SYNERGISM OF BSF-2/INTERLEUKIN 6 AND INTERLEUKIN 3 ON DEVELOPMENT OF MULTIPOTENTIAL HEMOPOIETIC PROGENITORS IN SERUM-FREE CULTURE

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The murine blast cell colony assay offers a unique method to study the mechanisms controlling the primitive hemopoietic progenitors (1). The progenitors for the blast cell colonies present in mice treated with 5-fluorouracil (5-FU)[1] probably represent the most primitive stem cells difinable in culture (2). Current studies have shown that IL-3 and granulocyte-macrophage CSF (GM-CSF) have direct action on the development of multipotential hemopoietic progenitors as well as committed progenitors (3, 4). Hemopoietin-1 (5, 6), which is probably identical to IL-1α (7), and a growth factor(s) produced by a murine adherent marrow cell line (8) have been reported as a new class of hemopoietic growth factors with synergistic activities on multilineage colony formation. These factors have been demonstrated to possess no capacity to stimulate hemopoietic colony formation by themselves but enhance the effects of other hemopoietic factors.

Human B cell stimulatory factor 2/interleukin 6 (BSF-2/IL-6) (9), identical to IFN-β-2 (10, 11), a 26-kD protein (12) hybridoma/plasmacytoma growth factor (13), has been demonstrated to act on the proliferation and differentiation of B cells at the late stage and induce the production of Ig. Recently, Ikebuchi et al. (14) presented evidence that human BSF-2 interacts with IL-3 on murine multipotential progenitors, and at least part of the effect results from a decrease in the G_0 period of the individual stem cells.

In this study, we examined the effects of human BSF-2 on the development of murine hemopoietic progenitors using serum-free and serum-containing culture systems.

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[1] *Abbreviations used in this paper:* B, erythroid bursts; BLAST, blast cell; BSF-2/IL-6, human B cell stimulatory factor 2/interleukin 6; EM, erythrocyte-megakaryocyte; Ep, erythropoietin; 5-FU, 5-fluorouracil; GEM, granulocyte-erythrocyte-macrophage; GEMM, granulocyte-erythrocyte-macrophage-megakaryocyte; GM, granulocyte-macrophage; GMM, granulocyte-macrophage-megakaryocyte; M, megakaryocyte; MAST, mast cell.

Materials and Methods

Cell Preparation. 10–15-wk-old female BDF_1 mice were obtained from the Shizuoka Experimental Animal Center (Shizuoka, Japan). Spleen cells were prepared by teasing the spleen in 3 ml of α medium (Flow Laboratories, Inc., Rockville, MD) in a 35-mm Lux suspension culture dish (5221R; Miles Laboratories, Inc., Naperville, IL), by repeated pipetting, and by passage through a 100-gauge wire mesh. Bone marrow cells were flushed from femurs into α medium by using a 26-gauge needle and single cell suspensions prepared in α medium.

5-FU (F. Hoffman-La Roche Co., Ltd., Basel, Switzerland) was administered through the tail vein of the mice at a dosage of 150 mg/kg body weight (15). Spleen cells were harvested 4 d after the 5-FU injection, and bone marrow cells were harvested 2 d after the treatment.

Colony-stimulating Factors and Antiserum. Human recombinant BSF-2 was prepared by expressing a cDNA for BSF-2 (9) in *Escherichia coli*, followed by further purification (16). Specific activity was determined as 3.6×10^9 U/g by using the BSF-2-responsive human B lymphoblastoid cell line, SKW6-CL4. Murine IL-3 was a generous gift by Dr. Ken-ichi Arai, DNAX, Research Institute of Molecular and Cellular Biology, Palo Alto, CA. Human recombinant erythropoietin (Ep) was kindly provided by Kirin-Amgen (Tokyo, Japan). Human IL-1α and anti-human IL-1α rabbit serum were generously provided by Dr. Yoshikatsu Hirai, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan. Specific activity of IL-1α was determined as 2×10^7 U/mg protein by using the lymphocyte activation assay and a 1:123,000 dilution of anti-IL-1α serum (OCT 303) neutralized the activity of 10 U (0.5 ng) of IL-1α (17). Serum-free pokeweed mitogen-stimulated murine spleen cell-conditioned medium (PWM-SCM) was prepared as described previously (1).

