

THE ESSENTIAL ROLE OF
B CELL STIMULATORY FACTOR 2 (BSF-2/IL-6)
FOR THE TERMINAL DIFFERENTIATION OF
B CELLS

BY ATSUSHI MURAGUCHI, TOSHIO HIRANO, BO TANG,
TADASHI MATSUDA, YASUHIRO HORII, KOICHI NAKAJIMA, AND
TADAMITSU KISHIMOTO

*From the Division of Immunology, Institute for Molecular and Cellular Biology, Osaka
University, Osaka 565, Japan*

Several interleukins are involved in the regulation of B cell maturation (1, 2). These interleukins are divided into three categories: (a) a factor mainly involved in the activation of resting B cells (B cell stimulatory factor 1 [BSF-1/IL-4]),¹ (b) a factor for the growth and maturation of activated B cells (B cell growth factor [BCGF-II/IL-5]), and (c) a factor for the terminal differentiation of B cells into antibody-secreting cells (BSF-2/IL-6). The cDNAs for these three interleukins have been cloned (3–6), and the recombinant molecules and specific antibodies against these molecules have become available, thus accelerating the studies on their physiological role in the B cell responses.

BSF-2 was originally described as a B cell differentiation factor that acts on activated B cells or B lymphoblastoid cell lines and induces Ig production (7, 8). The molecular cloning of the cDNA for BSF-2 has revealed that BSF-2 is identical with the molecules called IFN- β 2, 26-kD protein, and hybridoma plasmacytoma growth factor (HPGF), whose cDNAs have been cloned or whose partial amino acid sequence has been determined, essentially at the same time (9–11). The results suggest that the function of BSF-2 may not be restricted to B cells. Actually, it was shown that BSF-2 could stimulate liver cells to induce acute-phase proteins as hepatocyte-stimulating factor (HSF) (12, 13). However, BSF-2 did not have any antiviral activity (14, 15), although it has been called IFN- β 2 (10). Considering the variety of biological activities of BSF-2, it has been proposed that BSF-2 be called interleukin 6 (IL-6) (14, 16).

This study reports on the role of recombinant BSF-2 (rBSF-2) in the B cell response. The results show that rBSF-2 acts on normal activated B cells to induce Ig production in the absence of growth induction. However, rBSF-2 shows a potent growth activity on murine hybridoma cells. The observations made with

This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan.

¹Abbreviations used in this paper: BCGF, B cell growth factor; BSF, B cell stimulatory factor; HPGF, hybridoma plasmacytoma growth factor; HSF, hepatocyte-stimulating factor; MNC, mononuclear cells; RA, rheumatoid arthritis; SAC, *Staphylococcus aureus* Cowan I.

anti-BSF-2 antibody demonstrate that BSF-2 is one of the essential molecules in the PWM-induced Ig production in B cells.

Materials and Methods

Reagents. PWM was purchased from Sigma Chemical Co. (St. Louis, MO). Phytohemagglutinin (PHA) and *Staphylococcus aureus* Cowan I (SAC) were obtained from Pharmacia Fine Chemicals Co. (Uppsala, Sweden). Anti-human μ chain-specific antibody and anti-mouse μ chain-specific antibody were purchased from Cappel Laboratories (Cochranville, PA). rIL-1 β was kindly provided from Otsuka Pharmaceutical Co. Ltd. (Tokushima, Japan) and rIL-2 from Ajinomoto Co. Inc. (Tokyo, Japan).

Preparation of rBSF-2. rBSF-2 was prepared as described previously (15). Briefly, the plasmid pTBCDF-12 whose expression was under the control of *Escherichia coli trp* promoter was constructed using pT9-11 (17). rBSF-2 was produced as a fusion protein with a part of IL-2 which was further digested with kallikrein (EC 3.4.21.34) followed with aminopeptidase-P (EC 3.4.11.9) treatment to obtain the mature rBSF-2 protein. The rBSF-2 was purified by reverse-phase HPLC. The purity of rBSF-2 was >95%, as estimated by SDS-PAGE.

Preparation of Polyclonal Anti-BSF-2 Antibodies. Rabbits were immunized with 50 μ g of rBSF-2 in CFA at 2-wk intervals for 2 mo as described previously (15). The IgG fractions of the antiserum and preimmune serum were prepared by ion-exchange chromatography. F(ab')₂ fragments of the IgG fractions were prepared by a standard pepsin digestion method (18). The anti-BSF-2 antibody bound rBSF-2 and partially purified natural BSF-2 derived from bladder cell carcinoma T24, but did not bind rIL-1 β , rIL-2, IFN- β , rIFN- γ , or recombinant granulocyte colony stimulating factor (rG-CSF). Furthermore, anti-BSF-2 antibody inhibited the rBSF-2 or natural BSF-2-induced Ig production in the EBV-transformed B cell line, SKW6-CL4, as well as [³H]TdR uptake in the BSF-2-dependent murine hybridoma, MH60.BSF2 cells. However, it did not inhibit the antiviral activity of IFN- β as described elsewhere (15, and Hirano, T., M. Matsuda, M. Turner, et al., manuscript submitted for publication).

Cell Preparation. Heparinized peripheral blood was drawn from healthy donors. Tonsils were obtained at tonsillectomy from patients with chronic tonsillitis. The mononuclear cells (MNC) of peripheral blood or tonsillar cells were separated by the Ficoll-Paque gradient method (19). E-rosette-positive and -negative cells were separated from PBMC or tonsillar MNC by twice rosetting with 2-aminoethylisothiuronium bromide (AET)-treated sheep erythrocytes (E). E-rosette-positive cells were used as T cells. E-rosette-negative cells were further treated with anti-Leu-1 mAb (Becton Dickinson and Co., Sunnyvale, CA) and rabbit complement. Recovered cells, usually containing >90% sIg⁺ cells, <0.1% E⁺ cells and monocytes, were used as purified B cells. In some experiments, in vitro-activated B cells that had been incubated with either SAC or PWM were separated into high-density B (small B) cells and low-density B cells (B cell blasts) by using a discontinuous Percoll gradient centrifugation. $\sim 2 \times 10^7$ purified B cells were layered on top of the gradients, and the tubes were centrifuged at $1280 \times g$ for 15 min at 4 °C. B cells with low density and high density were recovered in fractions between 40/50% and 60/70%, respectively.

