200 U/mL alone or in combination with BSF-1 30 U/mL or IL 1 5 U/mL or IL 3 50 U/mL for six to seven days. Granulocytic clusters (10 to 50 cells) and colonies (>50 cells) were distinguished in cultures stimulated with G-CSF ± BSF-1. In IL 3-containing cultures only colonies were counted. The CFU-e number was evaluated at 2.5 to three days of incubation in preparations stained for benzidine to identify hemoglobin-containing cells. CFU-e were defined as clusters of more than eight normoblast-like hemoglobinized cells. Such clusters do not contain more than 20 normoblasts.

**BFU-e and CFU-Mix assay.** For assays of erythroid (BFU-e) and mixed erythroid (CFU-mix) progenitor cells, 4 x 10^6 cells/well were incubated with rEPO 2.0 U/mL in combination with IL 3 50 U/mL or BSF-1 30 U/mL for seven days and stained with benzidine. Such colonies generally contain more than 50 cells. Control cultures containing medium alone, IL 3, or BSF-1 alone were always included. The composition of mixed erythroid colonies was also evaluated in preparations stained for acetylcholinesterase to identify megakaryocytes. In these preparations erythroid colonies were identified by their typical morphology. Mixed erythroid colonies that contained erythroid cells and megakaryocytes and/or granulocytes were distinguished from pure erythroid colonies.

**CFU-Mk assay.** 4 x 10^6 cells/well were stimulated with BSF-1 30 U/mL, IL 3 1 to 100 U/mL alone or in combination with IL 1 5 U/mL, rEPO 2.0 U/mL or FS7-20.6.18-conditioned medium (CM) 5% as source of MkPA. Colonies were defined as aggregates of more than three large acetylcholinesterase-positive cells after seven to eight days of incubation. In cultures stimulated with EPO and IL 3 or BSF-1, mixed colonies containing megakaryocytes, erythroid cells, and/or granulocytic/monocytic cells were distinguished from pure megakaryocytic colonies.

**Use of anti-BSF-1 antibody.** In all hematopoietic progenitor cell assays described above, the specificity of the biological activity induced by added BSF-1 was confirmed by the addition of a monoclonal rat anti-BSF-1 antibody, designated as 11B11 to the cultures. The anti-BSF-1 antibody was used in dilutions of 1:500 of ascitic fluid in selected cultures and was added to the cultures together with BSF-1 or other growth factors during the whole incubation period.

**Statistical analysis.** All cultures on a single day were performed at least in duplicate, and colony counts of parallel cultures were pooled. The same experiments were repeated three to four times over a period of time, and the results were expressed as mean values of these different experiments. The differences between corresponding experiments have been examined by the two-tailed Dunnet test.

**RESULTS** The purpose of this study was to determine the effect of BSF-1, alone or together with other CSFs, on the growth of hematopoietic progenitor cells. Using adherent cell-depleted
Table 1. Hematopoietic Progenitor Cell Assays, Colony Types, and Factors Used

<table>
<thead>
<tr>
<th>Hematopoietic Progenitor Cell Type</th>
<th>Definition of In Vitro Colonies</th>
<th>Factors Used* for Stimulation</th>
<th>Staining Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU-GM: Restricted myelomonocytic progenitor cell</td>
<td>Clusters (10-50 cells) or colonies (&gt;50 cells) of granulocytes and/or macrophages, scored after 7 days of incubation</td>
<td>BSF-1 30 U/mL</td>
<td>May-Grünwald-Giemsa</td>
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<tr>
<td></td>
<td></td>
<td>G-CSF 10-200 U/mL</td>
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<td></td>
<td></td>
<td>IL 3 50 U/mL</td>
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<tr>
<td></td>
<td></td>
<td>IL 1 5 U/mL</td>
<td></td>
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<tr>
<td>CFU-e: Restricted erythroid progenitor cell</td>
<td>Hemoglobin-containing clusters of more than 8 cells, scored after 2.5-3 days of incubation</td>
<td>EPO 0.1-1.0 U/mL</td>
<td>Benzidine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BSF-1 30 U/mL</td>
<td>May-Grünwald-Giemsa</td>
</tr>
<tr>
<td>BFU-e: Restricted primitive erythroid progenitor cell</td>
<td>Hemoglobin-containing single or multiple colonies of more than 50 cells, scored after 7 days of incubation</td>
<td>EPO 2.0 U/mL</td>
<td>Benzidine</td>
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<tr>
<td></td>
<td></td>
<td>BSF-1 30 U/mL</td>
<td>May-Grünwald-Giemsa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL 3 50 U/mL</td>
<td></td>
</tr>
<tr>
<td>CFU-mix: Multipotent hemopoietic progenitor cell</td>
<td>Colonies consisting of erythroid and megakaryocytic and/or myelomonocytic cells, scored after 7 days of incubation</td>
<td>EPO 2.0 U/mL</td>
<td>Benzidine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BSF-1 30 U/mL</td>
<td>Acetylcholinesterase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL 3 50 U/mL</td>
<td>May-Grünwald-Giemsa</td>
</tr>
<tr>
<td>CFU-MK: Restricted megakaryocytic progenitor cell</td>
<td>Colonies of more than 3 megakaryocytes, scored after 7-8 days of incubation</td>
<td>IL 3 1-100 U/mL</td>
<td>Acetylcholinesterase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BSF-1 30 U/mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EPO 2.0 U/mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL 1 5 U/mL</td>
<td>FS7-20.16.8 CM 5%</td>
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*The factors listed were used alone or in combination with each other, as described in results.