Clonal Cell Cultures. Serum-free clonal culture was carried out in 35-mm Lux suspension culture plates by using a modification (18) of the technique described by Iscove et al. (19). Unless otherwise specified, the culture consisted of $4–6 \times 10^5$/ml spleen cells from normal mice, $1.0–1.5 \times 10^6$/ml spleen cells, or 4×10^4/ml bone marrow cells from 5-FU-treated mice, α medium (Flow Laboratories), 0.9% methylcellulose (Shinetsu Chemical Co., Tokyo, Japan), 1% crystallized deionized BSA (Calbiochem-Behring Corp., La Jolla, CA), 10^{-4} M mercaptoethanol (Eastman Organic Chemicals, Rochester, NY), 300 μg/ml of fully iron-saturated human transferrin (~98% pure, Sigma Chemical Co., St. Louis, MO), 160 μg/ml of soybean lecithin (Sigma Chemical Co.), 96 μg/ml of cholesterol (Nakarai Chemicals Ltd., Kyoto, Japan), and 80 ng/ml of BSF-2, 400 U/ml of IL-3 and/or 2 U/ml of Ep. The standard cultures contained 30% FCS (HyClone Laboratories, Inc., Logan, UT) instead of a combination of transferrin, lecithin, and cholesterol. Plates were incubated at 37°C in a humidified atmosphere flushed with 5% CO_2 in air.

Determination of Colony Types and Sizes. Colony types were determined by daily observation in situ on an inverted microscope. We used "mapping studies" to determine the full potential of colonies supported by BSF and/or IL-3 (2, 18). When a new colony appeared, its location on the plate was recorded and subsequent growth and differentiation followed every day. Colony type was classified according to the criteria described previously (1, 20, 21). Abbreviations for the colony types are GM, granulocyte-macrophage; B, erythroid bursts; M, megakaryocyte; EM, erythrocyte-megakaryocyte; GEM, granulocyte-erythrocyte-macrophage; GMM, granulocyte-macrophage-megakaryocyte; GEMM, granulocyte-erythrocyte-macrophage-megakaryocyte; MAST, mast cell; and BLAST, blast cell.

The size of small colonies (consisting of <500 cells) was determined by counting individual cells in situ with ×150 on an inverted microscope. Colonies consisting of >500 cells were individually lifted by an Eppendorf micropipet, and made into single cell suspensions. Colony size was estimated by using a counting chamber. In some experiments, colony types were determined by differential counting of the Cytospin preparations stained with May-Grunward-Giemsa.

Replating Experiments. To determine the potential of the blast cell colonies, we carried out replating experiments of individual blast cell colonies seen in cultures of spleen cells from normal or 5-FU-treated mice in the presence of 80 ng/ml of BSF-2. Individual blast cell colonies were picked up with an Eppendorf micropipet on an inverted microscope and plated in a secondary culture containing 2 U/ml of Ep and 10% PWM-SCM. Cultures of replated cells were incubated, and secondary colonies were scored in the same manner as primary cultures.

Results

Dose Response of Colony Formation to BSF-2. To determine the optimal concentration of BSF-2, we examined colony formation by 2×10^6 spleen cells of 5-FU-treated mice at BSF-2 concentrations from 0.6–160 ng/ml in the presence of FCS. The maximal number of total colonies was seen with 40–160 ng/ml of BSF-2 as shown in Fig. 1. Thus, BSF-2 at 80 ng/ml was used in subsequent studies.

