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Immunology

Characterization of the high-affinity cell-surface receptor for murine B-cell-stimulating factor 1

(lymphokines/polypeptide hormone/B-cell growth factor/interleukin 4)

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ABSTRACT **Radiolabeled recombinant murine B-cell-stimulatory factor 1 (BSF-1) was used to characterize receptors specific for this lymphokine on the surface of primary B and T cells and *in vitro* cell lines representing the B-cell, T-cell, mast cell, macrophage, and myelomonocytic lineages. BSF-1 binding was rapid and saturable at 4°C and 37°C with a slow dissociation rate. On all cell types examined, BSF-1 bound to a single class of high-affinity receptor (<2000 receptors per cell) with a K_a of 10^{10}–10^{11} M^{-1}. Receptor expression on resting primary B and T cells was low (<100 receptors per cell), whereas activation with lipopolysaccharide or Con A produced a 5- to 10-fold increase in receptor numbers. Among a panel of lymphokines and growth hormones, only unlabeled BSF-1 was able to compete for the binding of ^{125}I-labeled BSF-1. Affinity crosslinking experiments resulted in the identification on all cells tested of a receptor protein with an average M_r of 75,000.**

B-cell-stimulating factor 1 (BSF-1), also known as interleukin 4 (IL-4; ref. 1) and B-cell growth factor (2), was originally defined by its ability to facilitate the proliferative response of B cells to low concentrations of antibodies directed to surface immunoglobulin (3). More recently, purified BSF-1 has been found to stimulate the proliferation of several IL-2- and IL-3-dependent cell lines (4–6), to induce the expression of class II major histocompatibility complex molecules on resting B cells (7, 8), and to enhance the secretion of IgE and IgG1 by stimulated B cells (9, 10). These results suggest that BSF-1 is a growth factor and a differentiation factor and that it has effects on cell lineages (T-cell and hematopoietic) outside the B-lymphocyte compartment.

With the recent cloning and expression of the gene encoding murine BSF-1 (1, 5), it has been demonstrated conclusively that these multiple activities are mediated by a single molecule. In addition, the availability of purified recombinant BSF-1 has made it possible to undertake a search for specific receptors for this molecule on the plasma membrane of responding cells. This paper describes the use of recombinant murine BSF-1, radiolabeled with ^{125}I to high specific activity, to study the cellular distribution of the BSF-1 receptor as well as provide kinetic and structural characterization of the receptor. This initial characterization of the receptor for murine BSF-1 will lay the groundwork that will be necessary to fully understand how this single lymphokine, interacting with its receptor on cells of different lineages, may mediate such a diverse array of biological activities.

MATERIALS AND METHODS

Cell Preparations. FDC-P2, an IL-3-dependent murine bone marrow-derived line (11), and CTLL-2, an IL-2-dependent murine T-cell line (12), were maintained as described (4). 32D/IL-3 cells, which were developed from the IL-3-dependent 32Dcl-23 cell line (13), and FD.C/2 cells, which were developed from the IL-3-dependent FDC-P2 cell line, were kindly provided by James Watson (Auckland Medical School, Auckland, New Zealand). In addition to expressing IL-3 receptors, these cell lines have also been induced to express IL-2 receptors by culture in human IL-2 and were maintained as described (14, 15). All other cells listed in Table 1 were grown in RPMI 1640 medium containing 5–10% fetal bovine serum and antibiotics with or without 5 nM 2-mercaptoethanol and 1% sodium pyruvate.

B- and T-cell populations were each purified from spleens of female C57BL/6J mice 8–12 weeks of age (The Jackson Laboratory). B cells were purified as described (4) and were stimulated by culturing in RPMI 1640 medium containing 5% fetal bovine serum, penicillin, streptomycin, glutamine, 0.05 mM 2-mercaptoethanol, and 10 μg of lipopolysaccharide per ml at 2×10^6 cells per ml.

Splenic T cells were purified by using nylon wool as described (16) and were activated by culturing for 2 days in 16-mm wells at 2×10^6 cells per well in the presence of irradiated [3000 rads (1 rad = 0.01 gray)] unfractionated syngeneic spleen cells (2×10^6 cells per well) in RPMI 1640 medium containing 10% fetal bovine serum, 0.05 mM 2-mercaptoethanol, and 2.5 μg of Con A per ml. Activated T cells were harvested and dead cells were removed by discontinuous density gradient centrifugation over metrizamide.