and T cell-depleted bone marrow cells in a modified agar culture technique,26-38 no significant cell proliferation was observed without growth factors after seven to 14 days of incubation. Therefore, effects of constituent production of growth factors in situ could be neglected in our culture system. Stimulation with IL 3, EPO, or G-CSF led to a proliferation of myelomonocytic and megakaryocytic, erythroid and granulocytic colonies respectively, as had been previously described for these factors.12 In cultures stimulated with BSF-1 alone in concentrations of 30 to 1,000 U/mL, only a few small granulocyte/macrophage cell clusters and a few groups of two to three megakaryocytes developed. The effects of BSF-1 in combination with other growth factors is now described in the following sections.

**Proliferation of granulocyte-macrophage progenitor cells (CFU-GM).** First, to determine the maximal number of granulocyte/macrophage colonies that could be observed, IL 3, a powerful multi-CSF, was used as a growth factor. Stimulation with IL 3 (50 U/mL) induced a mean of 272 large colonies consisting of granulocytes and/or macrophages/20,000 cells plated in three different experiments. As described above, BSF-1 alone did not stimulate the proliferation of myeloid colonies; only four to seven granulocytic clusters of more than ten cells developed in these experiments.

We then tested the effects of BSF-1 together with G-CSF. After stimulation for seven days with various concentrations of G-CSF alone, a dose-dependent increase in the number of clusters (10 to 50 cells, Fig 1A) and of colonies (>50 cells, Fig 1B) consisting of mature granulocytes was observed. The addition of 30 U/mL of BSF-1 to various concentrations of G-CSF led to a significant increase in size and number of granulocytic clusters and colonies (Figs 1A and 1B). The use of higher concentrations of BSF-1 did not further increase the number of colonies derived from CFU-GM (Fig 2).

![Graph A](image1.png)

![Graph B](image2.png)

**Fig 1.** Role of BSF-1 in the stimulation of CFU-GM. Cultures were stimulated with various amounts of G-CSF alone or in combination with BSF-1 (30 U/mL) or IL 1 (5 U/mL). Clusters of 10 to 50 cells (A) and colonies of more than 50 cells (B) were counted after six days of incubation. Values represent the mean of three different experiments.
BSF-1 acts on hematopoietic progenitors of IL-1

200 U/mL (CFU-GM) or EPO 2.0 U/mL (BFU-e, CFU-mix, CFU-Mk).

Cultures were stimulated with G-CSF alone (Table 2). This result indicates that the enhancement effect of BSF-1 on colony formation (Figs 1A and 1B).

Colony formation was observed after six days (CFU-GM) or seven days (BFU-e, CFU-mix, CFU-Mk) of incubation. Values represent the mean of triplicate cultures of one typical experiment.

Morphologically, most of these colonies consist of less differentiated granulocytic cells as compared to cells in colonies stimulated with G-CSF alone. In addition, some colonies with monocyte-macrophage features appeared. The addition of IL-1 to G-CSF had no enhancing effect on granulocytic colony formation (Figs 1A and 1B).

