

B CELL-STIMULATORY FACTOR 1 (BSF-1) PROMOTES GROWTH OF HELPER T CELL LINES

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The activation, growth, and differentiation of B cells are modulated, in part, by T cell-derived lymphokines. One such lymphokine, B cell-stimulatory factor 1¹ (BSF-1) acts during several different phases of B cell activation and differentiation. Howard et al. (1) initially described BSF-1 as a lymphokine that synergized with anti-Ig in a B cell proliferation assay. It was later reported that BSF-1 induced small, resting B cells to proliferate in response to anti-Ig (2, 3), and increased the expression of class II MHC molecules (Ia antigens) on resting B cells (4, 5). BSF-1 also induces LPS-stimulated B cells to secrete IgG1 (6) and IgE (7).

Purified BSF-1 can be distinguished from IL-2 by its physicochemical properties (8–10). We purified BSF-1 from the supernatant (SN) of the Con A-stimulated alloreactive T cell line, PK 7.1 (11), by the procedures of Ohara et al. (10), and noted that the fractions containing BSF-1 also induced the proliferation of the IL-2-dependent helper type T cell line, HT-2. In this study, we show that this proliferation is caused by BSF-1, and not by contaminating IL-2. Furthermore, not all T cells or T cell lines are equally susceptible to the growth-promoting activity of BSF-1, since it appears to induce growth primarily of Th that induce activation and differentiation of B cells.

Materials and Methods

Animals. Female (C57BL/6 × DBA/2)F₁ (BDF₁) mice, 8–10 wk of age, were purchased from The Jackson Laboratory, Bar Harbor, ME. All animals were maintained in laminar flow hoods.

Cell Lines. The IL-2-dependent cell line, HT-2 (12), was the generous gift of Drs. Kappler and Marrack (National Jewish Hospital, Denver, CO), and was maintained in a 1:1 mixture of murine spleen cell ConA SN and RPMI-1640 medium containing 25 mM Hepes (Gibco Laboratories, Grand Island, NY), 10% heat-inactivated FCS (Hazleton

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¹ *Abbreviations used in this paper:* BCDF, B cell differentiation factor; BSF-1, B cell-stimulatory factor 1; BSS, balanced salt solution; DxS, dextran sulfate; GAMIg, goat anti-mouse Ig; GM-CSF, granulocyte/macrophage colony-stimulating factor; MLC, mixed lymphocyte culture; RAMIg, rabbit anti-mouse immunoglobulin; S, Sepharose; SN, supernatant; Ta, alloreactive T cells; Tc, cytotoxic T cells; TFA, trifluoroacetic acid; TMS-CpG, trimethylsilyl-coated controlled pore glass beads.

Dutchland, Denver, PA), penicillin (100 U/ml), streptomycin (100 $\mu\text{g}/\text{ml}$), gentamycin (10 $\mu\text{g}/\text{ml}$), L-glutamine (2 mM), 2-ME (5×10^{-5} M), and α -methyl mannoside (60 mM). Another IL-2-dependent T cell line, CTLL-2 (13), was obtained from the American Type Culture Collection (Rockville, MD) and was maintained in a 1:1 mixture of murine spleen cell Con A SN and RPMI 1640 containing 25 mM HEPES and 5% heat-inactivated FCS, penicillin (100 U/ml), streptomycin (100 $\mu\text{g}/\text{ml}$), gentamycin (10 $\mu\text{g}/\text{ml}$), L-glutamine (2 mM), sodium pyruvate (2 mM) and α -methyl-mannoside (60 mM). Six other T cell lines were maintained as described and included the alloreactive T cell line, PK 7.1-E10 (11); two long-term murine T cell clones of common origin, clone 96 and clone 29 (14), which are used as indicator cells in an IL-2 assay and one of which (clone 96) has been shown to possess cytotoxic activity against P815 mastocytoma cells (15), and the CBA anti-A.AL cytotoxic T cell lines L.13.D.4A, L.13.D.8, and L.13.D.17 (16). Th lines specific for KLH, BK-2, 8-5 (17), and A-12 were maintained by a modification of the method described by Kimoto and Fathman (18), by 7-d antigenic stimulation and rest cycles in cultures containing irradiated syngeneic spleen cells and murine Con A SN.

Reagents and mAbs. Affinity-purified anti-BSF-1 mAb 11B11 (19) and its isotype-matched control, 50C1 (anti-DNP), were kindly provided by Drs. Ohara and Paul (National Institutes of Health, Bethesda, MD). The anti-IL-2 mAb, DMS-1, which reacts with human and murine IL-2, was purchased from Genzyme (Boston, MA). Ascites fluid containing the anti-murine IL-2-R mAb, AMT-13, and its isotype-matched control, 23/7, were prepared as described previously (20). The AMT-13 ascites contained 0.5 mg/ml of antibody. rIL-2 was obtained from AmGen Biologicals (Thousand Oaks, CA).