Thymocytes were induced by culturing 5×10^6 cells per ml in RPMI 1640 medium, 10% fetal bovine serum, 0.05 mM 2-mercaptoethanol, and 2.5 μg of Con A per ml for 2 days. Activated thymocytes were harvested and dead cells were removed by discontinuous density gradient centrifugation on metrizamide.

Hormone Preparations. Human recombinant IL-2 was expressed in yeast under the control of the alcohol dehydrogenase 2 promoter, purified from the yeast medium to homogeneity by reversed-phase HPLC according to methods previously described (V. Price and D.L.U., unpublished data, refs. 17–19), and radiolabeled (20). IL-3 (CSF-2α), recombinant murine granulocyte/macrophage colony-stimulating factor (GM-CSF), and recombinant human IL-1α and IL-1β were obtained as described (18, 21, 22). Nerve growth factor, fibroblast growth factor, platelet-derived growth factor, and epidermal growth factor were obtained from Bethesda Research Laboratories. Human follicle-stimulating hormone, human luteinizing hormone, human thyroid-stimulating hormone, human growth hormone, and bovine insulin were obtained from Calbiochem–Behring.

Recombinant Murine BSF-1 Purification and Radiolabeling. Complementary DNA encoding murine BSF-1 was cloned from a cDNA library that had been prepared from sized mRNA isolated from EL-4 thymoma cells previously stimu-

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Abbreviations: BSF-1, B-cell-stimulating factor 1; IL, interleukin; GM-CSF, granulocyte/macrophage colony-stimulating factor.

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lated with phorbol 12-myristate 13-acetate. The BSF-1 cDNA was inserted into a plasmid where its transcription, translation, and secretion were under the control of the ADH2 promoter and α factor leader sequences. As a result, yeast transformed with this plasmid secreted murine BSF-1 into the yeast culture broth. Purification of BSF-1 was achieved by five sequential reversed-phase HPLC steps on C_4 and C_{18} derivatized silica (Vydac, separations group) with gradients of acetonitrile containing 0.1% trifluoroacetic acid or 1-propanol (60% 1-propanol in 0.9 M acetic acid/pyridine to pH 4.5) as described for the purification of other lymphokines (17–19). Following purification, BSF-1 concentrations were determined by fluorescamine analysis (23) of the purified protein with bovine serum albumin as a standard. BSF-1 activity was measured in either a B-cell or an FDC-P2 proliferation assay as described (4). The specific activity of purified recombinant murine BSF-1 was determined to be 2×10^5 units/μg.

Murine recombinant BSF-1 was radiolabeled by using the Enzymobead radioiodination reagent (Bio-Rad) essentially as described for murine recombinant GM-CSF (21). The specific activity of radiolabeled BSF-1 preparations was estimated to be $0.5–2.0 \times 10^{16}$ cpm/mmol based on a M_r of 49,000 and on a recovery of 40% from control experiments in which an aliquot of BSF-1 was spiked with ^{125}I-labeled BSF-1 (^{125}I-BSF-1) and put through the iodination protocol, with omission of ^{125}I. Bioactivity of ^{125}I-BSF-1 was determined in a B-cell proliferation assay (4).

Assay for Binding of ^{125}I-BSF-1 to Intact Cells. Binding assays (24) were performed essentially as described for murine ^{125}I-labeled GM-CSF (21). Sodium azide (0.2%) was included in all binding assays to inhibit internalization and degradation of ^{125}I-BSF-1 by cells at 37°C. Association and dissociation kinetic experiments were conducted as described (21).

Crosslinking to Intact Cells. Crosslinking experiments were performed essentially as described (21). Briefly, cells (4×10^7 per ml) were incubated with ^{125}I-BSF-1 (1.2×10^{-10} M) at 4°C in the presence and absence of a 50-fold or greater molar excess of unlabeled BSF-1 for 2 hr. The cells were then washed and bis(sulfosuccinimidyl)suberate was added to a final concentration of 0.1 mg/ml. After 30 min at 25°C, the cells were washed and resuspended in 100 μl of phosphate-buffered saline (PBS)/1% Triton containing 2 mM phenylmethylsulfonyl fluoride, 10 μM pepstatin, 10 μM leupeptin, 2 mM *o*-phenanthroline, and 2 mM EGTA to prevent proteolysis. Aliquots of the extract supernatants were analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis ($NaDodSO_4$/PAGE) (25).