Colony Formation Supported by BSF-2 or IL-3 in Serum-containing Culture. We compared the types and numbers of colonies formed in serum-containing culture by spleen cells of normal and 5-FU-treated mice supported by 80 ng/ml of BSF-2 with those supported by 400 U/ml of IL-3, which was shown to be the optimal concentration for the development of various types of colonies (3, 18, 22). The results are presented in Table I. The number of total colonies supported by BSF-2 was approximately one-fifth and one-half of those supported by IL-3 derived from spleen cells of normal mice and 5-FU-treated mice, respectively. Many different types of colonies including GM, M, GMM, MAST, and blast cell colonies were formed in the presence of IL-3. Serial observations of culture dishes containing spleen cells of normal or 5-FU-treated mice demonstrated that BSF-2 supported sequential appearance of blast cell colonies after day 7, similar to those supported by IL-3 or GM-CSF (4, 22). Most of the blast cell colonies showed terminal differentiation into GM, M, and GMM colonies until 16 d of incubation. However, some of them retained the morphological characteristics of blast cell colonies on day 16. BSF-2 did not support mast cell colony formation. Cytological analysis showed that the 11 randomly chosen mature day 16 colonies supported by BSF-2 derived from spleen cells of normal mice consisted of one macrophage colony, two megakaryocyte colonies, four neutrophil-macrophage colonies, and four neutrophil-macrophage-megakaryocyte colonies.

Replating Experiment of Blast Cell Colonies Supported by BSF-2. Since most of the colonies supported by BSF-2 revealed an early nature, we examined the potentiality of blast cell colonies grown in the presence of BSF-2 derived from spleen cells of normal mice and 5-FU-treated mice using replating experiments. Spleen cells (4×10^5) of normal mice were plated in a culture dish containing 80 ng/ml of BSF-2 and FCS. 17 colonies were seen in a culture dish until day 16. Seven of them were identified as small GM colonies on day 7. We individually lifted and replated 8 out of 10 blast cell colonies seen from day 7–15 into secondary culture dishes containing 2 U/ml of Ep and 10% PWM-SCM. The results are presented in Table II. Blast cell colonies revealed variable but high replating efficiencies. Five out of eight blast cell colonies yielded secondary multilineage colonies including GEM, GMM, and GEMM

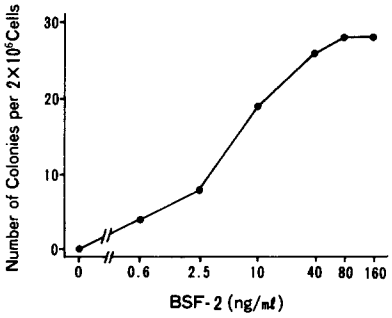

FIGURE 1. Dose response of colony formation by spleen cells of 5-FU-treated mice to BSF-2. The number of colonies derived from a total of 2×10^6 cells in two dishes was counted.

TABLE I

Colony Formation by Spleen Cells of Normal or 5-FU-treated Mice in Serum-containing Culture

Colony type	Normal mice			5-FU-treated mice		
	BSF-2	IL-3	None	BSF-2	IL-3	None
GM	13	42	0	5	12	0
M	4	23	0	6	6	0
GMM	6	19	0	7	30	0
MAST	0	79	0	0	9	0
BLAST	8	1	0	9	3	0
Total	31	164	0	27	60	0

Number of colonies was derived from a total of 1.6×10^6 spleen cells of normal mice and from a total of 4×10^6 spleen cells of 5-FU-treated mice in four dishes. BSF-2, 80 ng/ml; IL-3, 400 U/ml.

colonies. These results were comparable with those obtained by the replating experiment of blast cell colonies derived from spleen cells of 5-FU-treated mice in the presence of 80 ng/ml of BSF-2. These results suggested that BSF-2 supported the proliferation of multipotential progenitors preferentially in the presence of serum.