Preparation of IL-2 and BSF-1-containing SN. SN from PMA-stimulated EL-4 thymoma cells was prepared as described previously (21). Such SN contained high levels of IL-2 activity and negligible levels of BSF-1 activity.

SN from Con A-pulsed PK 7.1 cells were prepared as described previously (11). Such SNs contained greater than 10^3 U/ml of BSF-1 activity and were devoid of IL-2 activity.

BSF-1 Assay. BSF-1 activity was measured by a modification (22, 23) of the previously described anti-IgM costimulation assay (1). 5×10^4 small (1.083–1.222 g/ml), Percoll-fractionated B cells (23) were cultured for 3 d in a volume of 200 μl containing 10 μl of a 10% suspension of goat anti-mouse Ig coupled to Sepharose (S-GAMIG) (22, 24) and SN or purified lymphokines (20 μl). The cells were then pulsed with 1 $\mu\text{Ci}/\text{well}$ of [^3H]thymidine and harvested 16 h later. 1 U of BSF-1 was designated as the reciprocal of the dilution containing 50% of the maximal activity of a standard BSF-1-containing PK 7.1 SN.

IL-2 Assay. IL-2 activity was assayed by measuring the [^3H]thymidine uptake of IL-2-dependent HT-2 (12) or CTLL-2 (13) cell lines in response to IL-2- or to BSF-1-containing SN. One HT-2 line has been maintained in our laboratory for 3 yr and the other was recently obtained from Dr. Robert Coffman (DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA). For the assay, 20- μl aliquots/well of a 2.5×10^5 cells/ml suspension were added to 70 μl /well of different dilutions of the BSF-1 or IL-2-containing SN. Cells were pulsed with 1 $\mu\text{Ci}/\text{well}$ of [^3H]thymidine after 48 h of incubation at 37°C/5% CO₂ in air, and 16 h later the cells were harvested.

IL-2 Assay on T Cell Blasts. Murine Con A T cell blasts were prepared as described by Malek et al. (25). For the assay, the cells were cultured at a density of 2.5×10^5 cells/ml in 200 μl /well of assay medium containing various dilutions of the IL-2- or BSF-1-containing SN or the purified lymphokines. After 48 h the cells were pulsed with 1 $\mu\text{Ci}/\text{well}$ of [^3H]thymidine, and 16 h later the cells were harvested.

B Cell Growth Factor (BCGF) II Assay. BCGF-II activity was assayed by measuring the ability of the SN or purified lymphokines to synergize with dextran sulfate (DxS) in a B cell proliferation assay (26). B cells were not subjected to a Percoll density gradient fractionation before culture, since BCGF-II acts on low density B cells (23). Test SN were added on day 0, on day 3 cells were pulsed with [^3H]thymidine, and were harvested 16 h later.

BCDF for IgG1 (BCDF- γ) Assay. BCDF- γ activity was measured as described previously (6, 27), using B cells prepared as described for the BCGF-II assay. 20 $\mu\text{g}/\text{ml}$ LPS was

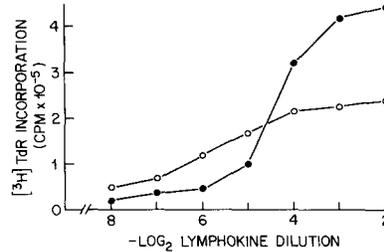


FIGURE 1. [³H]thymidine incorporation of HT-2 cells cultured in the presence of serial two-fold dilutions of IL-2-containing EL-4 SN (●) and the BSF-1-containing PK 7.1 SN (○). SNs were prepared, and [³H]thymidine incorporation assays were performed as described in Materials and Methods.

added at the initiation of culture and BSF-1 was added a day later. On day 6, the culture SN were assayed for IgG1 by a RIA.

BCDF for IgM (BCDF- μ) Assay. SN or purified lymphokines were tested for BCDF- μ activity by their ability to stimulate the secretion of IgM from the in vitro-adapted BCL₁ cell line, 3B3 (28). T cell SN were added on day 0, and on day 6 the culture SNs were assayed for IgM by a μ -specific RIA.

Gel Filtration. Gel filtration of the different SN was carried out at 4°C using a 2.5 × 50 cm Sephacryl S-200 column (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with Tris-buffered saline (50 mM, pH 7.4) containing 0.1% sodium azide. After chromatography, BSA was added to the different fractions at a final concentration of 1 mg/ml, and the fractions were dialyzed against 3 changes of PBS (3 × 1 liter) and once against RPMI-1640 (1 × 1 liter).