Determination of IL-1, IL-2, BSF-1/IL-4, BCGF-II/IL-5, BCGF, and HPGF Activities. All cultures were done using 96-well flat-bottomed microplates (2596; Costar, Cambridge, MA) in 0.2 ml of RPMI 1640 containing 10% FCS (Lot No. 5319; Gibco Laboratories, Grand Island, NY), 100 U/ml penicillin, 100 μ g/ml streptomycin, 2 mM L-glutamine, and 5×10^{-5} M 2-ME. To measure IL-1 activity, thymocytes from 7-wk-old BALB/c mice (2×10^6 /well) were cultured for 3 d with 1 μ g/ml PHA in the presence of test samples. For measuring IL-2 activity, 5×10^3 cells of the IL-2-dependent murine T cell line, MTH41.16 (19), were cultured with test samples for 24 h. To examine BSF-1/IL-4 activity, splenic B cells from 6-wk-old BALB/c mice (5×10^4 /well) or human tonsillar B cells (2×10^5 /well) were cultured with test samples for 3 d in the presence of 10 μ g/ml of anti- μ antibody (20, 21). For measuring BCGF-II/IL-5 activity, murine BCL-1 cells (10^5 /well), a kind gift

of Dr. E. S. Vitetta (University of Texas, Health Science Center, Dallas, TX), were cultured with test samples for 3 d as described previously (22). BCGF activity was measured by using SAC-activated B blast cells as described before (23). For measurements of HPGF activity, the BSF-2-dependent murine hybridoma clone, MH60.BSF2, was cultured at a cell concentration of 5×10^4 /ml with test samples for 48 h. MH60.BSF2 hybridoma was established as a clone producing mAb against human BSF-2 (Matsuda, M., et al., manuscript in preparation). Its growth was shown to be absolutely dependent on BSF-2, as described elsewhere (Hirano, T., M. Matsuda, M. Turner, et al., manuscript submitted for publication). All cultures were pulsed with $1 \mu\text{Ci}$ of [^3H]thymidine ([^3H]TdR) (15.1 Ci/mmol ; New England Nuclear, Boston MA) during the last 6 or 16 h, as indicated, and cells were harvested on glass filter paper by an automated cell harvester (Labo Mash Science Co., Tokyo, Japan). The radioactivity was measured with a Beckman scintillation counter (Beckman Instruments, Inc., Fullerton, CA). All assays were performed in triplicate cultures.

Measurement of BSF-2 Activity. EBV-transformed B cell lines, SKW6-CL-4 and SGB29, were cultured at 5×10^4 /ml with test samples in the microplates for 4 d and IgM and IgG were measured by ELISA as described previously (7).

PWM-induced Ig Production. MNC obtained from peripheral blood or tonsils or PWM-stimulated B blast cells were cultured at a cell density of 2×10^5 /well in the presence or absence of PWM together with various concentrations of test samples in the microplates for various periods as indicated. In some experiments, IgG fractions or F(ab')₂ fractions of antibodies were added to the cultures. Culture medium was replaced with fresh medium supplemented with test samples or antibodies every 4 d. The concentrations of Ig in the culture supernatant were determined by ELISA.

Results

rBSF-2 Induces Ig Production in a B Cell Line and in Activated Normal B Cells. rBSF-2 induced IgM production in an EBV-transformed B cell line, SKW6-CL-4 (Fig. 1 A), and IgG production in another cell line, SGB29 (Fig. 1 B) in a dose-dependent manner, but an equivalent amount of mock preparation did not cause Ig production. The maximum Ig production in those cells was observed at a concentration of 2–20 ng/ml of rBSF-2 and 50% of the maximum response was obtained at a concentration of around 0.2 ng/ml, the specific activity of rBSF-2 being estimated as $5 \times 10^6 \text{ U/mg}$. To examine the effect of rBSF-2 on Ig production of normal B cells, tonsillar MNC were cultured in the presence of varying concentrations of PWM together with rBSF-2 (1 ng/ml or 10 ng/ml) for 8 d and the amounts of Ig in the culture supernatants were determined. Representative data of three experiments with essentially similar results are shown in Fig. 2. The addition of rBSF-2 to the culture augmented IgM, IgG, and IgA productions 3–10-fold. Equivalent amounts of mock rBSF-2 preparation did not show any enhancing effect (data not shown). When rBSF-2 was added into the culture of peripheral blood MNC with PWM, a similar enhancement of PWM-induced Ig production was observed (Fig. 3). As shown in Fig. 3, kinetic studies with PWM-stimulated peripheral MNC in the presence or absence of rBSF-2 showed that rBSF-2 did not change the kinetics of the response but simply augmented the peak response, suggesting that rBSF-2 may enhance the process normally occurring in the response.

Then it was examined whether rBSF-2 acted directly on B cells. Purified B cells were stimulated with PWM in the presence of irradiated T cells for 3 d and B blast cells were obtained. These B blast cells were cultured with various concentrations of rBSF-2 for an additional 3 d and the concentrations of IgG in

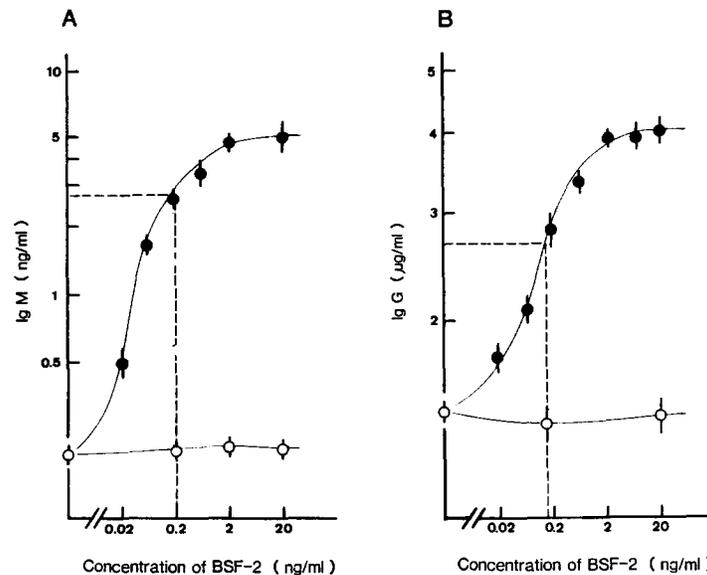


FIGURE 1. rBSF-2 induces Ig production in EBV-transformed B cell lines, SKW6-CL-4 and SGB29. SKW6-CL-4 (A) or SGB29 (B) cells were cultured with various concentrations of rBSF-2 (●) or mock preparation (○). Concentrations of IgM (SKW6-CL-4) or IgG (SGB29) in the culture supernatants were determined on day 4. Data are shown as means \pm SEM of the triplicate cultures.