Colony Formation Supported by BSF-2 or IL-3 in Serum-free Culture. Since FCS is potentially an endogenous source of various types of colony stimulating factors, we carried out studies using the serum-free culture technique (18). The number of total colonies formed by spleen cells of 5-FU-treated mice grown in the presence of 10% PWM-SCM in serum-free culture conditions was ~80% of that formed in serum-containing culture conditions (data not shown). Table III shows a comparison of

TABLE II

Replating Experiment of Blast Cell Colonies Supported by BSF-2 Derived from Spleen Cells of Normal Mice and 5-FU-treated Mice

Spleen cells from:	Colony number	Day of harvest	Colony size	Secondary colonies						Total replating efficiency
				GM	M	GEM	GMM	GEMM	Total	
Normal										%
mice	1	7	24	15	0	0	0	0	15	63
	2	9	76	38	1	1	0	12	52	68
	3	9	261	89	0	0	0	0	89	34
	4	9	294	173	4	2	0	1	180	61
	5	12	25	13	0	0	1	1	15	60
	6	12	30	22	0	0	0	0	22	73
	7	12	104	48	2	3	0	2	55	53
	8	15	53	31	0	5	0	1	37	70
5-FU-treated										
mice	1	15	246	102	1	0	13	14	130	53
	2	15	257	139	0	0	0	0	139	54
	3	16	167	101	0	3	0	2	106	63
	4	16	176	90	0	0	0	0	90	51
	5	16	207	145	0	0	0	1	146	71
	6	16	255	139	0	0	0	0	139	55
	7	17	95	66	0	0	0	0	66	69
	8	17	137	85	0	0	0	1	86	63

TABLE III
Colony Formation by Spleen Cells of Normal Mice or 5-FU-treated Mice in Serum-free Culture

Spleen cells from:	Colony type	Serum-free culture			Serum-containing culture
		BSF-2	IL-3	None	BSF-2
Normal mice	GM	0	49	0	78
	M	0	37	0	6
	GMM	0	29	0	18
	MAST	0	13	0	0
	BLAST	0	8	0	6
	Total	0	136	0	108
5-FU-treated mice	GM	0	10	0	18
	M	0	3	0	10
	GMM	0	12	0	10
	MAST	0	4	0	0
	BLAST	0	29	0	6
	Total	0	58	0	44

Number of colonies was derived from a total of 2.4×10^6 spleen cells of normal mice and from a total of 4×10^6 spleen cells of 5-FU-treated mice in four dishes. BSF-2, 80 ng/ml; IL-3, 400 U/ml.

colony formation supported by BSF-2 and IL-3 in serum-free culture. IL-3 supported the development of many different types of colonies by spleen cells of both normal mice and 5-FU-treated mice. In contrast, no colony growth was seen in the presence of BSF-2. These results suggest that BSF-2 does not support colony formation by itself and requires factor(s) present in the serum for colony formation.

Effect of Combination of BSF-2 and IL-3 in Serum-free Culture. Since IL-3 is one of the most important factor(s) on the proliferation and differentiation of the hemopoietic progenitors (23, 24), it is possible that IL-3 is a candidate cooperating with BSF-2 in the serum. We examined the effects of BSF-2 on the development of various types of progenitors supported by an optimal concentration of IL-3. First, spleen cells of normal mice were plated in culture dishes containing 2 U/ml of Ep, 400 U/ml of IL-3, and/or 80 ng/ml of BSF-2. As shown in Table IV, BSF-2 did not increase the number of total colonies or that of erythroid bursts supported by Ep. These results suggested that BSF-2 does not have a burst-promoting activity. BSF-2 did not augment the single lineage colony growth (B, M, mast cell colony) or oligolineage colony growth (GM, EM colony) supported by 400 U/ml of IL-3 and Ep, which suggests that BSF-2 does not influence the development of maturer progenitors. The sum of the numbers of GEM, GMM, GEMM, and blast cell colonies produced by spleen cells cultured with Ep, IL-3, and BSF-2 was similar to that produced by culture with Ep and IL-3. However, the majority of multipotential progenitors supported by BSF-2, IL-3, and Ep developed into GEMM colonies on day 16. On the other hand, in IL-3- and Ep-containing culture, half of them remained in the developmental stages of GMM and blast cell colonies on day 16 and mostly differentiated into GEMM colonies after day 16.