Purification of IL-2 and BSF-1. The initial step in the partial purification of IL-2 or BSF-1 was carried out as described (10, 29) by adsorption to trimethylsilyl-coated TMS-CpG and subsequent elution by a 50% acetonitrile/0.2 M NaCl/0.1% TFA solution. This procedure resulted in the adsorption of >95% of the BSF-1 or IL-2 activities from the SN with minimal contamination by BCGF-II and BCDF- μ activities. Reverse-phase HPLC was carried out as described previously (10), in a model 332 gradient liquid chromatograph (Beckman Instruments, Inc., Fullerton, CA) and an Altex Ultrasphere ODS (250 × 4.6 mm, 5 μ m) reverse-phase column (Altex Scientific, Berkeley, CA).

Results

HT-2 Growth-promoting Activity of PK 7.1 SN. The HT-2 cell line was derived from T cells that provide IA^d-restricted help to B cells for an anti-SRBC response. The requirement of HT-2 cells for the growth-promoting activity of IL-2 is the basis for the IL-2 assay used by many laboratories (12). However, when HT-2 cells were cultured with T cell SN lacking IL-2, namely, PK 7.1 (11), the HT-2 cells proliferated, as judged by both [³H]thymidine incorporation and by an increase in cell number. As shown in Fig. 1, the stimulation indexes obtained at maximal stimulatory concentrations of BSF-1-containing PK 7.1 SN were about 50% as high as those obtained with maximal stimulatory concentrations of the IL-2-containing EL-4 SN. Proliferation was observed at 24, 48, and 72 h using the [³H]thymidine incorporation assay, and was not affected by the inclusion of α -methyl-mannoside (20 mg/ml) in the medium (data not shown), suggesting that the proliferative activity of SN from Con A-pulsed PK 7.1 cells is not due to contaminating Con A.

Purification of IL-2 and BSF-1. When subjected to gel filtration on Sephacryl S-200 columns, the HT-2 growth-promoting activity of EL-4 cell SNs eluted with

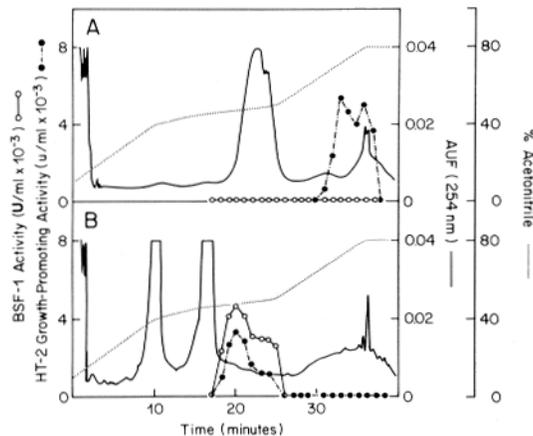


FIGURE 2. HPLC elution profiles of the HT-2 growth-promoting (●) and BSF-1 (○) activities from EL-4 (A) and PK 7.1 (B) cell SNs. SNs were adsorbed with TMS-CpG (Sepalyte, 40 μ m) and the IL-2 or BSF-1 activities eluted from the beads with 50% acetonitrile in 0.2 M NaCl/0.1% TFA. The eluates were lyophilized, reconstituted with distilled water and applied to a reverse-phase Altex Ultrasphere ODS column.

an apparent M_r of 30,000, in concordance with a previous report (30). The HT-2 growth-promoting activity of PK 7.1 SN, however, coeluted with BSF-1 and had an apparent M_r of 18,000. No IL-2 activity was detected in the PK 7.1 SN and no BSF-1 activity was detected in the EL-4 SN.²

To characterize further the HT-2 growth-promoting activity present in PK 7.1 SN, both HT-2 growth-promoting activity and BSF-1 activity were purified from PK 7.1 SN by TMS-CpG adsorption and reverse-phase HPLC, as described (10). IL-2 was purified from EL-4 SN by the same procedure. The beads adsorbed the HT-2 growth-promoting activities from both SN and the anti-Ig-mediated costimulator and BCDF- γ activities from the PK 7.1 SN. The 50% acetonitrile eluates that contained all three activities were then subjected to reverse-phase HPLC, as described in Materials and Methods. As shown in Fig. 2A, the IL-2 activity from the EL-4 SN eluted at 60–80% acetonitrile. No BSF-1 activity was detected in the original EL-4 SNs, nor in any of the HPLC fractions. Using a system similar to the one described here, the BSF-1 activity has been reported to elute at acetonitrile concentrations of 47–49% (10, 31). As shown in Fig. 2B, both the BSF-1 and the HT-2 growth-promoting activities present in the PK 7.1 SN coeluted at 47–50% acetonitrile. No HT-2 growth-promoting activity was detected in those fractions in which IL-2 should have eluted (60–80% acetonitrile). The fractions containing the BSF-1 activity were, as expected, also positive for BCDF- γ activity, according to previous reports by our group (6) and other (32, 33) that BSF-1 and BCDF- γ are identical. No BCGF-II or BCDF- μ activities were detected in these fractions.