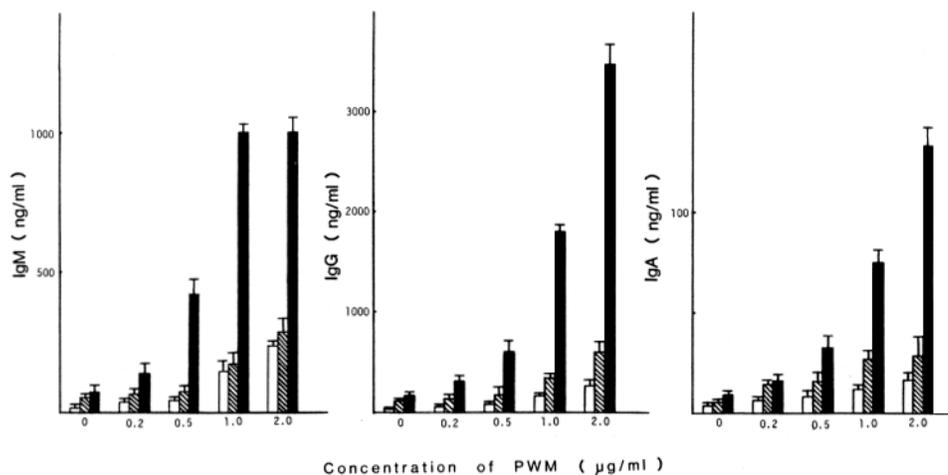


FIGURE 2. Enhancing effects of rBSF-2 on PWM-induced Ig production in tonsillar MNC. Tonsillar MNC were cultured with medium (□), 1 ng/ml (▨), or 10 ng/ml (■) of rBSF-2 in the absence or presence of various concentrations of PWM. Amounts of IgM, IgG, or IgA in the supernatant after 8 d culture were determined by ELISA. Data are shown as means \pm SEM of the triplicate cultures.

the culture supernatants were determined. As shown in Fig. 4A, rBSF-2 induced IgG production in activated B cells in a dose-dependent manner, but had no effect on unstimulated B cells. The equivalent amount of mock preparation did not induce Ig production in PWM-stimulated B blasts (data not shown). Contrary

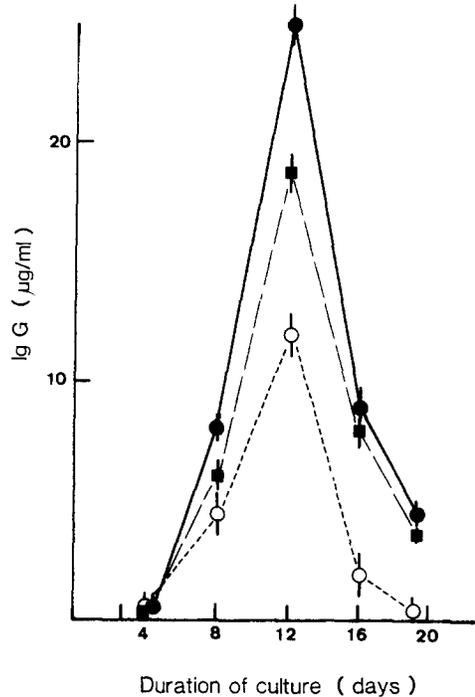


FIGURE 3. Enhancing effects of rBSF-2 on PWM-induced Ig production of PBMC. PBMC were cultured in the presence of PWM without (○) or with 2 ng/ml (■) or 20 ng/ml (●) of rBSF-2 for various days as indicated. The concentrations of IgG in the culture supernatant were determined. Results are shown as means \pm SEM of the triplicate cultures by ELISA.

to this, rBSF-2 did not induce [3 H]TdR uptake in activated B cells, whereas rIL-2 promoted cell proliferation of the same activated B cell population (Fig. 4B).

Effects of Anti-BSF-2 Antibody on the PWM-induced Ig Production. The role of BSF-2 in PWM-induced Ig production was further demonstrated using the antibody specific to BSF-2. Peripheral blood MNC were cultured with PWM together with various concentrations of anti-BSF-2 for 8 d. As shown in Fig. 5A, the PWM-induced IgG production was inhibited up to 80% by the addition of anti-BSF-2 antibody. The inhibition was also caused by the F(ab')₂ fragment of anti-BSF-2 antibody, indicating that the inhibition was not mediated through the Fc receptor. However, anti-BSF-2 antibody had no effect on PWM-induced [3 H]TdR uptake, which was measured on days 3 and 7 (Fig. 5B).

To examine whether the inhibition of PWM-induced Ig production by anti-BSF-2 can be overcome with rBSF-2, peripheral blood MNC were cultured with PWM and anti-BSF-2 (50 μ g/ml) in the presence of different concentrations of rBSF-2 for 7 d. The levels of IgG in the culture supernatants were determined by ELISA. As shown in Fig. 6, the inhibitory effect of anti-BSF-2 was reversed by the addition of rBSF-2 in a dose-dependent manner.

To examine on which stage of PWM-induced Ig production BSF-2 exerts its effects, anti-BSF-2 antibody was added either at the initiation or on the 4th day of the culture. The amounts of IgG produced were measured on day 8. As shown in Fig. 7, anti-BSF-2 antibody was effective even when added on day 4 of the culture.