To study the precise effect of BSF-2 on the development of multipotential progen-

TABLE IV

Effect of BSF-2 on Colony Formation by Spleen Cells of Normal Mice Supported by 400 U/ml of IL-3 and/or 2 U/ml of Ep in Serum-free Culture

Colony type	Ep	Ep + BSF-2	Ep + IL-3	Ep + IL-3 + BSF-2	None
GM	0	0	27	34	0
B	19	24	71	63	0
M	0	0	17	19	0
EM	2	0	29	21	0
GEM	0	0	7	7	0
GMM	0	0	15	6	0
GEMM	0	0	19	35	0
MAST	0	0	23	27	0
BLAST	0	0	9	0	0
Total	21	24	217	212	0

Number of colonies was derived from a total of 1.6×10^6 spleen cells of normal mice in four dishes. BSF-2, 80 ng/ml; IL-3, 400 U/ml; Ep, 2 U/ml.

itors, we used spleen cells of 5-FU-treated mice in which primitive cells were enriched. The results are presented in Table V. The addition of BSF-2 to the culture containing 400 U/ml of IL-3 resulted in a significant increase in the number of total colonies, including many GMM colonies. BSF-2 augmented the IL-3-dependent colony growth by bone narrow cells of 5-FU-treated mice as well. The combination of BSF-2 and 400 U/ml of IL-3 yielded 87 colonies (26 GM, 21 M, 33 GMM, 2 MAST, 5 BLAST), and IL-3 alone yielded 33 colonies (18 GM, 8 GMM, 4 MAST, 3 BLAST) from 1.2×10^5 cells in three dishes. No colony growth was seen in culture with BSF-2 alone. These results suggested that BSF-2 acted synergistically with IL-3 on the development of multipotential progenitors, not on that of the maturer progenitors.

Since BSF-2 was prepared by expressing a cDNA for BSF-2 in *E. coli*, it is possible that the effects of BSF-2 resulted from endotoxin present in BSF-2 samples. Therefore, we examined whether the action of BSF-2 was abolished by polymyxin B. Addition of polymyxin B (10 μg/ml) did not remove synergism of BSF-2 and IL-3 (data not shown).

Effect of BSF-2 on Colony Formation Supported by suboptimal Concentrations of IL-3 in Serum-free Culture. Next, we examined the mechanism of synergism between BSF-2

TABLE V

Effect of BSF-2 on Colony Formation by Spleen Cells of 5-FU-treated Mice Supported by 400 U/ml of IL-3 in Serum-free Culture

Colony type	BSF-2	IL-3	BSF-2 + IL-3	None
GM	0	2	28	0
M	0	1	5	0
GMM	0	11	61	0
MAST	0	2	1	0
BLAST	0	7	2	0
Total	0	23	97	0

Number of colonies was derived from a total of 6×10^6 spleen cells of 5-FU-treated mice in four dishes. BSF-2, 80 ng/ml; IL-3, 400 U/ml.

and IL-3. We plated spleen cells of 5-FU-treated mice in the presence of 80 ng/ml of BSF-2 and/or 1 or 4 U/ml of IL-3. The results are presented in Table VI. As shown in the previous sections, no colony growth was seen in culture with BSF-2 alone. Very low concentrations of IL-3 supported the growth of zero or two colonies. On the other hand, a combination of BSF-2 and IL-3 resulted in a significant increase in the number of colonies. Similar results were obtained in the culture containing bone marrow cells of 5-FU-treated mice. The combination of 80 ng/ml of BSF-2 and 4 U/ml of IL-3 supported the formation of seven colonies (six M, one GMM) from 1.2×10^5 cells in three dishes, while each factor alone failed to support the colony growth. It is possible that new colonies are formed in the culture with IL-3 alone and not with the combination of BSF-2 and IL-3 after day 16 of incubation, since Ikebuchi et al. (14) reported that human BSF-2 shortens the time course of appearance of colonies. Therefore, cultures containing spleen cells of 5-FU-treated mice were maintained until day 30, and sequential observations were carried out. A significant number of new colonies was seen in the presence of BSF-2 and IL-3 after day 16 of incubation; whereas, few new colonies grew with IL-3 alone (Table VI).