Data Analysis. Curve fitting of binding and kinetic data was done by using RS/1 (Bolt, Beranek, and Newman, Boston), a commercially available data processing package running on a VAX 11/750 under the VMS operating system, and kinetic data were analyzed as described (21).

RESULTS

Recombinant murine BSF-1 was purified to homogeneity by reversed-phase HPLC and iodinated to high specific activity (in the range of 1×10^{16} cpm/mmol). Such preparations were stable for at least 2 weeks when stored at 4°C in 0.05 M $NaPO_4$ (pH 7.2) containing 0.01% bovine serum albumin and 0.02% sodium azide and retained 100% of their biological activity (data not shown).

Recombinant murine ^{125}I-BSF-1 was shown to exhibit specific binding to the factor-dependent bone marrow-derived cell line FDC-P2. Preliminary experiments indicated that binding was saturable and required ≈60 min to reach equilibrium at either 37°C or 4°C, with the rate of approach to equilibrium being only slightly slower at 4°C (data not shown). A low level of nonspecific binding of ^{125}I-BSF-1 was seen that remained constant over the time course. Fig. 1*A* shows the association kinetics at 37°C of ^{125}I-BSF-1 with FDC-P2 at three different concentrations of ligand. The data show that the final equilibrium amount bound to cells and the rate of approach to equilibrium are dependent on the initial concentration of ^{125}I-BSF-1 in the medium. The curves passing through the data are best-fit single exponential time-dependency curves (26), consistent with the presence of a single population of receptors on these cells. The *Inset* (Fig. 1*B*) shows the dependence of the pseudo first-order forward rate constant (determined by curve fitting the data of Fig. 1*A*) on the concentration of ^{125}I-BSF-1 in the medium. For a bimolecular reaction such data should fit a straight line, with the slope being the forward rate constant and the intercept on the ordinate being the reverse rate constant (26). The data shown in the *Inset* generate values of $4.3 \pm 0.6 \times 10^8$ $M^{-1}\cdot min^{-1}$ for the forward rate constant and 0.107 ± 0.004 min^{-1} for the reverse rate constant. Similar values were

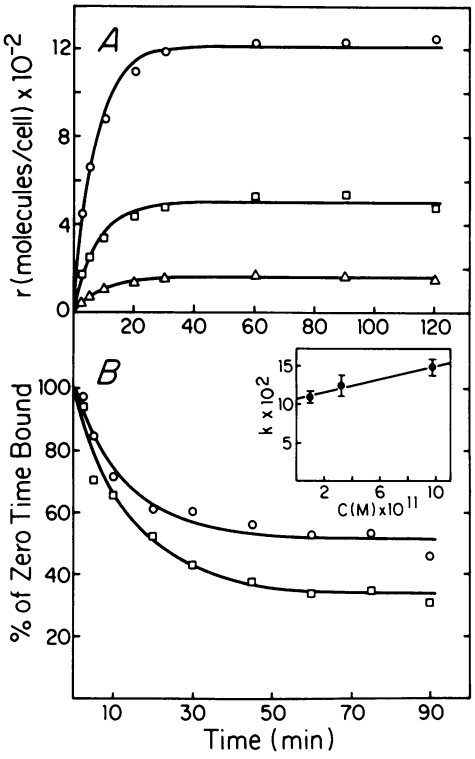

FIG. 1. Association and dissociation kinetics of ^{125}I-BSF-1 with FDC-P2 cells at 37°C. (*A*) FDC-P2 cells (1.33×10^7 cells per ml) were incubated with 9.75×10^{-11} M (○), 3.20×10^{-11} M (□), or 9.60×10^{-12} M (△) ^{125}I-BSF-1 at 37°C. At the time points indicated, aliquots were removed and assayed for binding. Nonspecific binding was measured in the presence of a 100-fold molar excess of unlabeled BSF-1 at each concentration of ^{125}I-BSF-1. The continuous curves passing through the data were calculated from the best fit parameter values using a single exponential term (24). Infinite time binding and association rate constants, respectively, for each curve were ○, 1211.4 ± 21.9 molecules per cell and 0.15 ± 0.01 $M^{-1}\cdot min^{-1}$; □, 505.2 ± 13.8 molecules per cell and 0.12 ± 0.01 $M^{-1}\cdot min^{-1}$; △, 164.2 ± 3.0 molecules per cell and 0.11 ± 0.01 $M^{-1}\cdot min^{-1}$. A plot of the association rate constant calculated from each curve versus the molar concentration of ^{125}I-BSF-1 initially present in the medium is shown in the *Inset* (in *B*). (*B*) FDC-P2 (2.3×10^7 cells) were incubated with ^{125}I-BSF-1 (2.2×10^{-10} M) in 0.7 ml of binding medium for 60 min at 37°C. The cells were then divided, harvested by centrifugation, and resuspended in either binding medium alone (○) or binding medium containing 7.5×10^{-10} M unlabeled BSF-1 (□). The cells were maintained at 37°C, and aliquots were removed at various time points and assayed for binding. Free ^{125}I-BSF-1 concentration during the dissociation incubation was 1.5×10^{-12} M.