Effects of rBSF-2 on a Variety of Cells Responsive to Different Kinds of Interleukins. As shown in Table I, rBSF-2 did not show any IL-1 or IL-2 activity. This

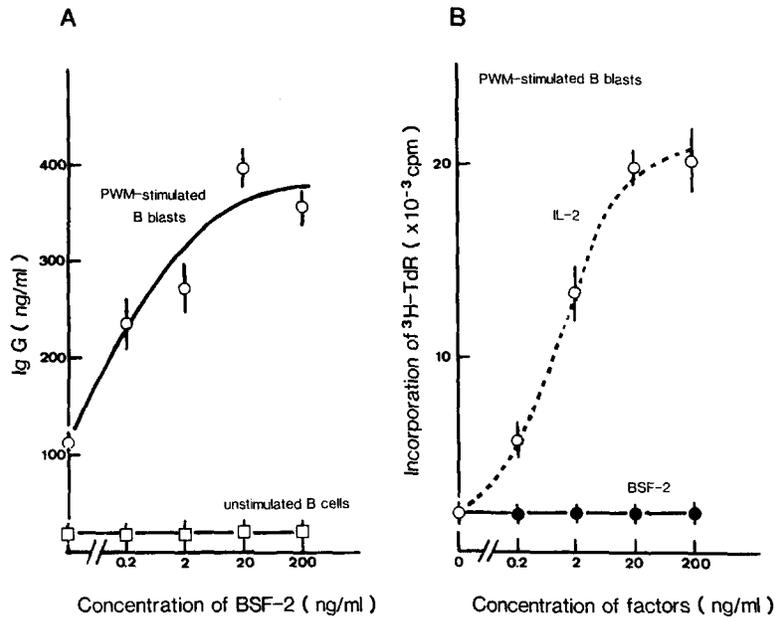


FIGURE 4. Effects of rBSF-2 on PWM-stimulated B blast cells. PWM-stimulated B blast cells (○) or unstimulated B cells (□) were cultured with various concentrations of rBSF-2 for 3 d. The concentrations of IgG in the culture supernatant were determined by ELISA (A). PWM-stimulated B blast cells were cultured with various concentrations of rBSF-2 (●) or rIL-2 (○) for 3 d and [³H]TdR uptake was measured (B). Results are shown as means ± SEM of the triplicate cultures.

was demonstrated by a costimulatory assay using PHA-stimulated murine thymocytes or examining proliferation-inducing ability in the murine IL-2-dependent T cell line, MTH41.16. rBSF-2 did not induce any increase of [³H]TdR uptake of anti-IgM-stimulated human or murine B cells or murine BCGF-II/IL-5-responsive cell line, BCL-1. In addition, rBSF-2 did not cause any proliferation in SAC-activated B cells.

BSF-2 Is a Potent Growth Factor for Hybridoma Cells. As shown in Fig. 8, rBSF-2 induced [³H]TdR uptake of murine hybridoma, MH60.BSF2 cells, in a dose-dependent manner. The maximum response was observed at a concentration of 20 pg/ml of rBSF-2 and the half-maximum response at a concentration of ~5 pg/ml of rBSF-2, indicating that the amount of BSF-2 required for the growth of MH60.BSF2 is 40 times less than that for Ig induction in SKW6-CL-4 cells.

Discussion

The process through which antigen-stimulated B cells mature into antibody-forming cells could be dissected into three stages; activation, proliferation, and differentiation. Several interleukins have been demonstrated to be involved in each phase of the B cell responses (1, 2) and BSF-2 has been classified as a factor that mainly induces terminal differentiation of B cells (2, 8). In this report, using rBSF-2, it was confirmed that BSF-2 in fact induces Ig production in PWM-activated B cells without any effect on cell proliferation. The most important result in this study is that anti-BSF-2 antibody almost completely inhibited PWM-

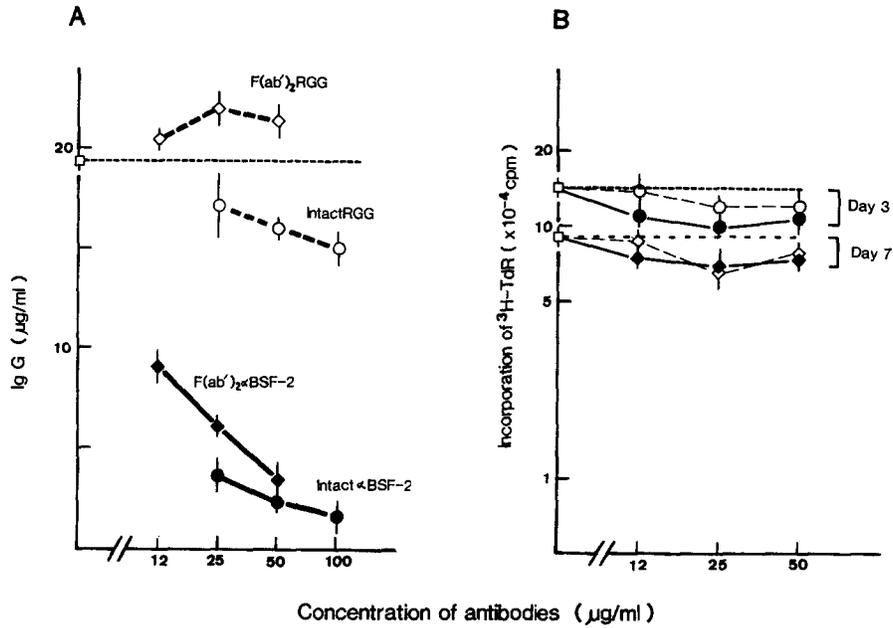


FIGURE 5. Effect of anti-BSF-2 antibodies on PWM-induced Ig production and cell proliferation of PBMC. PBMC were cultured in the presence of PWM with or without various concentrations of IgG fraction or F(ab')₂ fraction of rabbit IgG(RGG) (○, ◇) or anti-BSF-2 (αBSF-2) antibody (●, ◆). (A) The concentrations of IgG in the culture supernatants were determined on day 8. (B) Incorporation of [³H]TdR by the cultured cells was measured on day 3 and day 7. Results are shown as means ± SEM.