Effect of Anti-BSF-1 mAb, 11B11. The above results indicated that the material containing the HT-2 growth-promoting activity and isolated from the PK 7.1 SN was different in size and polarity from the material containing the IL-2

² Several sublines of EL-4 cells have been generated in our laboratory. After stimulation with PMA, some lines secrete only IL-2 and others secrete both IL-2 and BSF-1.

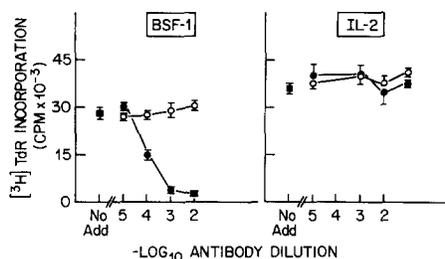


FIGURE 3. Inhibition of HT-2 growth-promoting activity of purified BSF-1 but not purified IL-2 by mAb to BSF-1. Constant amounts of BSF-1 or IL-2 were preincubated for 30 min at room temperature with the indicated dilutions of a 1 mg/ml stock of monoclonal anti-BSF-1, 11B11 (●) or isotype-matched control antibodies, 50C1 (○). HT-2 cells were then added and proliferation was measured. Results represent the mean of triplicate experiments \pm SEM. [^3H]Thymidine incorporation of cells cultured in the absence of added lymphokines was $2,765 \pm 254$ cpm.

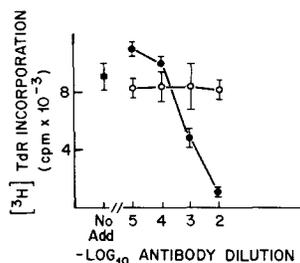


FIGURE 4. Inhibition of anti-Ig costimulator activity of BSF-1 by mAb to BSF-1. The indicated dilution of a 1 mg/ml stock of mAbs 11B11 (●) or 50C1 control (○) were preincubated at room temperature for 60 min with constant amounts of BSF-1. GAMIG-S and aliquots from a small B cell suspension were added, and BSF-1 activity was assayed as described in Materials and Methods. Results represent the mean of triplicate experiments \pm SEM. [^3H]Thymidine incorporation of cells cultured in the absence of added lymphokines was 737 ± 298 cpm.

activity isolated from the EL-4 SN. The former was similar to BSF-1 based on its physicochemical properties. We, therefore, postulated that BSF-1 had IL-2-like growth-promoting activity on HT-2 cells. To test this hypothesis and to disprove the possibility of contamination of BSF-1 by an IL-2 species with physicochemical properties similar to those of BSF-1, we tested the effects of soluble anti-BSF-1 mAbs (11B11), previously reported to inhibit BSF-1 activity (19), on both the BSF-1 and HT-2 growth-promoting activities of the purified BSF-1 and IL-2 preparations from PK 7.1 and EL-4 SN, respectively. As shown in Fig. 3A, the HT-2 growth-promoting activity purified from PK 7.1 SN was readily inhibitable by 11B11 antibody, but not by an isotype-matched anti-DNP antibody (50C1). In contrast, the HT-2 growth-promoting activity of the EL-4-derived IL-2 was not affected by 11B11 (or the control antibody) at any of the antibody concentrations tested (Fig. 3B).

The mAb 11B11 completely blocked the anti-Ig-mediated costimulator activity of the PK 7.1-derived BSF-1 at the same concentrations that inhibited the BSF-1-mediated proliferation of HT-2 cells (Fig. 4). A similar experiment could not be performed with EL-4-derived IL-2 since it did not show any anti-Ig-mediated costimulatory activity on B cells.²

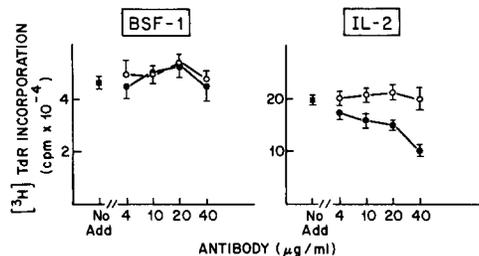


FIGURE 5. Inhibition of IL-2 but not BSF-1-mediated proliferation of HT-2 cells by mAb to IL-2. Several dilutions of monoclonal anti-IL-2, DMS-1 (●), or normal mouse IgG control (○) were preincubated with constant amounts of purified BSF-1 or IL-2 for 60 min at 37°C. Aliquots of a HT-2 cell suspension were added and proliferation measured as described in Materials and Methods. Results represent the mean of triplicate experiments \pm SEM. [3 H]Thymidine incorporation of cells cultured in the absence of added lymphokines was $2,452 \pm 281$ cpm.