When cultures were stimulated with G-CSF and BSF-1 and incubated for 14 days, we observed development of macrophage colonies, whereas in control cultures containing medium alone, G-CSF or BSF-1 alone, no colony formation of any kind could be detected at this time (data not shown). The granulocytic colonies that had been present at seven days of culture have by 14 days degenerated and are no longer visible. The appearance of macrophage colonies at 14 days suggests that BSF-1 in the presence of G-CSF also can induce a commitment and proliferation of CFU-GM among the monocyte-macrophage cell lineage.

Monoclonal anti-BSF-1 antibody blocked the enhancing effect of BSF-1 on the formation of granulocytic colonies. In such cultures the number of colonies was reduced to values obtained with G-CSF alone (Table 2). This result indicates that the active principal in the BSF-1 preparations is, indeed, BSF-1.

Proliferation of primitive erythroid (BFU-e) and multipotent (CFU-mix) progenitor cells.

In cultures with BSF-1 alone or with 0.1 U/mL EPO ± BSF-1, only nonhemoglobinized basophilic normoblasts were observed, whereas in the presence of 1.0 U/mL EPO, BSF-1 enhanced the proliferation of fully hemoglobinized erythroid colonies. These data demonstrate that BSF-1 has an enhancing effect on the in vitro proliferation of mature erythroid progenitor cells and resembles the effects described for EPA.22 As shown in Table 2, the enhancing activity of BSF-1 on BFU-e was abrogated by anti-BSF-1 antibody.

Proliferation of primitive erythroid (BFU-e) and multipotent (CFU-mix) progenitor cells. In contrast to BFU-e, BFU-e were stimulated with optimal (1.0 U/mL) or suboptimal (0.1 U/mL) amounts of recombinant erythropoietin (rEPO), as previously determined in titration experiments (data not shown), and numbers of erythroid clusters were evaluated in benzidine-stained preparations at 2.5 to three days of incubation (Fig 3). A few cell clusters consisting of nonhemoglobinized basophilic normoblasts developed in the absence of exogenously added EPO, most probably under the influence of the small amounts of EPO that are present in FCS. BSF-1 significantly enhanced the effect of 0.1 U/mL and 1.0 U/mL of EPO on BFU-e proliferation induced; the background proliferation of BFU-e without exogeneous EPO was also increased by BSF-1 (Fig 3).

In cultures with BSF-1 alone or with 0.1 U/mL EPO ± BSF-1, only nonhemoglobinized basophilic normoblasts were observed, whereas in the presence of 1.0 U/mL EPO, BSF-1 enhanced the proliferation of fully hemoglobinized erythroid clusters. These data demonstrate that BSF-1 has an enhancing effect on the in vitro proliferation of mature erythroid progenitor cells and resembles the effects described for EPA.22 As shown in Table 2, the enhancing activity of BSF-1 on BFU-e was abrogated by anti-BSF-1 antibody.

Proliferation of primitive erythroid (BFU-e) and multipotent (CFU-mix) progenitor cells. In contrast to BFU-e, BFU-e were stimulated with colony formation at seven days of culture, at which time clusters arising from BFU-e have degenerated. Under our culture conditions rEPO alone had almost no colony-stimulating activity on BFU-e (Fig 4). In cultures containing IL-3 alone, no hemoglobin-containing erythroid cells or morphologically recognizable normoblasts could be detected. Only pure granulocyte-macrophage and megakaryocyte colonies and colonies consisting of both of these cell types were observed. Thus a commitment to the erythroid cell line was not observed as a result of stimulation by IL-3 alone (data not shown). However, combination of EPO with IL-3 led to the proliferation of pure erythroid colonies and of mixed erythroid colonies (Fig 4). When BSF-1 was combined with EPO, a similar effect on BFU-e and CFU-mix was observed; both pure erythroid and mixed erythroid colonies were induced. Thus IL-3 and BSF-1 had comparable activities in allowing proliferation of BFU-e and of multipotent progenitor cells in the presence of EPO, as assessed in four different experiments (Fig 4). The effect of BSF-1 on BFU-e and CFU-mix was clearly detectable with BSF-1 10 U/mL and was not significantly enhanced with higher concentrations (Fig 2).