We previously demonstrated that the multilineage colonies appeared smaller in lower concentrations of IL-3 than in higher concentrations of IL-3 (22). Based on these results, we examined whether BSF-2 was capable of enlarging the GMM colonies supported by 40 U/ml of IL-3 derived from spleen cells of 5-FU-treated mice. When a colony appeared to have matured and did not change its morphology or size for 3 d, it was lifted from the methylcellulose medium by use of an Eppendorf micropipet for the determination of colony size and morphological examination. The results in Fig. 2 show a significant difference in the size of GMM colonies between the BSF-2 and IL-3 group and the IL-3 group ($p < 0.001$) according to Student's t test on logarithms of the cell numbers of individual colonies, although BSF-2 alone failed to support the colony growth. By contrast, there was no difference in the size of the mast cell colonies between the two groups. These results suggested that BSF-2 enhanced the sensitivity of multipotential hemopoietic progenitors to a low concentration of IL-3.

Effect of Anti-IL-1α Serum on Colony Formation Supported by BSF-2 and IL-3. Since Hemopoietin-1, probably identical to IL-1α (7), has been demonstrated to possess

TABLE VI

Effect of BSF-2 on Colony Formation Supported by 1 U/ml or 4 U/ml of IL-3 in Serum-free Culture

Colony type	GM	M	GMM	MAST	BLAST	Total
Exp. 1						
BSF-2(80 ng/ml)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
IL-3(1 U/ml)	0(1)	0(0)	0(0)	0(0)	0(0)	0(1)
IL-3(1 U/ml) + BSF-2(80 ng/ml)	1(1)	3(3)	3(4)	0(0)	1(1)	8(9)
Exp. 2						
BSF-2(80 ng/ml)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
IL-3(4 U/ml)	0(0)	0(0)	0(0)	2(2)	0(0)	2(2)
IL-3(4 U/ml) + BSF-2(80 ng/ml)	3(6)	6(7)	12(22)	0(0)	1(0)	22(35)

Numbers of colonies derived from a total of 3×10^6 (Exp. 1) and 3.6×10^6 (Exp. 2) spleen cells of 5-FU-treated mice in three dishes were counted until day 16 and numbers of colonies in parentheses until day 30.

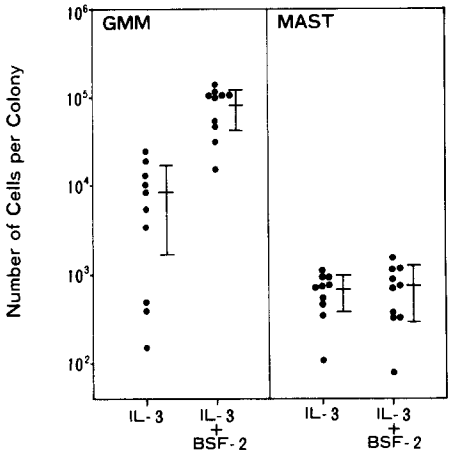

FIGURE 2. Effect of BSF-2 on size of GMM and mast cell colonies supported by 40 U/ml of IL-3. Mean values and SD for 10 GMM or 10 mast cell colonies are indicated. BSF-2; 80 ng/ml.