Anti-IL-2 mAb Does Not Block the HT-2 Growth-promoting Activity of BSF-1. To obtain additional evidence that the HT-2 growth-promoting activity of purified BSF-1 was not due to contaminating IL-2, and to investigate whether the BSF-1-mediated proliferation of HT-2 cells was due to autocrine production and utilization of IL-2 in response to BSF-1, soluble anti-IL-2 mAb (DMS-1) was added to cultures of HT-2 cells containing purified BSF-1 or rIL-2. The anti-IL-2 mAb failed to inhibit the proliferation caused by BSF-1, whereas the same concentrations of antibody inhibited the rIL-2-mediated proliferation of HT-2 cells by 50–60% (Fig. 5). These results suggest that BSF-1 purified from PK 7.1 SN has both growth-promoting activity on HT-2 cells and anti-Ig-mediated costimulatory activity on resting B cells, and that neither activity can be accounted for by contaminating or autocrine IL-2.

Anti-IL-2-R mAb Blocks the Growth-promoting Activity of IL-2 but not BSF-1 on HT-2 Cells. Although the above results indicate that the HT-2 growth-promoting activity of BSF-1 was not due to IL-2 contamination or mediated by autocrine IL-2 production, the proliferative signal given by BSF-1 might have resulted from interaction with IL-2-R. To test this possibility, the anti-IL-2-R antibody, AMT-13, previously shown to inhibit IL-2 binding and IL-2-mediated proliferation in activated murine T cells (20), was added to cultures of HT-2 cells stimulated with rIL-2 or BSF-1. AMT-13 antibodies blocked (by $\sim 70\%$) the proliferation caused by rIL-2 but did not affect the proliferation mediated by BSF-1 (Fig. 6). These results suggest that BSF-1 and IL-2 do not bind to the same receptor, or at least the same epitope on the IL-2-R on HT-2 cells, even though both induce their growth. The nature of the BSF-1 receptor on HT-2 cells is not known.

Morphology of HT-2 Cells Cultured with BSF-1 or rIL-2. When HT-2 cells were cultured with purified BSF-1, they showed striking changes in morphology (Fig. 7A) when compared with HT-2 cells cultured with IL-2 (Fig. 7B). While the cells cultured with IL-2 usually maintained a rounded shape, grew in suspension, and formed small clusters, the cells cultured in BSF-1 acquired a flattened appearance, produced numerous dendritic-like cytoplasmic projections, and attached to the surface of the culture flask. The percentage of cells undergoing

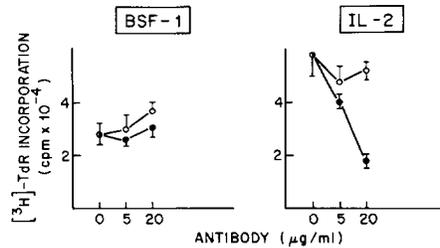


FIGURE 6. Inhibition of IL-2 but not BSF-1-mediated proliferation of HT-2 cells by mAb to the murine IL-2-R (AMT-13). The indicated amounts of ascites fluid containing the mAbs AMT-13 (●) or 23/7 control (○) were preincubated for 60 min at 37°C with equal volumes of a 2.5×10^5 cells/ml suspension of HT-2 cells. The mixtures were then added to constant amounts of BSF-1 or IL-2 and proliferation was measured as described in Materials and Methods. Results represent the mean of triplicate experiments \pm SEM. [^3H]Thymidine incorporation of cells cultured in the absence of added lymphokines was $6,952 \pm 1,968$ cpm.

such morphological changes in the BSF-1-containing cultures was dependent upon the concentration and time of exposure to BSF-1. Morphologic alterations were evident as early as 12 h after exposure, although they did not reach a maximum until 48 h. Compared with the proliferative activity of BSF-1, higher concentrations of BSF-1 were required to induce morphologic changes in the HT-2 cells, since lower concentrations of BSF-1 that promoted proliferation failed to induce significant morphological alterations.

BSF-1 has Growth-promoting Activity on Other Subsets of T Cells. The growth-promoting activity of BSF-1 on HT-2 cells prompted us to investigate its effects on a variety of T cell lines and blasts. The population of T cells to be tested was incubated with the purified BSF-1 or IL-2 preparations for 24–48 h, and then was assayed for [^3H]thymidine incorporation. T cell lines included: (a) a second HT-2 line obtained from DNAX; (b) the cytotoxic, IL-2-dependent T cell line, CTLL-2; (c) the long-term alloreactive T cell clone, PK 7.1-E10 (11); (d) a long-term murine T cell line (with cytotoxic activity to P815 mastocytoma cells [14, 15]), clone 96, and another long-term T cell line of similar origin, clone 29 (14); (e) three CTL lines (16); (f) three KLH-specific Th lines (reference 17 and unpublished observations). The antigen-specific Th all induced IgM secretion in B cells when cultured with TNP-KLH (data not shown); and (g) Con A-activated spleen and peripheral T cell blasts (Fig. 8). As shown in Fig. 8, the IL-2 preparation supported the growth of all the T cell lines tested. In contrast, the BSF-1 preparation supported the growth of the Th populations (C–E) and both HT-2 cell lines (A–B), but induced only a modest proliferation of Con A T cell blasts (M), and no significant proliferation of the other T cell lines, including cytotoxic, alloreactive, and long-term T cell lines (Fig. 8 F–L). These results suggest that BSF-1 may act as a growth factor for only some T cell subsets. Based on the T cells tested to date, it appears that BSF-1 acts primarily on T cells that provide help to B cells. It will be necessary to test the effect of BSF-1 on additional T cell lines and clones to determine if additional subsets of cells are responsive.