The mixed colonies, induced by EPO together with IL-3 or BSF-1, consisted of erythroid cells, megakaryocytes, and not clearly identifiable blast-like mononuclear cells. In the presence of IL-3, mixed colonies also contained granulocytes and macrophages, whereas BSF-1 together with EPO did not induce significant differentiation to the granulocytic cell lineage. In experiments in which the effect of the anti-BSF-1 antibody was examined, the effect of BSF-1 on BFU-e and CFU-mix was completely blocked by 11B11 (Table 2).

Proliferation of megakaryocyte progenitor cells (CFU-Mk). When bone marrow cells were incubated with BSF-1 alone, a few groups of three to four megakaryocytes and an increased number of single megakaryocytes could be identi-
were either unstimulated or stimulated with EPO alone or in combination with BSF-1 (30 U/mL). Erythroid clusters were counted in benzidine-stained preparations after 2.5 to three days of incubation. Values represent the mean of three different experiments.

**Fig 5A.** IL 3 stimulates a substantial number of megakaryocyte colonies (Fig 5A). IL 3 stimulates a substantial number of megakaryocyte colonies (Fig 5A). Recently a megakaryocyte-potentiating activity has been described that enhances megakaryocyte colony formation induced by IL 3. Therefore several growth factors were tested for a megakaryocyte potentiating activity (Mk-PA) in costimulation with BSF-1 and with IL 3. IL 1, EPO, and the supernatant of the T cell hybridoma FS7-20.6.18, which has recently been shown to be a source of Mk-PA, all significantly increased the number of megakaryocytic colonies obtained with BSF-1 (Fig 5A). This megakaryocyte-potentiating activity was also reflected in an increased cell number of the individual megakaryocyte colonies. IL 1 alone had no influence on megakaryocyte proliferation, whereas FS7-20.6.18-conditioned medium and EPO induced a small number of megakaryocyte colonies comparable to BSF-1. In cultures stimulated with BSF-1 and EPO, 14 of 63 megakaryocyte colonies shown in Fig 5A also contained erythrocytes. IL 1, FS7-20.6.18-CM, and EPO also significantly (P < 0.05) increased the number and size of megakaryocyte colonies obtained with IL 3 (10 U/mL or 100 U/mL). In the presence of EPO and IL 3, 25 of 139 colonies shown in Fig 5B contained both erythroid cells and megakaryocytes. These data demonstrate that the same potentiating factors have synergistic activities with both BSF-1 and IL 3, although at a different quantitative level. This suggests a Mk-CSF-like activity for BSF-1. The stimulating potency of BSF-1 on CFU-Mk was similar to its effects on CFU-GM and BFU-e (Fig 2). To determine whether BSF-1 also has Mk potentiating activity, we tested the capacity of BSF-1 to enhance the number of megakaryocyte colonies that appear in response to IL 3 (Table 3). Even at suboptimal concentrations of IL 3, BSF-1 had no additive or synergistic effect on CFU-Mk proliferation, suggesting that BSF-1 lack Mk potentiating activity.

**Table 2. Effect of Anti-BSF-1 Antibody on the Number of Colonies Arising from Proliferation of Hematopoietic Precursor Cells**

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>CFU-GM</th>
<th>CFU-e</th>
<th>BFU-e and CFU-mix</th>
<th>CFU-Mk</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSF-1</td>
<td>3/0*</td>
<td>2/0</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>G-CSF 50</td>
<td>20/6</td>
<td>ND</td>
<td>rEPO 1.0</td>
<td>55</td>
</tr>
<tr>
<td>G-CSF 50 + BSF-1</td>
<td>53/34</td>
<td>30/9</td>
<td>BSF-1</td>
<td>9</td>
</tr>
<tr>
<td>G-CSF 100</td>
<td>30/12</td>
<td>ND</td>
<td>BSF-1 + rEPO 1.0</td>
<td>89</td>
</tr>
<tr>
<td>G-CSF 100 + BSF-1</td>
<td>53/43</td>
<td>23/12</td>
<td>rEPO + IL 3</td>
<td>31</td>
</tr>
</tbody>
</table>

*The first value represents the number of clusters of 10-50 cells, the second value represents the number of colonies of >50 cells.
†Pure and mixed erythroid colonies were counted together in this experiment.
‡T-CM = FS7-20.6.18 CM 5%.

