Brief Definitive Report

SEROLOGICAL, BIOCHEMICAL, AND FUNCTIONAL IDENTITY OF B CELL–STIMULATORY FACTOR 1 AND B CELL DIFFERENTIATION FACTOR FOR IgG1

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Activation, growth, and differentiation of B lymphocytes is controlled by binding of antigen to membrane Ig receptors, by interaction with antigen-specific, major histocompatibility antigen (MHC)-restricted helper T cells, and by the action of a series of soluble products derived from lymphocytes (lymphokines) and monocytes (monokines) (1–6). Three responses of B cells that are mediated by lymphokines are (*a*) induction of increased levels of class II MHC molecules (Ia antigens) on resting cells (7, 8), (*b*) stimulation of B cell entry into the S phase of the cell cycle in the presence of low concentrations of anti-Ig antibodies (5, 9), and (*c*) expression and secretion of IgG1 in the presence of lipopolysaccharide (LPS) (10, 11). The former two responses are controlled by B cell stimulatory factor (BSF-1) (previously, B cell growth factor or BCGF I), a protein with an M_r of 20,000 and pI of 6.3–6.6 and 7.4–7.6 (12, 13). BSF-1 has recently been purified to homogeneity (13) and a monoclonal anti-BSF-1 antibody has been prepared (14). The latter response is due to the action of a lymphokine, termed B cell differentiation factor for IgG (BCDF-γ) (10, 11), which has an M_r (10, 11) and pI (11, 12) similar to that of BSF-1. In this report, we provide evidence that BCDF-γ and BSF-1 are the same lymphokine.

Materials and Methods

Purification of BSF-1. BSF-1 was purified by reverse phase high performance liquid chromatography (HPLC) of serum-free supernatants (SN) from EL-4 cells (5, 13). HPLC-purified BSF-1 is enriched 2,500-fold and lacks interleukin 1 (IL-1), IL-2, IL-3, and γ interferon (IFN-γ) activity (13). For some experiments, HPLC-purified BSF-1 was separated by preparative electrophoresis on sodium dodecyl sulfate–polyacrylamide gels (SDS-PAGE). BSF-1 was also purified by affinity chromatography of SN using a monoclonal anti-BSF-1 antibody (11B11) (14) conjugated to Sepharose. The affinity-purified BSF-1 was similar in purity to the material prepared by HPLC.

Lymphokine Assays. (*a*) BSF-1 activity was measured by its capacity to costimulate [^{3}H]-

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thymidine uptake by resting BALB/c or (C57BL/6 × DBA/2)F_1 (BDF_1) B cells (5) cultured with either a soluble monoclonal rat anti-mouse IgM antibody (Bet2) (15) or with Sepharose coupled to affinity-purified goat anti-mouse Ig (S-GAMIg) or goat anti-mouse δ (S-GAM-δ) (16). BSF-1 preparations were added at the initiation of culture and [^{3}H]-thymidine (1 μCi/well) was added for the last 16 h of the 72 h culture period. BSF-1 activity was reported either as a stimulation index [^{3}H]thymidine uptake in the presence of BSF-1/[^{3}H]thymidine uptake in its absence) or in units (U), where 1 U represents the amount of BSF-1 required for half-maximal stimulation induced by a standard preparation of the BSF-1-containing EL-4 SN.

(*b*) BCDF-γ activity was measured by culturing T cell–depleted BDF_1 spleen cells in 96-well Costar plates for 16 h in medium containing 20 μg/ml of LPS (10). Purified BSF-1 or unfractionated SN were added after 16 h and the cells were incubated for an additional 5 d. On the 6th d, the cell SN were harvested and assayed in a solid phase radioimmunoassay (RIA) for the presence of secreted IgG1 and IgG3 (10).

(*c*) BCDF-μ activity was determined using the in vitro–adapted clone of BCL_1 cells (3B3) (17). Factors to be tested for BCDF-μ activity were added at the initiation of the 6-d culture and the cells were incubated at 37°C in an atmosphere of 5% CO_2. The cell SN were harvested and assayed by a solid phase RIA for the presence of secreted IgM (17).