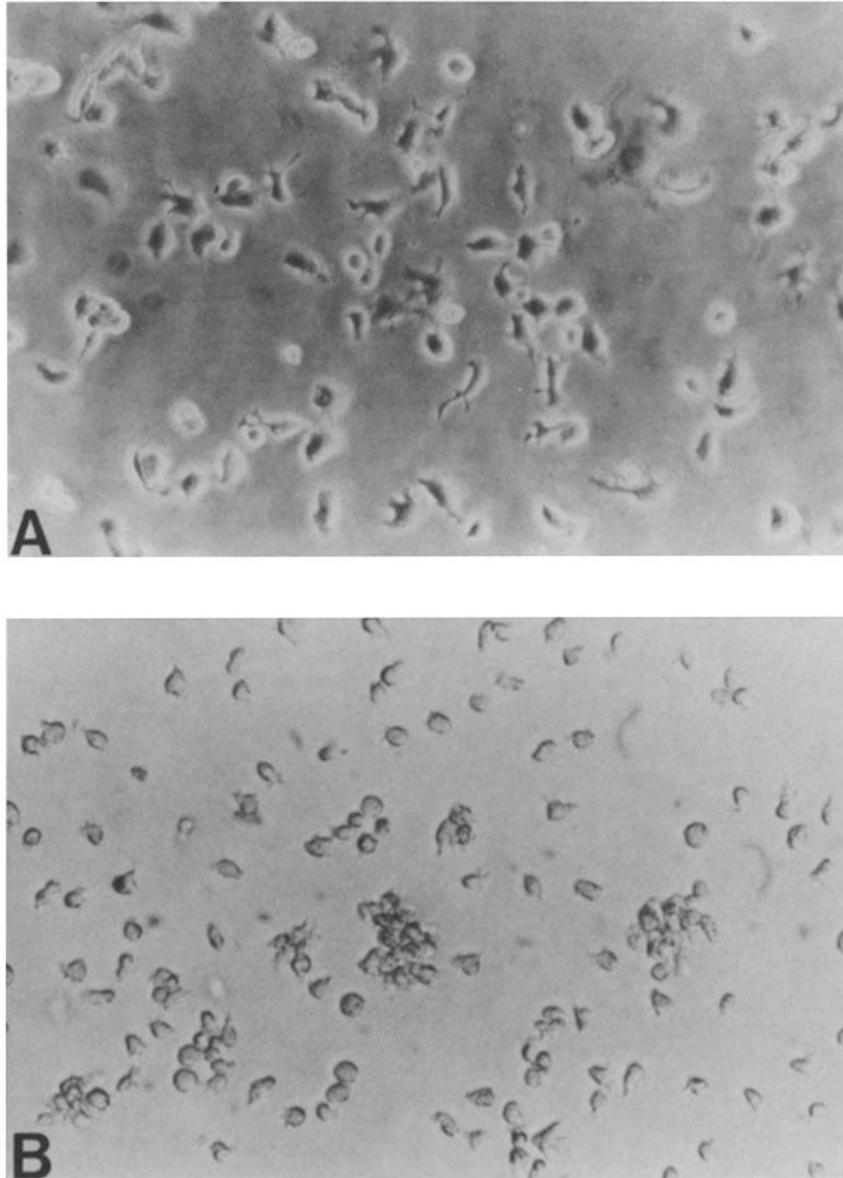


FIGURE 7. Morphology of HT-2 cells grown in the presence of BSF-1 (A) or IL-2 (B). HT-2 cells were grown in 25-cm² tissue culture flasks in RPMI-1640 10% FBS medium with purified BSF-1 or IL-2. Microphotographs were taken after 48 h of culture using an inverted phase contrast microscope.