obtained on the IL-2-dependent T-cell line CTLL. The ratio of these parameters gives a range of values for the affinity constant of the radiolabeled BSF-1 preparation for its receptor of $3.3–4.7 \times 10^9\ M^{-1}$.

Fig. 1*B* shows the dissociation of ^{125}I-BSF-1 from FDC-P2 cells at 37°C. The experiment is designed, as originally described by Demeyts *et al.* (27), to test whether the BSF-1 receptor exhibits any cooperative properties. A complex kinetic pattern was revealed that was characterized by a fast and slowly dissociating component similar to that observed for a number of receptor systems (21, 24, 26–30). The dissociation rate constants of the fast component measured in this experiment were $7.7 \pm 1.4 \times 10^{-2}\ min^{-1}$ in medium alone and $7.2 \pm 1.2 \times 10^{-2}\ min^{-1}$ in the presence of 7.5×10^{-10} M unlabeled BSF-1, whereas in both cases the rate constant of the slow component was $<10^{-2}\ min^{-1}$. Comparison of the fast rates of dissociation in medium alone, when a fraction of the receptors is occupied, with that in the presence of unlabeled BSF-1, where almost all the receptors are occupied, revealed no significant sensitivity to receptor occupancy, suggesting that BSF-1 receptors on FDC-P2 cells are noncooperative.

Fig. 2 illustrates typical equilibrium binding data for ^{125}I-BSF-1 at 37°C to FDC-P2 cells (*A*) and CTLL cells (*B*). In both cases, display of the data in the Scatchard coordinate system (31) yielded a straight line, indicating a single class of high-affinity binding sites for BSF-1. Nonspecific binding increased linearly with increasing concentration and did not exceed 2% of the total cpm added. For FDC-P2 cells, the calculated apparent K_a was $2.55 \pm 0.51 \times 10^{10}\ M^{-1}$ with 1740 $\pm$ 280 specific binding sites per cell (average from four binding experiments), and for CTLL cells the calculated apparent K_a was $1.63 \pm 0.37 \times 10^{10}\ M^{-1}$ with 1130 $\pm$ 320 specific binding sites per cell (average from four binding experiments). Similar experiments with FDC-P2 carried out at 4°C showed an apparent K_a of $1.88 \pm 0.01 \times 10^{10}\ M^{-1}$ with 2110 $\pm$ 60 specific sites per cell, values that are not significantly different from those obtained at 37°C. Inhibition of binding of ^{125}I-BSF-1 to cells with unlabeled BSF-1 yielded an inhibition constant of $3.36 \pm 0.26 \times 10^{10}\ M^{-1}$ on FDC-P2 and $2.06 \pm 0.18 \times 10^{10}\ M^{-1}$ on CTLL (data not shown). These values are only slightly higher than the K_a of the radiolabeled material as measured by equilibrium binding, showing that radiolabeling produces little loss in affinity for the receptor. The iodinated reagent is thus a valid probe for characterization of receptor binding properties.