Results

BCDF-γ Activity of Purified BSF-1. Preparations of HPLC-purified BSF-1 and affinity-purified BSF-1 were tested for their activity in the BSF-1, BCDF-γ, and BCDF-μ assays. 2–10 U/ml of BSF-1 in conjunction with S-GAMIg or S-GAM-δ caused striking increases in [^{3}H]thymidine uptake and induction of IgG1 secretion and parallel inhibition of IgG3 secretion in LPS-stimulated cells (Fig. 1). The increases in IgG1 secretion and decreases in IgG3 secretion caused by BSF-

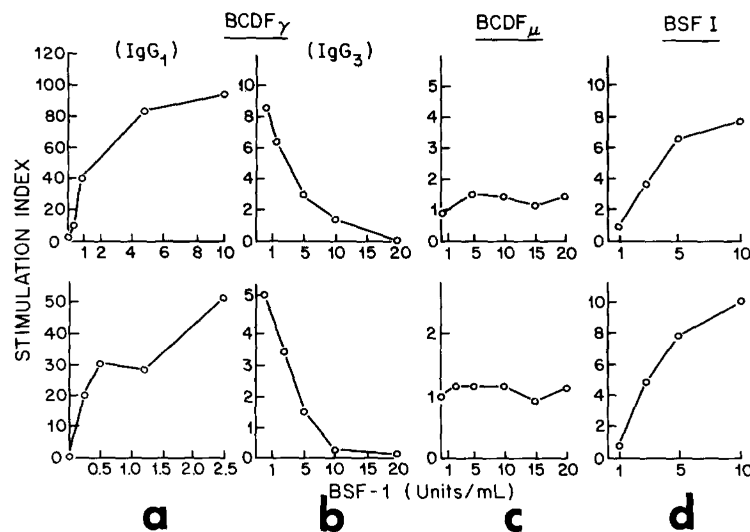

FIGURE 1. BSF-1 purified by HPLC (*top*) or affinity chromatography on a monoclonal anti-BSF-1 Sepharose column (*bottom*) induced an increase in IgG1 secretion (*a*) and a decrease in IgG3 secretion (*b*) in LPS-stimulated murine B cells. It did not induce IgM secretion in BCL_1 cells (*c*). As a positive control, it caused an increase in the BSF-1-mediated incorporation of thymidine in S-GAMIg-stimulated cells (*d*). Background values in the absence of BSF-1: IgM (BCDF-μ), 34 ± 6 ng/ml; IgG1 (BCDF-γ), 235 ± 16 ng/ml; IgG3 (BCDF-γ), 1,800 ± 400 ng/ml; [^{3}H]thymidine (BSF-1), 2,458 ± 350 cpm/10^5 cells. The stimulation indices using optimal amounts of control PK 7.1 SN (2–5 μl) were: BCDF-γ (IgG1, 94; IgG3, −40); BCDF-μ, 5; BSF-1, 14. This is a representative experiment out of six for BCDF-γ (IgG1), four for BCDFγ (IgG3), eight for BCDF-μ, and five for BSF-1.

1 preparation were similar in magnitude to those obtained using the SN of the concanavalin A–pulsed, alloreactive T cell clone PK 7.1 (18), the standard source of BCDF-γ (see Fig. 1 legend). It should be noted, however, that the majority of the cells producing IgG1 and IgG3 are different (19). Neither HPLC-purified BSF-1 nor affinity-purified BSF-1 had any activity in the BCDF-μ assay (Fig. 1), although the PK 7.1 SN was positive (see legend, Fig. 1). The effluent from the anti-BSF-1 affinity column was devoid of both BSF-1 and BCDF-γ activity but contained IL-2 and BCDF-μ activity (data not shown). HPLC-purified BSF-1 was further purified by SDS-PAGE. Extracts of eight individual bands, migrating with apparent M_r of slightly more than 20,000 to 14,000, were tested for BSF-1 and BCDF-γ activity. Fig. 2 illustrates a strong quantitative correlation between BSF-1 and BCDF-γ activities, with the three bands of M_r 20,000 having substantial activity in both assays. No BSF-1 or BCDF-γ activity was found in the lower molecular weight bands.