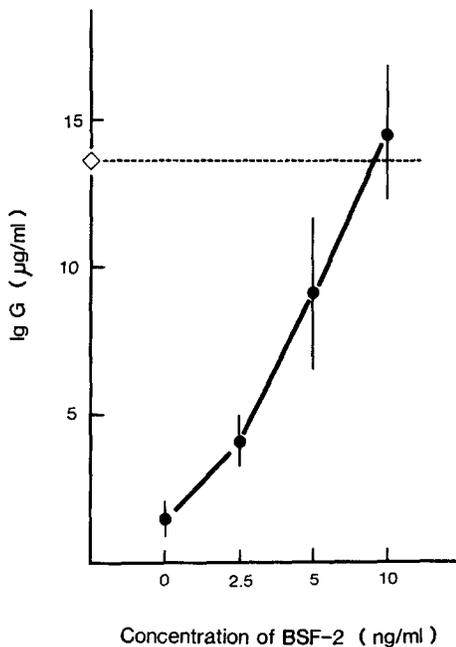


FIGURE 6. Reverse effect of rBSF-2 on the anti-BSF-2-induced inhibition of PWM-stimulated Ig production. PBMC (10⁵/well) were cultured with PWM (◇) or PWM plus anti-BSF-2 antibody (50 µg/ml) in the presence of various concentrations of rBSF-2 (●) for 7 d. The concentrations of IgG in the culture supernatants were determined by ELISA. Data are shown as means ± SEM.

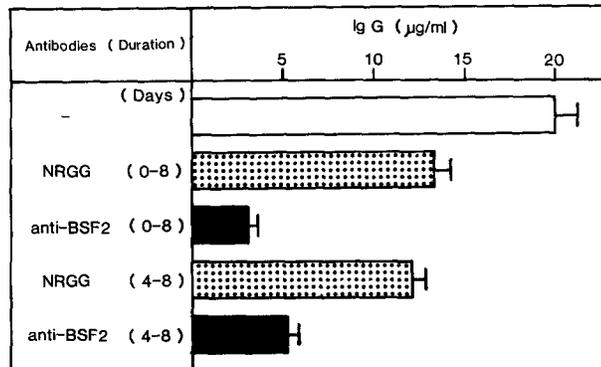


FIGURE 7. Effect of the addition time of anti-BSF-2 antibody on its inhibitory effect on PWM-induced Ig production of PBMC. PBMC were cultured in the presence of PWM. Normal rabbit IgG (NRGG) or anti-BSF-2 antibody was added to the culture on day 0 or day 4 and the concentrations of IgG were determined on day 8. Results are shown as means \pm SEM.

TABLE I
Effects of rBSF-2 on a Variety of Cells Responsive to Different Kinds of Interleukins

Assay	Target cells	Negative control	Positive control	^3H Thymidine incorporation after adding recombinant BSF-2		
				0.2 ng/ml	2 ng/ml	20 ng/ml
				<i>cpm</i>		
IL-1*	Mouse thymocytes	7,500 \pm 800	201,000 \pm 6,300	9,200 \pm 600	8,600 \pm 500	9,900 \pm 700
IL-2 [‡]	MTH 41.16	2,900 \pm 400	98,900 \pm 2,700	2,900 \pm 100	3,800 \pm 200	3,500 \pm 200
BSF-1/IL-4 [§]	Murine anti- μ -stimulated B cells	3,200 \pm 200	38,600 \pm 2,000	2,200 \pm 100	1,800 \pm 200	2,900 \pm 300
	Human anti- μ -stimulated B cells	1,500 \pm 200	72,000 \pm 1,800	1,600 \pm 200	1,500 \pm 100	1,900 \pm 200
BCGF-II/IL-5 [¶]	Murine BCL-1 cells	200 \pm 100	9,200 \pm 800	400 \pm 100	200 \pm 100	200 \pm 100
BCGF [¶]	Human SAC-stimulated B cells	1,100 \pm 200	9,500 \pm 800	1,200 \pm 200	1,100 \pm 100	900 \pm 100

* BALB/c (6 wk) thymocytes (2×10^6 /well) were stimulated for 3 d with rBSF-2 or 10 U/ml of rIL-1 in the presence of PHA-P (1 $\mu\text{g}/\text{ml}$).

[‡] IL-2-dependent murine cell line, MTH41.16 cells, were stimulated for 24 h with rBSF2 or 10 U/ml of rIL-2.

[§] BALB/c splenic B cells (5×10^4 /well) or human tonsillar B cells (10^5 /well) were stimulated for 3 d with rBSF-2 or 20 μl of PMA-stimulated EL4 supernatant (EL4-sup) (for murine B cells) or 40 μl of PHA-stimulated human T cell supernatant (T-sup) (for human B cells).

[¶] Murine BCL-1 cells were stimulated for 3 d with rBSF-2 or 20 μl of EL4-sup.

[¶] Human SAC-stimulated B blasts were stimulated for 3 d with rBSF-2 or 40 μl of T-sup. Data shown as means \pm SEM of triplicate cultures.

induced Ig production, indicating that BSF-2 is one of the essential factors for PWM-induced Ig production in B cells. Anti-BSF-2 antibody was effective even when added on the fourth day of a total 8-d culture, indicating that BSF-2 acts on the late phase of the B cell response rather than the early activation phase. This is in complete agreement with the previous reports demonstrating that BSF-2 is a late acting factor for the Ig induction in SAC-activated B cells (24, 25). In accordance with this data, Taga et al. (26) previously reported that BSF-2-receptor was expressed on the activated B cells but not on resting B cells. As reported previously with purified BSF-2 (7), rBSF-2 has no effect on the growth of anti-IgM, SAC, or PWM-stimulated B cells. Furthermore, anti-BSF-2 antibody, which suppressed PWM-induced Ig production, did not inhibit PWM-induced cell proliferation, indicating that BSF-2 is not involved in the PWM-induced proliferation of B cells. The results also excluded the possibility that the inhibitory effect of anti-BSF-2 on Ig production was due to nonspecific cytotoxic effect on activated B cells.