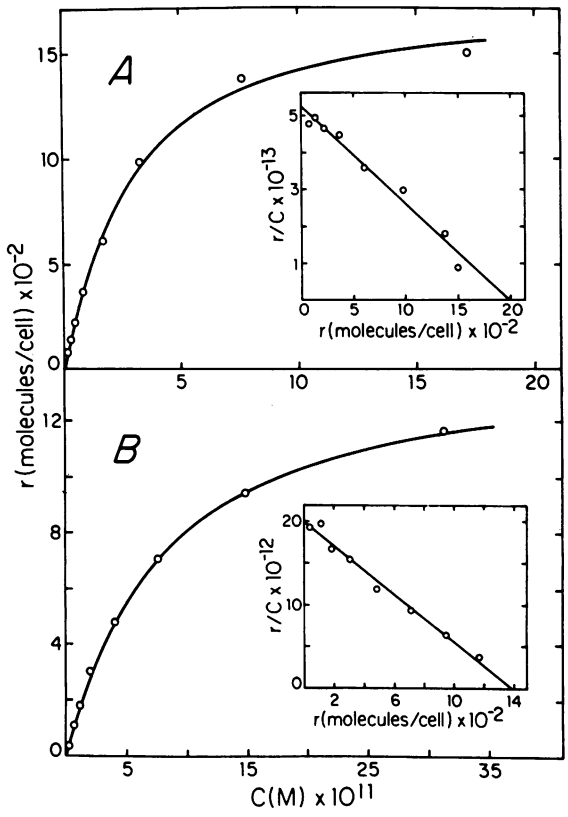

FIG. 2. Equilibrium binding of ^{125}I-BSF-1 to FDC-P2 and CTLL cells. FDC-P2 (*A*) and CTLL (*B*) (1.33×10^7 cells per ml) were incubated with various concentrations of ^{125}I-BSF-1 (specific radioactivity, 1.1×10^{16} cpm/mmol) for 1 hr at 37°C and assayed for binding. Data are corrected for nonspecific binding (1.03×10^{12} and 7.80×10^{11} molecules per cell per M for *A* and *B*, respectively), measured in the presence of a 100-fold molar excess of unlabeled BSF-1. (*Insets*) Scatchard representations of specific binding replotted from *A* and *B*.

A comparison of the values obtained for the affinity constant of ^{125}I-BSF-1 binding to FDC-P2 shows that the average value obtained by equilibrium binding ($2.55 \pm 0.51 \times 10^{10}\ M^{-1}$) is somewhat higher than that obtained by analysis of association kinetics ($4.0 \pm 0.7 \times 10^9\ M^{-1}$). This discrepancy is most likely due to inaccurate assessment of the reverse rate constant in the association kinetic experiment due to the marked biphasic nature of the dissociation kinetics (see Fig. 1*B*).

The specificity of ^{125}I-BSF-1 binding was examined by testing a number of purified lymphokines and other polypeptide hormones for their ability to compete with ^{125}I-BSF-1 for binding to its receptor on FDC-P2 cells. As shown in Fig. 3, recombinant murine BSF-1 (column b) eliminated >95% of ^{125}I-BSF-1 binding, whereas none of the other lymphokines or hormones tested, including IL-2 and IL-3, exhibited any ability to compete, even when present at concentrations that were 1000-fold greater (on a molar basis) than that of ^{125}I-BSF-1 (columns c–o). IL-2 also did not inhibit binding of ^{125}I-BSF-1 to CTLL cells (which express IL-2 receptors) nor