Depletion of BSF-1 and BCDF-γ from T Cell Supernatants with a Monoclonal Anti-BSF-1 Antibody Coupled to Sepharose. We next determined whether BSF-1 was the principal molecular species with BCDF-γ activity in the standard PK 7.1 SN (10). Since the IgG1-inducing factor produced by both PK 7.1 SN (10) and a T cell hybridoma (11) has been reported to have an M_r of 20,000 and, in the case of the T cell hybridoma (11), pIs similar to those of BSF-1 (12), we subjected SN from induced PK 7.1 cells to affinity chromatography using Sepharose conjugated to 11B11. As shown in Fig. 3, the column removed BSF-1 and BCDF-γ but not BCDF-μ activity from PK 7.1 SN.

Ability of Monoclonal Anti-BSF-1 to Block BCDFγ Activity. 11B11 ascitic fluid,

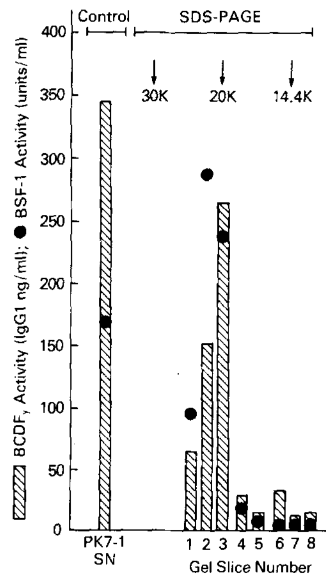

FIGURE 2. BCDF-γ (▨) and BSF-1 (●) activity of HPLC-purified BSF-1 fractionated on 20% SDS-PAGE. The gel was stained with Coomassie Blue, and individual bands were cut from the gel and eluted in phosphate-buffered saline containing 0.1% SDS. BCDF-γ activity was measured using 2 μl of extract. BSF-1 activity was measured using dilutions of extract from 1:10 to 1:100 and is reported in units. The background response in the BCDF-γ assay was 3 ± 0.4 ng/ml IgG. The experiment was repeated three times with similar results.

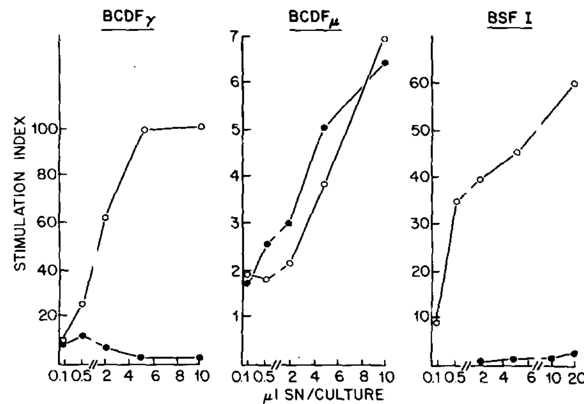

FIGURE 3. The monoclonal anti-BSF-1 antibody (11B11) coupled to Sepharose depleted BSF-1 and BCDF-γ activity from PK 7.1 SN, but had no effect on BCDF-μ activity. Briefly, 5.0 ml of PK 7.1 SN were passed over 1.0 ml of Sepharose anti-BSF-1. The material not adhering to the columns (●) was assayed for BCDF-γ, BCDF-μ, and BSF-1 activities. In each case, the assay was compared with the PK 7.1 SN before passage over the affinity column (○). In the absence of PK 7.1 SN, the amount of IgM secreted in the BCDF-μ assay was 30 ± 6 ng/ml and the amount of IgG1 secreted in the BCDF-γ assay was 255 ± 18 ng/ml. [^{3}H]-thymidine incorporation per 10^5 cells was 3,424 ± 186 cpm in the presence of S-GAM-δ. This is a representative experiment out of three performed. Similar results were obtained in the BSF-1 assay using S-GAMIg (two experiments).