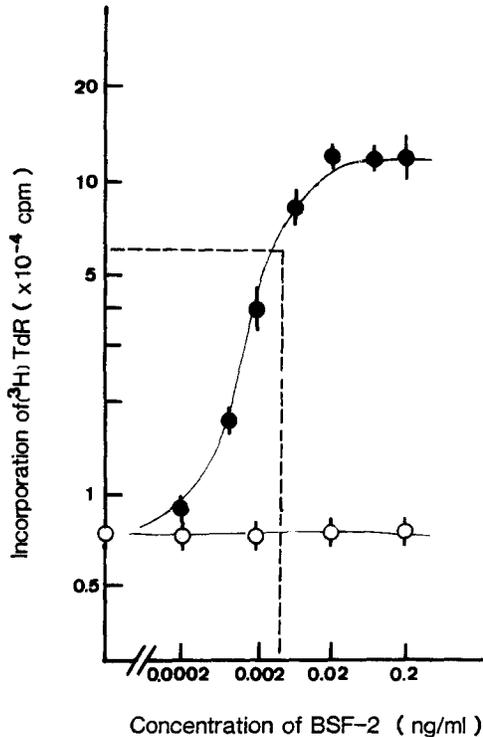


FIGURE 8. rBSF-2 induces cell growth of the murine hybridoma clone, MH60.BSF2. MH60.BSF2 cells were cultured with various concentrations of rBSF-2 (●) or mock preparation (○) and ^3H TdR uptake was measured on day 2. Data are shown as means \pm SEM of the triplicate cultures.

These functional properties of BSF-2 are quite different from those of BSF-1/IL-4 (3, 4, 27), BCGF II/IL-5 (5) and neuroleukin (28). BSF-1/IL-4 activates resting B cells (29) and induces proliferation of B cells together with anti-IgM (4, 20). BSF-1/IL-4 can induce IgG1 and IgE production in murine LPS-stimulated B blast cells (3, 30). However, the activity of BSF-1/IL-4 to induce Ig production was demonstrated to be exerted on resting B cells (31). BSF-1/IL-4 is thus considered an activation factor. In fact, the receptors for BSF-1/IL-4 were shown to be expressed on resting B cells, in contrast to BSF-2 receptors which are expressed only on activated B cells (26, 32, 33, 34). BCGF-II/IL-5 promotes proliferation of dextran sulfate-stimulated B cells and B lymphoma cell line, BCL-1, and induces Ig production in activated B cells (5, 35, 36). Therefore, BCGF-II/IL-5 is a factor with growth and differentiation activities. This is in contrast to the function of BSF-2, which induces only Ig production in B cells without any growth activity. Neuroleukin was demonstrated to be one of the essential factors for PWM-induced Ig production, but its effect was restricted in an early phase of B cell response, since antineuroleukin antibody inhibited PWM-induced Ig production only when it was added at the initiation of culture (28). Neuroleukin may directly act on B cells but the presence of T cells and monocytes is essential for neuroleukin-induced Ig secretion in B cells, indicating that several other factors such as BSF-2 are required for the process of B cell differentiation. In any case, the studies with antineuroleukin and anti-BSF-2 antibodies indicate that both factors are involved in the early and late stages of the PWM-induced B cell response, respectively.

rBSF-2 enhanced PWM-induced IgM, IgG, and IgA production, suggesting that BSF-2 has no preferential effect on certain isotypes of Ig in contrast to BSF-1/IL-4, IFN- γ , and BCGF-II/IL-5 which display preferential effect on certain classes and/or subclasses of murine Ig; BSF-1/IL-4 for IgG1 and IgE, IFN- γ for IgG2a (30, 37), and BCGF-II/IL-5 for IgA (38). However, BSF-2 might have preferential effect on a specific isotype of Ig, such as IgG2b. Al-Balaghi et al. (39) reported that synovial fluid of patients with rheumatoid arthritis (RA) contained factor(s) that preferentially induced IgG2b in LPS-stimulated murine B cells. They called the active factor BCDF and demonstrated that BCDF enhanced PWM-induced Ig production in human peripheral blood MNC without any effect on cell proliferation. They also showed that BCDF was directly effective on activated B cells. The functional properties of synovia-derived BCDF and BSF-2 seem to be similar. In fact, we recently found that synovial fluid obtained from affected joints of RA patients contains BSF-2 (Hirano, T., M. Matsuda, M. Turner, et al., manuscript submitted for publication). These findings suggest that BSF-2 might have preferential effect on the expression of IgG2b and this possibility remains to be determined.

The data presented in this report indicate that BSF-2 has no growth-inducing activity in normal activated B cells. However, rBSF-2 was a potent growth factor for a murine hybridoma cell line, MH60.BSF2, in complete agreement with the results obtained by Van Damme et al. (11) and Poupart et al. (14). rBSF-2 induced 50% of the maximum [^3H]TdR uptake of MH60.BSF2 at a concentration of ~ 5 pg/ml, the specific activity being calculated as 2×10^8 U/mg. The specific activity of rBSF-2 estimated on the basis of the Ig-inducing activity of EBV-transformed B lymphoblastoid cell line, SKW6-CL-4, is 5×10^6 U/mg. Van Snick et al. (40) also showed that hybridoma cells required 200 times lower BSF-2 than that required for plasmacytoma growth. The reasons why hybridoma cells are more sensitive to BSF-2 than plasmacytomas or B cell lines is not known. This could be explained by the assumption that the degree of the saturation of the receptor molecules with BSF-2 required for its functional expression might be different among a variety of cells. Alternatively, the presence of two classes of receptors with different affinity would cause the different sensitivity to BSF-2. This seems unlikely since the analysis of the BSF-2-receptor on various cells showed the presence of only a single class of receptor with high affinity (26).

In this study, the essential role of BSF-2 in Ig induction was demonstrated in PWM-stimulated B cells using rBSF2 and anti-BSF2 antibody. It should be examined whether the results obtained with mitogen-stimulated B cells could be applied to the antigen-specific antibody response.