Depletion of B cells has no influence on the effect of BSF-1 on hematopoietic progenitor cells. Hematopoietic growth factors can exert their specific activity directly on the progenitor cell, or they can induce other cells like macrophages or T lymphocytes to release other growth factors that activate the particular target cell. To avoid these indirect effects, adherent cells and T lymphocytes were depleted in all experiments prior to the agar culture. Recently it has been described that activated B cells also can produce cytokines that affect in vitro stem cell proliferation. Since BSF-1 is a known B cell activation factor, we attempted to exclude the possibility that the activity of BSF-1 on hematopoietic progenitor cells might be caused by an indirect effect on B lymphocytes in our bone marrow population. For this purpose we additionally depleted bone marrow cells of pre-B and B cells by incubation with anti-B220 antibody (RA33A16.1) and complement. The effects of BSF-1 and other growth factors on hematopoietic progenitor cells was tested on bone marrow cells depleted of B cells, adherent cells, and T cells and compared with cells depleted only of adherent cells and T cells. As shown in one typical experiment in Table 4, the number of colonies per culture increased after depletion of B220+ cells, probably due to an enrichment of hematopoietic progenitor cells. The activity of BSF-1 on all progenitor cells tested was not affected by the depletion of B and pre-B cells from the bone marrow.

**Fig 3.** Role of BSF-1 in the stimulation of CFU-e. Cultures were either unstimulated or stimulated with EPO alone or in combination with BSF-1 (30 U/mL). Erythroid clusters were counted in benzidine-stained preparations after 2.5 to three days of incubation. Values represent the mean of three different experiments.

**DISCUSSION**

BSF-1 was originally described as a lymphokine with multiple actions on B lymphocytes. Recently it was...
BSF-1 ACTS ON HEMATOPOIETIC PROGENITORS

reported that the proliferation of T cell lines and mast cell lines is also affected by BSF-1. The results presented in this study further extend the known activities of BSF-1 and provide evidence that it stimulates a broad spectrum of hematopoietic progenitor cells. Proliferative effects were detected on granulocyte-macrophage, erythroid, megakaryocyte, and multipotent progenitor cells. Such a wide range of activities was previously ascribed only to IL-3. In contrast to IL-3, however, the hematopoietic proliferative activities of BSF-1 were generally dependent on the presence of other factors.

A stimulatory effect of BSF-1 on proliferation of CFU-GM could only be detected in combination with G-CSF. Murine G-CSF at low-to-medium concentrations stimulates only a portion of the CFU-GM population to form colonies of small, mature granulocytes. G-CSF is also a potent granulocytic differentiation factor and it can initiate proliferation of multipotent and erythroid progenitor cells but is unable to sustain continued proliferation of these cells to result in colony formation. The synergistic effect of BSF-1 with G-CSF could be explained by one of several mechanisms. It is possible that (1) BSF-1 enhances responsiveness of CFU-

...
GM to G-CSF, possibly by increasing the expression of receptors for G-CSF; (2) BSF-1 prevents terminal differentiation of progeny of CFU-GM and augments, therefore, their proliferation; or (3) G-CSF induces responsiveness of myeloid cells to BSF-1. In preliminary experiments we observed that BSF-1 can sustain the survival of CFU-GM in agar or liquid culture over several days (data not shown). Furthermore, a proliferation of macrophage colonies is also induced by BSF-1 in presence of G-CSF, a factor predominantly specific for cells of the granulocyte lineage.44 These observations and the finding that the myeloid cell line DA-1 expresses BSF-1 receptors45 support the possibility that BSF-1 directly stimulates CFU-GM.