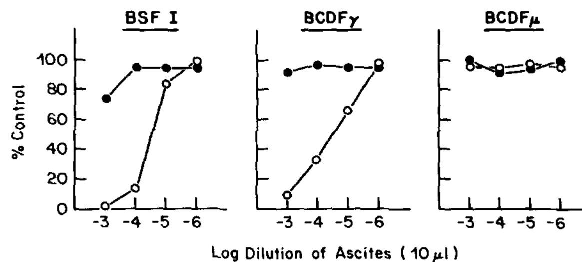

FIGURE 4. Monoclonal anti-BSF-1 blocks BSF-1 and BCDF-γ activities, but not the BCDF-μ activity of PK 7.1 SN. 10-μl dilutions of ascitic fluid containing monoclonal rat anti-BSF-1 (11B11) or anti-DNP (50Cl) antibody were added to cultures in which the BSF-1, BCDF-γ, and BCDF-μ activities of PK 7.1 SN were tested. The percent of the control response obtained with the PK 7.1 SN in the presence of 50Cl ascites (●) was compared with that using the 11B11 ascites (○). Neither ascites had any stimulatory or inhibitory effect in the assays when added in the absence of PK 7.1 SN (data not shown). In the absence of PK 7.1 SN, the background values were IgG1 (BCDF-γ), 232 ± 88 ng/ml; IgM (BCDF-μl), 45 ± 6 ng/ml; [^{3}H]thymidine (BSF-1), 4,088 ± 135 cpm/10^5 cells. This is a representative experiment out of four performed.

prepared in nude mice, was tested for its ability to inhibit the activity of PK 7.1 SN in the BSF-1 and BCDF-γ assays. Both functions were fully inhibited by 10 μl of a 1:1000 dilution of 11B11 ascitic fluid but not by a control ascitic fluid containing a monoclonal anti-DNP antibody (50C1) (Fig. 4). In contrast, the BCDF-μ activity of PK 7.1 SN was not inhibited by 11B11 ascitic fluid.

Discussion

The observations that the induction of IgG1 secretion and inhibition of IgG3 secretion in LPS-stimulated B cells are functions of BSF-1 indicate a need to reexamine the mechanism of action of this lymphokine. Recent evidence (7, 8)

has established that BSF-1 is a potent activation factor for resting B cells. In contrast, BSF-1 has little or no capacity to sustain proliferation of B cell blasts after treatment with anti-Ig (9) or anti-μ plus BSF-1 (20).

The present findings, taken together with results of past studies, suggest that BSF-1 is a pleiotropic activation factor. Thus, it appears to have differential effects on resting B cells and on a subset of LPS-stimulated B cells. These divergent effects could result from different biochemical events initiated by the lymphokine. Alternatively, a similar biochemical event induced by BSF-1 could lead to distinct pathways of gene activation or suppression that depend on the state of differentiation of the target cell. To explore these alternatives, it will be important to determine whether BSF-1 regulates the expression of other classes of immunoglobulin in activated B cells and whether the biochemical events induced by BSF-1 in resting and activated B cells are the same or different.

Summary

By three criteria, we have demonstrated that B cell stimulatory factor (BSF-1) and B cell differentiation factor (BCDF-γ) are the same lymphokine. Highly purified preparations of high performance liquid chromatography–purified or affinity-purified BSF-1 had BCDF-γ activity but not BCDF-μ activity. A monoclonal anti-BSF-1 antibody coupled to Sepharose depleted both BSF-1 and BCDF-γ activity but not BCDF-μ activity from two different T cell supernatants. Soluble monoclonal anti-BSF-1 blocked the BSF-1 and BCDF-γ but not the BCDF-μ responses. These results suggest that BSF-1 acts on both resting and activated B cells to induce different effects.

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References

1. Dutton, R. W., P. Campbell, E. Chan, J. Hirst, H. Hoffman, J. Kettman, J. Lesley, M. McCarthy, R. I. Mishell, D. J. Raidt, and D. Vann. 1971. *In* Cellular Interactions in the Immune Response. S. Cohen, G. Cudknowicz, and R. T. McCluskey, editors. S. Karger AG, Basel, Switzerland, 31–46.
2. Andersson, J., M. H. Schreier, and F. Melchers. 1980. T cell-dependent B cell stimulation is H-2 restricted and antigen dependent only at the resting B cell level. *Proc. Natl. Acad. Sci. USA.* 77:1612.
3. Asano, Y., A. Singer, and R. J. Hodes. 1981. Role of the major histocompatibility complex in T cell activation of B cell subpopulations. Major histocompatibility complex-restricted and unrestricted B cell responses are mediated by distinct B cell subpopulations. *J. Exp. Med.* 154:1100.
4. Julius, M. H., J. M. Chiller, and C. L. Sidman. 1982. Major histocompatibility complex-restricted cellular interactions determining B cell activation. *Eur. J. Immunol.* 12:627.
5. Howard, M., J. Farrar, B. Hilfiker, K. Johnson, K. Takatsu, T. Hamaoka, and W. E.