synergism with IL-3 (6), we examined whether the antiserum against human IL-1α absorbed the synergistic effect of BSF-2 using serum-free culture system. We therefore added the anti-IL-1α serum to the culture dishes containing spleen cells of 5-FU-treated mice with 40 U/ml of IL-3 and 80 ng/ml of BSF-2 or 2 ng/ml of IL-1α. The results are presented in Fig. 3. IL-1α did not support colony formation by itself but increased the number of total colonies grown in the presence of IL-3. The effect of 2 ng/ml of IL-1α on the IL-3-dependent colony growth, comparable with that of 20 ng/ml of IL-1α (data not shown), was less effective than that of BSF-2 as reported by Ikebuchi et al. (14). A 1:2,000 dilution of anti-IL-1α serum reduced the colony growth supported by IL-3 and IL-1α to the level seen in culture with IL-3 alone. On the other hand, the synergistic action between BSF-2 and IL-3 was not precipitated by the addition of antiserum.

Discussion

As previously indicated, IL-3 has been shown to support proliferation and differentiation of multipotential progenitors as well as committed progenitors to single lineages (3). Murine GM-CSF also has been demonstrated to possess direct effects on not only GM progenitors but also multipotential progenitors, and the primitive cells responding to GM-CSF are subpopulations of multipotential progenitors supported

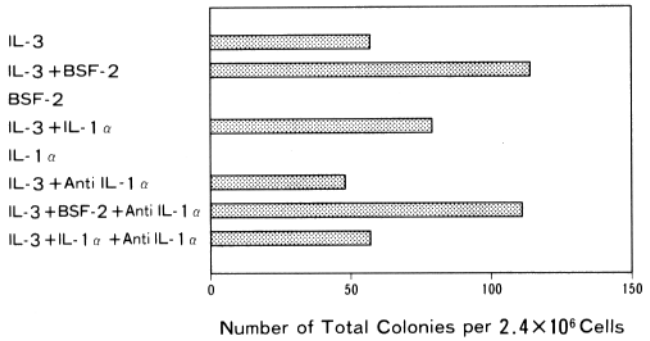

FIGURE 3. Effect of anti-IL-1α serum on colony formation supported by IL-3 and BSF-2 or IL-1α. The number of colonies derived from a total of 2.4×10^6 spleen cells of 5-FU-treated mice was counted. IL-3, 40 U/ml; BSF-2, 80 ng/ml; IL-1α, 2 ng/ml; Anti-IL-1α serum, 1:2,000.

by IL-3 (4). On the other hand, hemopoietin-1 produced no hemopoietic colonies by itself in the presence of FBS but acted synergistically with CSF-1 in the generation of mononuclear phagocytic precursor cells (5) and with IL-3 on development of multipotential hemopoietic cells (6). Quesenberry et al. (8) reported a growth factor(s) in the medium conditioned by murine marrow-adherent cell line, TC-1, which has shown multilineage synergistic activities with IL-3, GM-CSF, and CSF-1 on bone marrow cells of normal and 5-FU-treated mice using a serum-containing culture condition. They showed that this factor differs from hemopoietin-1, and the major synergistic effect is on the size rather than on the number of colonies.

The present study demonstrated that BSF-2 supports multipotential blast cell colony formation by spleen cells of normal and 5-FU-treated mice in the presence of serum. These results were consistent with those reported by Ikebuchi et al. (14). However, use of a serum-free culture method showed BSF-2 alone has no effect on colony growth, suggesting that BSF-2 fails to support colony formation by itself. Combination of BSF-2 and IL-3 in serum-free culture resulted in a significant increase of colony formation by spleen cells and bone marrow cells of 5-FU-treated mice in comparison with IL-3 alone; whereas, no effect by the addition of BSF-2 was seen on the number of single or oligolineage colonies by spleen cells of normal mice supported by IL-3 and/or Ep. These results suggested that BSF-2 and IL-3 act synergistically on the multipotential progenitors and not on that of the maturer progenitors. Addition of BSF-2 to culture with a low concentration of IL-3 with little effect on colony formation resulted in a significant increase in the number of colonies. Synergistic activities of BSF-2 and a low concentration of IL-3 was also seen on the size of GMM colonies. These results suggested that BSF-2 enhances the susceptibility of multipotential progenitors to IL-3.