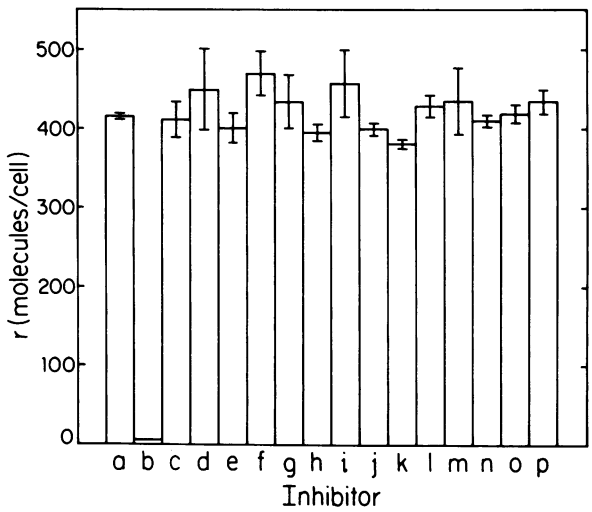

FIG. 3. Specificity of ^{125}I-BSF-1 binding to FDC-P2 cells. FDC-P2 cells (9.0×10^6 cells per ml) were incubated with ^{125}I-BSF-1 (2.5×10^{-11} M) and the following unlabeled proteins at the concentrations indicated: a, none; b, recombinant murine BSF-1, 5×10^{-9} M; c, recombinant human IL-2, 5×10^{-7} M; d, human IL-1α, 5×10^{-7} M; e, human IL-1β, 5×10^{-7} M; f, CSF-2α (IL-3), 7.5×10^{-9} M; g, recombinant murine GM-CSF, 7.5×10^{-7} M; h, epidermal growth factor, 3 μg/ml; i, fibroblast growth factor, 1 μg/ml; j, nerve growth factor, 2 μg/ml; k, insulin, 1.1×10^{-7} M; l, luteinizing hormone (human), 1 μg/ml; m, growth hormone (human), 1.7×10^{-7} M; n, thyroid-stimulating hormone, 1 μg/ml; o, follicle-stimulating hormone, 1 μg/ml; p, anti-LFA-1 (M17/5.2), 15 μg/ml. Concentrations of partially pure hormone preparations are given in μg/ml of total protein. Incubation was for 1 hr at 37°C and binding was assayed. Data are expressed as ±SEM.

did BSF-1 inhibit binding of ^{125}I-labeled IL-2 to these cells (data not shown). In addition, an anti-LFA-1 monoclonal antibody, M17/5.2 (Hybritech, La Jolla, CA) directed against the α chain of LFA-1, did not inhibit ^{125}I-BSF-1 binding even at a concentration of 15 μg/ml (Fig. 3, column p).

A number of primary cells and continuous cell lines of murine origin were examined for their ability to bind ^{125}I-BSF-1 (Table 1). In all cases, complete binding curves were done over a range of ^{125}I-BSF-1 concentrations and receptor numbers per cell were generated by Scatchard analysis of the data. The high-affinity constant of BSF-1 binding, combined with the very high specific activity of the radiolabeled molecule, allowed receptor levels of <100 per cell to be reproducibly measured. The number of receptors is particularly low on resting B and T cells but these populations appear to increase their receptor numbers by 5- to 10-fold upon stimulation with lipopolysaccharide or Con A (Table 1). Although all of the murine lines tested displayed BSF-1 receptors, neither a human B lymphoma (RPMI-1788) nor a human T lymphoma (CEM) bound any murine ^{125}I-BSF-1.

Further characterization of the BSF-1 receptor on five of these cell lines, FDC-P2, CTLL, 32D, P815, and BCL-1, was carried out by affinity crosslinking. Fig. 4 shows a NaDodSO$_4$/PAGE analysis run under reducing conditions of ^{125}I-BSF-1 crosslinked to FDC-P2 cells. One intense crosslinked band was observed with an approximate M_r of 124,000 (lane b). Controls showed that ^{125}I-BSF-1 alone (lane a) or a sample incubated in the presence of excess unlabeled BSF-1 (lane c) exhibited no crosslinked species. Recombinant murine BSF-1 exhibits a very heterogeneous size due to yeast glycosylation (H. Sassenfeld, unpublished data) and runs on NaDodSO$_4$/PAGE with a M_r of 49,700 ± 4300. From three separate crosslinking experiments, run on five different polyacrylamide gels (8%, 10%, and 10–20% linear gradient) an average M_r for the crosslinked species on FDC-P2 cells was calculated to be 124,700 ± 13,300. After subtraction of the molecular weight of BSF-1, the crosslinked species would represent a membrane protein of M_r 75,000. The other four cell types examined, CTLL, 32D, P815, and BCL-1, all exhibited a similar crosslinked band that fell into the same weight range as that on FDC-P2 (data not shown). An identical pattern was obtained when NaDodSO$_4$/PAGE analysis was carried out under nonreducing conditions. In addition, although cells were routinely extracted in the presence of a cocktail of protease inhibitors, no apparent degradation of the crosslinked species was observed if extraction was done in the total absence of protease inhibitors.