Paul. 1982. Identification of a T cell-derived B cell growth factor distinct from interleukin-2. *J. Exp. Med.* 155:914.

6. Noelle, R. J., E. C. Snow, J. W. Uhr, and E. S. Vitetta. 1983. Activation of antigen-specific B cells: role of T cells, cytokines, and antigen in induction of growth and differentiation. *Proc. Natl. Acad. Sci. USA.* 80:6628.
7. Noelle, R., P. H. Krammer, J. Ohara, J. W. Uhr, and E. S. Vitetta. 1984. Increased expression of Ia antigens on resting B cells: a new role for B cell growth factor. *Proc. Natl. Acad. Sci. USA.* 81:6149.
8. Roehm, N. W., H. J. Liebson, A. Zlotnik, J. Kappler, P. Marrack, and J. C. Cambier. 1984. Interleukin-induced increase in Ia expression by normal mouse B cells. *J. Exp. Med.* 160:679.
9. Oliver, K., R. J. Noelle, J. W. Uhr, P. H. Krammer, and E. S. Vitetta. 1985. B cell growth factor (B cell growth factor I or B cell-stimulating factor, provisional 1) is a differentiation factor for resting B cells and may not induce cell growth. *Proc. Natl. Acad. Sci. USA.* 82:2465.
10. Vitetta, E. S., K. Brooks, Y.-W. Chen, P. Isakson, S. Jones, J. Layton, G. C. Mishra, E. Pure, E. Weiss, C. Word, D. Yuan, P. Tucker, J. W. Uhr, and P. H. Krammer. 1984. T cell-derived lymphokines that induce IgM and IgG secretion in activated murine B cells. *Immunol. Rev.* 78:137.
11. Sideras, P., S. Bergstedt-Lindqvist, and E. Severinson. 1985. Partial biochemical characterization of IgG_1-inducing factor. *Eur. J. Immunol.* 15:593.
12. Farrar, J. J., M. Howard, J. Fuller-Farrar, and W. E. Paul. 1983. Biochemical and physiochemical characterization of mouse B cell growth factor: a lymphokine distinct from interleukin-2. *J. Immunol.* 131:1838.
13. Ohara, J., I. Lahet, J. Inman, and W. E. Paul. 1985. Partial purification of murine BSF-1. *J. Immunol.* In press.
14. Ohara, J., and W. E. Paul. 1985. Production of a monoclonal antibody to and molecular characterization of B cell stimulation factor-1. *Nature* (*Lond.*). 315:333.
15. Kung, J. T., S. O. Sharrow, D. G. Sieckmann, R. Lieberman, and W. E. Paul. 1981. A mouse IgM allotypic determinant (IgH-6.5) recognized by a monoclonal rat antibody. *J. Immunol.* 127:873.
16. Layton, J. E., P. H. Krammer, T. Hamaoka, J. W. Uhr, and E. S. Vitetta. 1985. Small and large B cell subsets respond differently to T cell-derived B cell growth and differentiation factors. *J. Mol. Cell. Immunol.* In press.
17. Brooks, K., D. Yuan, J. W. Uhr, P. H. Krammer, and E. S. Vitetta. 1983. Lymphokine-induced IgM secretion by clones of neoplastic B cells. *Nature* 302:825.
18. Krammer, P. H., M. Dy, L. Hultner, P. Isakson, U. Kees, M. L. Lohmann-Mathes, F. Marcucci, A. Michnay, E. Pure, A. Schimpl, F. Staber, E. S. Vitetta, and M. Waller. 1982. Production of lymphokines by murine T cells grown in limiting dilution and long-term cultures. *In* Isolation, Characterization and Utilization of T Lymphocyte Clones. F. Fitch, G. Fathman, editors, Academic Press, Inc., New York. 253–262.
19. Layton, J. E., E. S. Vitetta, J. W. Uhr, and P. H. Krammer. 1984. Clonal analysis of B cells induced to secrete IgG by T cell-derived lymphokine(s). *J. Exp. Med.* 160:1850.
20. 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.