Discussion

The major finding to emerge from these studies is that BSF-1, in addition to its known effects on B cells, also induces the proliferation of some subsets of T cells such as HT-2 cells and antigen-specific Th (that provide help to B cells). Since IL-2 plays a central role in the proliferation of T cells (reviewed in

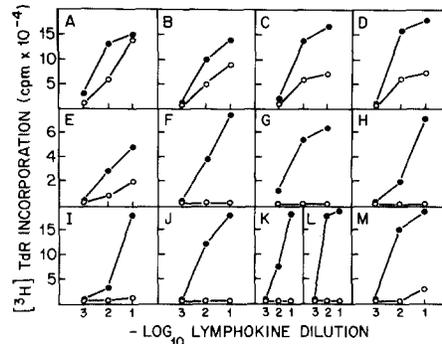


FIGURE 8. Thymidine incorporation of different T cell lines and blasts in response to IL-2 (●) or BSF-1 (○). (A) HT-2 (maintained in our laboratory); (B) HT-2 (provided by Dr. R. Coffman, DNAX); (C) KLH-specific Th, 8-5; (D) KLH-specific Th line, A-12; (E) KLH-specific Th line BK-2; (F) the alloreactive T cell line, PK 7.1-E10; (G) the long-term murine T cell clone with cytotoxic activity to P815 mastocytoma cells, clone 96; and (H) its related clone 29; and the cytotoxic T cell lines: (I) CTLL-2; (J) L.13.D.4A; (K) L.13.D.8; (L) L.73.D.17; and (M) Con A-stimulated peripheral T cell blasts.

references 34 and 35), it was essential to show that the growth-promoting activity of BSF-1 was not due to the presence of contaminating IL-2. To this end, it was shown that (a) PK 7.1 SNs, which lack IL-2 activity, support the proliferation of HT-2 cells ~50% as well as IL-2-containing EL-4 SNs; (b) The HT-2 growth-promoting activity present in PK 7.1 SNs copurifies with BSF-1 by both gel filtration and reverse-phase HPLC; (c) The proliferation of HT-2 cells in response to purified BSF-1, but not to IL-2, is inhibited by anti-BSF-1 mAbs. The same antibodies block the anti-Ig-mediated costimulatory activity of BSF-1; (d) anti-IL-2 mAbs block the HT-2 growth-promoting activity of IL-2 but not BSF-1; (e) anti-IL-2R mAbs block the HT-2 growth-promoting activity of IL-2 but not BSF-1; and (f) BSF-1 preparations that support the growth of HT-2 cells do not induce significant proliferation of other IL-2-dependent T cell lines, with the exception of antigen-specific Th lines. In contrast, IL-2 supports the growth of all the T cell lines and normal T cell blasts tested.

We have chosen the PK 7.1 SN as a source of BSF-1 because of its paucity of IL-2 activity and its high levels of BSF-1 activity (11). Other T cell lines or clones that fail to secrete IL-2 but secrete BSF-1 and/or other lymphokines that act on B cells have been described (36-39). In the last year, there have been several reports describing the partial purification of BSF-1 (10, 31, 32), and the cloning and expression of the BSF-1 cDNA (33). In these reports, no T cell growth-promoting activity associated with BSF-1 was described. One possible explanation is that two groups (31, 32) used cytotoxic, IL-2-dependent T cell lines (CSP 2.1 and CTLL-2, respectively) as indicator cells in their IL-2 assays. All cytotoxic or alloreactive T cell lines tested to date in our system have also failed to proliferate significantly in response to BSF-1. Paradoxically, however, Ohara et al. (10) used HT-2 cells in their IL-2 assay and could not detect any HT-2 growth-promoting activity associated with their purified BSF-1. This observation could be explained by the fact that they used cloned IL-2-dependent T cells from the HT-2 line. During the cloning process, the cells might have lost responsiveness to BSF-1, while retaining responsiveness to IL-2. Another possible explanation is that the

HT-2 growth-promoting activity associated with PK 7.1-derived BSF-1 is related to its cell source. However, this possibility appears unlikely since we have recently purified BSF-1 from BSF-1-containing SN of EL-4 cells, and found that it behaves identically to that isolated from PK 7.1 SNs (unpublished observations).

If BSF-1 acts primarily on Th (L3T4⁺, Lyt-2⁻), then the observation that BSF-1 induced only modest proliferation of Con A-activated T cells could be explained by the fact that Con A induces the preferential proliferation of Lyt-2⁺ cells (40). In support of this possibility is the finding that immunofluorescence staining of Con A-activated lymph node blasts revealed that <10% of the cells were L3T4⁺ (data not shown).

Presumably, the biological effects of BSF-1 on T cells are mediated by its binding to specific receptors on the surface of activated T cells analogous to other hormones (35). However, BSF-1 receptors have not yet been characterized. Recent results from our laboratory (41) have shown that antibodies directed against the α chain of LFA-1 mimic the biological effects of BSF-1 on B cells. However, it is not yet known whether LFA-1 is the receptor for BSF-1, or another molecule that can signal resting B cells in a similar manner. It is of interest that immunofluorescence staining of HT-2 cells with anti-LFA-1 antibodies has revealed that >99% of the cells express LFA-1.