Summary

The role of recombinant B cell stimulatory factor 2 (BSF-2/IL-6) in the regulation of growth and differentiation of B cells was investigated. rBSF-2 at 200 pg/ml could induce 50% of the maximum Ig production in B lymphoblastoid cell lines, the specific activity being estimated as 5×10^6 U/mg. rBSF-2 augmented PWM-induced IgM, IgG, and IgA production in mononuclear cells (MNC); the effect was exerted by directly acting on PWM-induced B blast cells to induce Ig production. However, rBSF-2 did not induce any growth of activated

B cells. In contrast, rBSF-2 showed a potent growth activity on a murine hybridoma clone, MH60.BSF2. The concentration required for half-maximal [³H]TdR uptake was ~5 pg/ml, which was 40 times less than that required for Ig induction in a B cell line. Anti-BSF-2 antibody inhibited PWM-induced Ig production in MNC, but not PWM-induced proliferation. The antibody was effective even when added on day 4 of an 8-d culture, indicating that BSF-2 is one of the essential late-acting factors in PWM-induced Ig production.

We thank Dr. E. Barsumian for his critical review and Ms. K. Kubota, J. Shibuya, and M. Kawata for their secretarial assistance.

Received for publication 2 September 1987 and in revised form 2 November 1987.

References

1. Howard, M., and W. E. Paul. 1983. Regulation of B-cell growth and differentiation by soluble factors. *Annu. Rev. Immunol.* 1:307.
2. Kishimoto, T. 1985. Factors affecting B cell growth and differentiation. *Annu. Rev. Immunol.* 3:135.
3. Noma, Y., P. Sideras, T. Naito, S. Bergstedt-Lindquist, C. Azuma, E. Severinson, T. Tanabe, T. Kinashi, F. Matsuda, Y. Yaoita, and T. Honjo. 1986. Cloning of cDNA encoding the murine IgG1 induction factor by a novel strategy using SP6 promoter. *Nature (Lond.)* 319:640.
4. Lee, F., T. Yokota, T. Otsuka, P. Meyerson, D. Villaret, R. Coffman, T. Mosmann, D. Rennick, N. Roeham, C. Smith, A. Zlotnik, and K. Arai. 1986. Isolation and characterization of a mouse interleukin cDNA clone that expresses B-cell stimulatory factor 1 activities and T-cell and mast-cell-stimulating activities. *Proc. Natl. Acad. Sci. USA.* 83:2061.
5. Kinashi, T., N. Harada, E. Severinson, T. Tanabe, P. Sideras, M. Konishi, C. Azuma, A. Tominaga, S. Bergstedt-Lindquist, M. Takahashi, F. Matsuda, Y. Yaoita, K. Takatsu, and T. Honjo. 1986. Cloning of complementary DNA encoding T-cell replacing factor and identity with B-cell growth factor II. *Nature (Lond.)* 324:70.
6. Hirano, T., K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S. Kashiwamura, K. Nakajima, K. Koyama, A. Iwamatsu, S. Tsunasawa, F. Sakiyama, H. Matsui, Y. Takahara, T. Taniguchi, and T. Kishimoto. 1986. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature (Lond.)* 324:73.
7. Hirano, T., T. Taga, N. Nakano, K. Yasukawa, S. Kashiwamura, K. Shimizu, K. Nakajima, K. H. Pyun, and T. Kishimoto. 1985. Purification to homogeneity and characterization of human B cell differentiation factor (BCDF or BSFp-2). *Proc. Natl. Acad. Sci. USA.* 82:5490.
8. Kishimoto, T., and T. Hirano. 1988. Molecular regulation of B lymphocyte response. *Annu. Rev. Immunol.* In press.
9. Haegeman, G., J. Content, G. Volckaert, R. Derynck, J. Tavernier, and W. Fiers. 1986. Structural analysis of the sequence encoding for an inducible 26-kd protein in human fibroblasts. *Eur. J. Biochem.* 159:625.
10. Zilberstein, A., R. Ruggieri, J. H. Korn, and M. Revel. 1986. Structure and expression of cDNA and genes for human interferon- β 2, a distinct species inducible by growth-stimulatory cytokines. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:2529.
11. Van Damme, J., G. Opdenakker, R. J. Simpson, M. R. Rubira, S. Cayphas, A. Vink, A. Billiau, and J. Van Snick. 1987. Identification of the human 26-kD protein,