Hemopoietin-1, probably identical to IL-1α (7), was reported to act synergistically with IL-3 on development of multipotential hemopoietic cells (6). Our serum-free culture study demonstrated that IL-1α also failed to support colony formation by itself but enhanced the IL-3-dependent colony growth. However, synergistic action of BSF-2 was more effective than that of IL-1α and was not absorbed by the addition of anti-IL-1α serum, suggesting that potentiating activity of BSF-2 was distinguishable from that of IL-1α.

BSF-2 supported multipotential blast cell colony formation and differentiation of hemopoietic progenitors into neutrophil, macrophage, and megakaryocyte lineage in the presence of serum. Serum-free culture demonstrated that BSF-2 requires factor(s) present in serum for the manifestation of the activities. Combination of BSF-2 and IL-3 suggested that IL-3 is a candidate for synergistic action with BSF-2. The level of IL-3 in our FBS was <1 U/ml, according to the measurement using an IL-3-dependent cell line, 32 D-cl line (data not shown). Addition of 1 U/ml of IL-3 to serum-free culture containing BSF-2 failed to restore the number of total colonies to the level obtained by serum-containing culture with BSF-2 alone, when spleen cells of 5-FU-treated mice were used (data not shown). Therefore, some factors other than IL-3 in FBS may also act with BSF-2 synergistically. It may be related to the results that BSF-2 supported formation of small GM colonies until day 7 from spleen cells of normal mice in a serum-containing culture condition.

BSF-2 may possess several functions on hemopoiesis. One of them is that BSF-2 may advance the maturation of multipotential progenitors, because BSF-2 hastened

the differentiation of multipotential progenitors in the culture with IL-3 and Ep when target cells were spleen cells of normal mice. It appears to be related to the evidence described by Ikebuchi et al. (14), that the combination of BSF-2 and IL-3 hastened the rate of colony appearance relative to that observed with either factor alone. However, augmentation of BSF-2 on number and size of colonies supported by low IL-3 cannot be interpreted by this mechanism. Another mechanism of synergism will be proposed that BSF-2 enhances the sensitivity of multipotential progenitors to IL-3. If the activities of IL-3 on hemopoietic progenitors are mediated by receptors, it may suggest that BSF-2 increases the numbers of receptors to IL-3 or upregulates the affinity of receptors to IL-3 on the multipotential progenitors. Further studies are required.

Summary

We investigated the effects of B cell stimulatory factor 2/interleukin 6 (BSF-2/IL-6) on the development of murine hemopoietic progenitors using serum-containing culture and serum-free culture. In serum-containing culture, BSF-2 mainly supported multipotential blast cell colonies from spleen cells of normal and 5-fluorouracil (5-FU)-treated mice. In serum-free culture, no colony growth was seen in the presence of BSF-2. Addition of BSF-2 to the serum-free culture containing IL-3 resulted in a significant increase in the number of colonies formed from multipotential progenitors in spleen cells and bone marrow cells of 5-FU-treated mice, whereas no effects were seen on the number of single or oligolineage colonies formed by the spleen cells of normal mice. These results suggested that BSF-2 and IL-3 act synergistically on the multipotential progenitors but not on the maturer progenitors. When BSF-2 was added to a culture containing low concentrations of IL-3 (1 U/ml, 4 U/ml), which had little effect on colony formation, the number of total colonies formed by the spleen cells and bone marrow cells of 5-FU-treated mice increased significantly. The combination of BSF-2 and 40 U/ml of IL-3 resulted in a significant enlargement of GMM colonies. Thus, BSF-2 appears to enhance the sensitivity of multipotential hemopoietic progenitors to IL-3.

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