BSF-1, in contrast to IL-2, induced striking morphologic alterations of HT-2 cells, namely, increased adherence to plastic and the appearance of elongated cytoplasmic projections. These phenotypic changes may depend on cytoskeletal alterations. Such alterations may play a role in facilitating T-B cell interaction and the delivery of T cell help to B cells.

The capacity of BSF-1 to induce proliferation of Th, but not of the cytotoxic/alloreactive type (Tc/Ta), is provocative. However, it should be emphasized that the negative results using clones of Tc and Ta could be due to their production and use of BSF-1, thereby abrogating their requirement for exogenous BSF-1. A possible physiological role for BSF-1 is that it acts on both Th and B cells during Th-B cell interaction. Noelle et al. (4) and Roehm et al. (5) have reported that BSF-1 increases the levels of class II MHC molecules (Ia antigens) on the surface of resting B cells. This increase in expression of Ia antigens may enhance the recognition of processed antigen and Ia by the Th (2). A proliferative signal mediated by BSF-1 to specific Th could enhance proliferation of the specific Th in the T-B cluster. Such Th cells might subsequently form new conjugates with additional specific B cells not yet stimulated.

It is not yet known whether the Th subset(s) that secretes BSF-1 also bears receptors for it, or whether different subsets secrete and bind the ligand or both. In this regard, Mosmann et al. (39) have recently shown the existence of two different types of murine Th clones based on their patterns of lymphokine secretion. Type 1 Th cells produce IL-2, IFN- γ , GM-CSF, and IL-3, whereas Type 2 Th cells produce BSF-1, a mast cell growth factor distinct from IL-3, and a T cell growth factor distinct from IL-2. The effect of BSF-1 on the proliferation of such clones was not investigated. Nevertheless, the presence of a T cell growth factor activity, different from IL-2 in the SNs of those clones also producing BSF-1 (assayed by proliferation of HT-2 cells), suggests that this T cell growth factor activity might be due to BSF-1.

Arthur and Mason (42) have also recently reported that inducer/helper T cells in the rat can be separated into two functional subsets based on reactivity with an mAb (MRC OX-22) that recognizes high molecular weight forms of the rat leukocyte-common antigen. One subset (OX-22⁺) proliferates well in mixed lymphocyte culture (MLC), responds to Con A, and produces high levels of IL-2 after stimulation; this subset would be expected to play a role in cell-mediated immunity. The other subset (OX-22⁻) that proliferates poorly in response to MLC and Con A, produces low levels of IL-2 but provides effective help for B cell responses; this subset presumably plays an important role in the induction of humoral responses. Based on these data, the rat CD4⁺, OX-22⁻ subset would be expected to produce (and also respond to) a lymphokine analogous to murine BSF-1. The effects of BSF-1 on B cell activation and differentiation coupled to its preferential growth-promoting activity on Th cells could provide the means, as suggested by Arthur and Mason (42), for an independent regulation of cellular and humoral immune responses.

During the past year, the complexity of the biologic activities of BSF-1 has become evident (2-7). Until now, the activity of BSF-1 had been exclusively associated with B cells. This report provides evidence that BSF-1 also acts on some T cell subpopulations.

Summary

T cell-derived supernatants (SN) that contain B cell-stimulatory factor 1 (BSF-1) and lack IL-2 promote the growth of the IL-2-dependent T cell line, HT-2, as well as three other clones or lines of T cells that can provide help to B cells. The BSF-1 purified from these SNs promotes growth of HT-2 cells ~50% as effectively as purified IL-2. A potential involvement for contaminating IL-2 in the BSF-1 preparations was excluded by the demonstration that anti-BSF-1 mAbs blocked the BSF-1-induced growth of HT-2 cells; in contrast, these antibodies did not block the IL-2-induced proliferation of the HT-2 cells. In addition, anti-IL-2 mAbs or anti-IL-2-R antibodies blocked the HT-2 growth-promoting activity of purified IL-2, but not BSF-1. Finally, BSF-1 promoted only a very modest growth of Con A-induced T cell blasts, and failed to induce significant growth in seven other cytotoxic, alloreactive, and long-term T cell lines. Taken together, these results indicate that in addition to its known effects on resting and LPS-stimulated B cells, BSF-1 can promote growth of certain subsets of activated T cells, in particular, those that provide help to B cells.

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Note added in proof: Lee et al. (43) have recently generated a murine cDNA clone for BSF-1. Monkey COS cells transfected with this cDNA produce BSF-1. This BSF-1 has Ia-inducing activity, anti-Ig costimulatory activity, BCDF- γ /BCDF- ϵ activity, mast cell stimulatory activity, and HT-2 growth promoting activity. Our results are in agreement with those of Lee et al.

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