Clear evidence for a direct effect of BSF-1 on progenitor cells is provided by its activity on BFU-e and CFU-mix. It is generally accepted that EPO represents the growth factor for the more mature erythroid progenitor cell, CFU-e, whereas primitive erythroid progenitor cells, BFU-e, require another activity, frequently designated as “burst-promoting activity,” for proliferation. This activity has previously been shown to be provided by IL 3,15 erythroid potentiating activity (EPA),23 and to some extent by G-CSF44 and GM-CSF,17,19,20 IL 3 promotes proliferation of BFU-e and CFU-mix. Progeny of such IL 3-stimulated BFU-e and CFU-mix can then be induced by EPO to further growth and differentiation into mature erythrocytes. However, EPO does not appear to be necessary for initial proliferation of the immature progenitors.13,22,34 Therefore it has been suggested that EPO receptors are expressed on intermediate cells between BFU-e and CFU-e. We demonstrate here that BSF-1 and EPO together can also stimulate the growth of BFU-e to form erythroid colonies and of CFU-mix with a predominant differentiation toward erythroid and megakaryocytic cells. Since EPO by itself is believed to have no capacity to stimulate BFU-e and CFU-mix, BSF-1, like IL 3, must be another primary stimulatory factor that prepares the progenitor cells to respond to EPO. In contrast to IL 3, however, BSF-1 alone does not appear to stimulate sustained proliferation of BFU-e and of CFU-mix, but EPO and BSF-1 are both required during the entire culture period for optimal growth of erythroid colonies. It can therefore be assumed that BSF-1 works mainly as an activation factor on BFU-e and CFU-mix and renders them sensitive to EPO, possibly by inducing the expression of functional EPO receptors.

In addition to its activity on BFU-e, BSF-1 enhances the proliferation of CFU-e in the presence of suboptimal and of optimal amounts of EPO. The enhancing effect of BSF-1 on the response of CFU-e and BFU-e to EPO resembles the activity of EPA on human and mouse erythroid progenitors. EPA has recently been purified from a HTLV-II-infected human T cell line and has been molecularly cloned.19,24 A comparison of the cDNA sequences of EPA25 and the human analogue of BSF-16 demonstrates that these are distinct entities. EPA also appears to differ from BSF-1 in that its described activities are limited to erythroid lineage cells.21

In assays for megakaryocytic progenitor cells, we found that BSF-1 exerted striking effects on colony formation. Previous studies had indicated that murine CFU-Mk are regulated in vitro by Mk-CSF and Mk potentiating activity. Mk-CSF appears to act directly on CFU-Mk cells and is now considered to be identical with IL 3.26, GM-CSF has also some activity on CFU-Mk.18 Mk potentiating activity (MkPA) is less well defined; it enhances the number and size of Mk colonies in the presence of IL 3 and acts on immature megakaryocytes rather than on CFU-Mk. MkPA is found in several crude supernatants from bone, bone marrow, lung and spleen cells,47 and in supernatants of the macrophage line P388D126; recently a T cell hybridoma designated FS7-20.6.18 has been described to produce MkPA.27

IL 3 by itself stimulates the production of a large number of Mk colonies. By contrast, BSF-1 alone induced only sparse small groups of megakaryocytes. Nevertheless both IL 3 and BSF-1 show synergy with the MkPA found in FS7-20.6.18 CM. Furthermore we showed that IL 1 and EPO synergize with IL 3 and thus should be regarded as having Mk-PA. Both these agents also synergize with BSF-1. This suggests that BSF-1, although stimulating by itself the appearance of only a small number of Mk colonies, shares some activity with IL 3 on CFU-Mk. The finding that BSF-1 and IL 3 do not synergize with one another also supports the concept that

### Table 3. Lack of Synergy Between IL 3 and BSF-1 on CFU-Mk

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>BFU-e/CFU-mix</th>
<th>Number of Megakaryocytic Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ B Cells</td>
<td>- B Cells</td>
</tr>
<tr>
<td>IL 3 None</td>
<td>1.5 ± 1.4*</td>
<td>11.6 ± 5.7</td>
</tr>
<tr>
<td>1 U</td>
<td>44.5 ± 17.5</td>
<td>51.5 ± 16.4</td>
</tr>
<tr>
<td>5 U</td>
<td>78.0 ± 27.8</td>
<td>73.6 ± 22.0</td>
</tr>
<tr>
<td>10 U</td>
<td>87.0 ± 22.8</td>
<td>78.5 ± 25.0</td>
</tr>
<tr>
<td>100 U</td>
<td>83.0 ± 21.0</td>
<td>82.5 ± 17.7</td>
</tr>
</tbody>
</table>

*Values represent the mean number ± SD of three different experiments.