- interferon $\beta 2$ (IFN- $\beta 2$), as a B cell hybridoma/plasmacytoma growth factor induced by interleukin 1 and tumor necrosis factor. *J. Exp. Med.* 165:914.
12. Andus, T., T. Geiger, T. Hirano, H. Northoff, U. Ganter, J. Bauer, T. Kishimoto, and P. C. Heinrich. 1987. Recombinant human B cell stimulatory factor 2 (BSF-2/IFN- $\beta 2$) regulates β -fibrinogen and albumin mRNA levels in Fao-9 cells. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 221:18.
 13. Gauldie, J., C. Richards, D. Harnish, P. Lansdorp, and H. Baumann. 1987. Interferon $\beta 2$ /BSF-2 shares identity with monocyte derived hepatocyte stimulating factor (HSF) and regulates the major acute phase protein response in liver cells. *Proc. Natl. Acad. Sci. USA.* 84:7251.
 14. Poupart, P., P. Vandenabeele, S. Cayphas, J. V. Snick, G. Haegeman, V. Kruys, W. Fiers, and J. Content. 1987. B cell growth modulating and differentiating activity of recombinant human 26-kd protein (BSF-2, HuIFN- $\beta 2$, HPGF). *EMBO (Eur. Mol. Biol. Organ.) J.* 6:1219.
 15. Hirano, T., T. Matsuda, K. Hosoi, A. Okano, H. Matsui, and T. Kishimoto. 1988. Absence of antiviral activity in recombinant B cell stimulatory factor 2 (BSF-2). *Immunol. Lett.* In press.
 16. Yasukawa, K., T. Hirano, Y. Watanabe, K. Muratani, T. Matsuda, S. Nakai, and T. Kishimoto. 1987. Structure and expression of human B cell stimulatory factor-2 (BSF-2/IL-6) gene. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:2939.
 17. Sato, T., H. Matsui, S. Shibahara, T. Kobayashi, Y. Morinaga, N. Kashima, S. Yamasaki, J. Hamuro, and T. Taniguchi. 1987. New approaches for the high-level expression of human interleukin-2 cDNA in *Escherichia coli*. *J. Biochem.* 101:525.
 18. Nisonoff, A., F. C. Wissler, L. N. Lipman, and D. L. Woernley. 1960. Separation of univalent fragments from the bivalent rabbit antibody molecule by reduction of disulfide bonds. *Arch. Biochem. Biophys.* 89:230.
 19. Hirano, T., K. Fujimoto, T. Teranishi, N. Nishino, K. Onoue, S. Maeda, and K. Shimada. 1984. Phorbol ester increases the level of interleukin 2 mRNA in mitogen-stimulated human lymphocytes. *J. Immunol.* 132:2165.
 20. Howard, M., J. Farrar, M. Hilfiker, B. Johnson, K. Takatsu, T. Hamaoka, and W. E. Paul. 1982. Identification of a T cell-derived B cell growth factor distinct from IL-2. *J. Exp. Med.* 155:914.
 21. Muraguchi, A., J. L. Butler, J. H. Kehrl, and A. S. Fauci. 1983. Differential sensitivity of human B cell subsets to activation signals delivered by anti- μ -antibody and proliferative signals delivered by a monoclonal B cell growth factor. *J. Exp. Med.* 157:530.
 22. Shimizu, K., T. Hirano, K. Ishibashi, N. Nakano, T. Taga, K. Sugamura, Y. Yamamura, and T. Kishimoto. 1985. immortalization of BCGDF (BCGFII)- and BCGDF-producing T cells by human T cell leukemia virus (HTLV) and characterization of human BCGDF (BCGFII). *J. Immunol.* 134:1728.
 23. Muraguchi, A., and A. S. Fauci. 1982. Proliferative responses of normal human B lymphocytes. Development of an assay system for B cell growth factor (BCGF). *J. Immunol.* 129:1104.
 24. Hirano, T., T. Teranishi, B. H. Lin, and K. Onoue. 1984. Human helper T cell factor(s). IV. Demonstration of a human late-acting B cell differentiation factor acting on *Staphylococcus aureus* Cowan I-stimulated B cells. *J. Immunol.* 133:798.
 25. Nakagawa, T., N. Nakagawa, H. Goldstein, D. J. Volkman, and A. S. Fauci. 1986. Demonstration that human B cells respond differently to interleukin 2 and B cell differentiation factor based on their stages of maturation. *J. Immunol.* 137:3175.
 26. Taga, T., Y. Kawanishi, R. R. Hardy, T. Hirano, and T. Kishimoto. 1987. Receptors for B cell stimulatory factor-2 (BSF-2): quantitation, specificity, distribution and regulation of the expression. *J. Exp. Med.* 166:967.

27. Paul, W. E., and J. Ohara. 1987. B cell stimulatory factor 1/interleukin 4. *Annu. Rev. Immunol.* 5:429.
28. Gurney, M. E., B. R. Apatoff, G. T. Spear, M. J. Baumel, J. P. Antel, M. B. Bania, and A. T. Reder. 1986. Neuroleukin: a lymphokine product of lectin-stimulated T cells. *Science (Wash. DC)*. 234:574.
29. Rabin, E. M., J. Ohara, and W. E. Paul. 1985. B cell stimulatory factor (BSF)-1 activates resting B cells. *Proc. Natl. Acad. Sci. USA*. 82:2935.
30. Coffman, R. L., J. Ohara, M. W. Bond, J. Carty, E. Zlotnick, and W. E. Paul. 1981. B cell stimulatory factor 1 enhances the IgE response of lipopolysaccharide-activated B cells. *J. Immunol.* 136:4538.
31. Snapper, C. M., and W. E. Paul. 1987. B cell stimulatory factor-1 (interleukin 4) prepares resting murine B cells to secrete IgG1 upon subsequent stimulation with bacterial lipopolysaccharide. *J. Immunol.* 139:10.
32. Ohara, J., and W. E. Paul. 1987. Receptors for B-cell stimulatory factor 1 expressed on cells of haematopoietic lineage. *Nature (Lond.)*. 325:537.
33. Park, L. S., D. Friend, K. Grabstein, and D. L. Urdal. 1987. Characterization of the high-affinity cell-surface receptor for murine B-cell-stimulating factor 1. *Proc. Natl. Acad. Sci. USA*. 84:1669-1673.
34. Nakajima, K., T. Hirano, K. Koyama, and T. Kishimoto. 1987. Detection of receptors of murine B cell stimulatory factor 1 (BSF-1): presence of functional receptors on CBA/N splenic B cells. *J. Immunol.* 139:774.
35. Swain, S. L., M. Howard, J. Kappler, P. Marrack, J. Watson, R. Booth, G. D. Wetzel, and R. W. Dutton. 1983. Evidence for two distinct classes of murine B cell growth factor with activities in different functional assays. *J. Exp. Med.* 158:822.
36. Harada, N., T. Takahashi, M. Matsumoto, T. Kinashi, J. Ohara, Y. Kikuchi, N. Koyama, E. Severison, Y. Yaoita, T. Honjo, N. Yamaguchi, A. Tominaga, and K. Takatsu. 1987. Production of a monoclonal antibody to and its use in the molecular characterization of murine T cell-replacing factor (TRF) and B cell growth factor II (BCGF II). *Proc. Natl. Acad. Sci. USA*. 84:4581.
37. Snapper, C. M., and W. E. Paul. 1987. Interferon- γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science (Wash. DC)*. 236:944.
38. Mosmann, T. R., and R. L. Coffman. 1987. Two types of mouse helper T-cell clone. *Immunol. Today*. 8:223.
39. Al-Balaghi, S., H. Ström, and E. Möller. 1984. B cell differentiation factor in synovial fluid of patients with rheumatoid arthritis. *Immunol. Rev.* 78:7.
40. Van Snick, J., A. Vink, S. Cayphas, and C. Uyttenhove. 1987. Interleukin-HP1, a T cell-derived hybridoma growth factor that supports the in vitro growth of murine plasmacytomas. *J. Exp. Med.* 165:641.