### Table 4. Depletion of B220⁺ Cells Does Not Inhibit Hemopoietic Progenitor Cell Proliferation Induced by BSF-1

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>CFU-GM</th>
<th>BFU-e/CFU-mix</th>
<th>CFU-Mk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ B Cells</td>
<td>- B Cells</td>
<td>+ B Cells</td>
</tr>
<tr>
<td>G-CSF 50</td>
<td>10/0</td>
<td>21/0</td>
<td>EPO</td>
</tr>
<tr>
<td>G-CSF 200</td>
<td>16/9</td>
<td>28/14</td>
<td>EPO + BSF-1</td>
</tr>
<tr>
<td>G-CSF 50 + BSF-1</td>
<td>29/10</td>
<td>42/25</td>
<td>EPO + IL 3</td>
</tr>
<tr>
<td>G-CSF 200 + BSF-1</td>
<td>25/22</td>
<td>34/66</td>
<td>BSF-1 + EPO</td>
</tr>
<tr>
<td>IL 3</td>
<td>ND/66</td>
<td>ND/135</td>
<td>IL 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IL 3 + T-CM</td>
</tr>
</tbody>
</table>

*The first value represents the number of clusters (10-40 cells); the second value represents the number of colonies (>50 cells).
†The first value represents the number of BFU-e; the second value represents the number of mixed erythroid colonies.
‡T-CM = FS7-20.6.18 CM.
they have a similar activity. Thus we would identify BSF-1 as having some Mk-CSF activity.

The MkPA of pure IL-1 and EPO have not previously been described. However, supernatants of the P388D1 cell line, a major component of which is IL-1, have been described to enhance Mk colony formation.26 Our experiments using recombinant IL-1 confirm that the megakaryocyte-enhancing activity in P388D1 supernatant is most likely IL-1. Crude preparations of EPO have been used as primary stimulus of CFU-Mk49 and as potentiating factor together with IL-3 (D. Geissler, manuscript in preparation). In another study a small level of Mk stimulatory activity was found in only one of four preparations of biochemically purified EPO.49 We here present clear evidence that purified, recombinant EPO has an effect on megakaryocyte colony formation in the presence of IL-3 or BSF-1. Small but constant numbers of Mk colonies were also detected in cultures stimulated with EPO alone, demonstrating a direct effect of EPO at least on a subpopulation of CFU-Mk.

In conclusion, BSF-1 affects the in vitro proliferation of myeloid, erythroid, megakaryocyte, and multipotent progenitor cells. The nature of action on these hematopoietic cells is not yet known. It can be assumed that BSF-1 works directly on the progenitor cell population, since the depletion of adherent cells, T cells, and B cells did not result in a diminished colony proliferation. Active cell proliferation and colony formation, however, can only be observed when BSF-1 is combined with an additional factor, a property that has also been observed for BSF-1 as B cell25 and mast cell-stimulating factor9,11 and in its ability to stimulate proliferation by resting T cells.50 The factors costimulating with BSF-1 are G-CSF for CFU-GM; EPO for CFU-e, BFU-e, and CFU-mix; and factors with MkPA for CFU-Mk proliferation. All these proteins are regarded to influence predominantly later stages of hemopoiesis, whereas certain progenitor cells (BFU-e, CFU-mix, and most CFU-Mk) do not respond to their respective factors alone. However, the combination of these factors with BSF-1 results in a clonal proliferation and colony formation in vitro, suggesting a primary effect of BSF-1 on such progenitor cells. It can therefore be hypothesized that BSF-1 represents an activation factor for early hemopoietic progenitor cells and prepares the progenitor cells to respond to specific growth or differentiation factors. The effects of BSF-1 on hematopoietic progenitor cells are reminiscent of a recently described pre-B cell stimulatory activity that has synergistic effects with IL-3, GM-CSF, and M-CSF.9 However, anti-BSF-1 antibody does not neutralize this activity, suggesting that this pre-B cell stimulating factor and BSF-1 are different entities.31

The concepts of action of BSF-1 on hematopoietic progenitor cells are clearly in keeping with the capacity of BSF-1 to act on resting B cells to enhance their subsequent responsiveness to anti-IgM antibodies2,3 and to LPS and with the costimulatory action of BSF-1 and phorbol esters to render T cells responsive to interleukin 2 (IL-2).50 However, BSF-1 also possesses clear growth-promoting activities on T cells50 and B cells.52 It stimulates DNA synthesis in T cell blasts and certain long-term T cell lines10 to enter the S phase of the cell cycle by an IL-2-independent mechanism. BSF-1 may prove to act as both a physiologic activation factor and growth factor in the regulation by T cells of hematopoietic cell growth.

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