Table 1. Cellular distribution of murine BSF-1 receptors

Designation	Characteristics	BSF-1 bound, molecules per cell
Primary cell		
B cell	Resting	65 ± 5
	LPS blast	320 ± 65
Thymocyte	Resting	45 ± 5
	Con A blast	165 ± 5
Splenic T cell	Resting	60 ± 5
	Con A blast	590 ± 10
Bone marrow		20 ± 5
Cell line		
FDC-P2	Bone marrow-derived, IL-3-dependent	1740 ± 280
32D/IL-3	Bone marrow-derived, IL-3-dependent	1590 ± 300
FD.C/2	Bone marrow-derived, IL-2-dependent	3520 ± 140
CTLL	IL-2-dependent T cell	1130 ± 320
LSTRA	T-lymphocytic leukemia	115 ± 15
EL4	T lymphoma	140 ± 10
BCL-1	B-cell tumor	440 ± 30
Wehi 279.1	B-cell lymphoma	110 ± 10
P815	Mastocytoma	1040 ± 30
Wehi 3	Myelomonocyte tumor	185 ± 15
P388D$_1$	Macrophage tumor	270 ± 50
RPMI-1788	Human B lymphoma	0
CEM	Human T lymphoma	0

Binding experiments were conducted as described in the legend to Fig. 2. With each cell line, Scatchard analysis of at least two separate sets of binding data was performed. All primary cells and *in vitro* cell lines are of murine origin unless otherwise indicated.

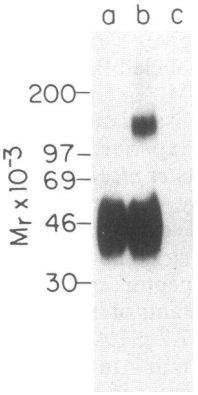

FIG. 4. Characterization of the murine BSF-1 receptor by affinity crosslinking. Cells (20×10^6) were incubated with ^{125}I-BSF-1 (1.2×10^{-10} M) in the presence and absence of unlabeled BSF-1 for 2 hr at 4°C. Cells were harvested, washed, crosslinked for 30 min at 25°C with bis(sulfosuccinimidyl)suberate (0.1 mg/ml), and extracted with PBS/1% Triton containing a cocktail of protease inhibitors. Aliquots corresponding to 0.5×10^6 cells were boiled for 3 min in sample buffer containing 2% NaDodSO$_4$ and 5% 2-mercaptoethanol and subjected to electrophoresis on a 10–20% gradient gel. Shown is ^{125}I-BSF-1 (specific radioactivity, 1.1×10^{16} cpm/mmol) alone (lane a) or incubated with and crosslinked to FDC-P2 cells in the absence (lane b) or presence (lane c) of unlabeled BSF-1.

DISCUSSION

Recombinant murine BSF-1 was radiolabeled with ^{125}I to high specific activity and used to characterize the specific receptor for this lymphokine on the IL-3-dependent FDC-P2 and IL-2-dependent CTLL cell lines. Both lines have been shown to proliferate in response to purified BSF-1 (4). In addition, we found BSF-1 receptors expressed, at varying levels, on cell lines representing the B-cell, T-cell, mast cell, macrophage, and myelomonocytic lineages, a cellular distribution of the BSF-1 receptor molecule that reinforces the observation that this lymphokine has a broad array of activities. The level of expression of BSF-1 receptors on all of the cell lines tested was quite low, reminiscent of results with a number of different lymphokines, including IL-3 (CSF-2α) (28, 32), granulocyte CSF (33), GM-CSF (21, 30, 34, 35), and IL-1 (29). The low number of receptors was particularly striking on resting B and T cells, which both expressed <100 receptors per cell. Though expression of receptors on these populations is consistent with the demonstration that resting lymphocytes are a major target of BSF-1 (7, 8), the extremely low number expressed demonstrates that BSF-1 can exert its biological effects with few molecules bound per cell. In fact, it has been shown that $1–5 \times 10^{-12}$ M BSF-1 is sufficient to induce 50% maximal thymidine incorporation in either a B-cell or FDC-P2 proliferation assay (4), whereas the concentration of BSF-1 required to produce 50% maximal receptor occupancy is $\approx 3 \times 10^{-11}$ M based on the affinity of unmodified BSF-1 for FDC-P2 cells. This concentration is an order of magnitude

higher than that required to produce biological effects, reminiscent of many other systems, including GM-CSF (21), IL-3 (28), and IL-1 (29), in which only fractional receptor occupancy is required to produce a maximal biological effect. As has been described (21), these three factors and BSF-1 also share a common pattern of ligand dissociation, in which a slowly exchanging component was observed that might reflect a subpopulation of receptors that can effectively bind ligand irreversibly and perhaps mediate a biological effect through this interaction even at extremely low levels of receptor expression. It is interesting to note in this light that activation of B and T cells with lipopolysaccharide and Con A, respectively, which enhances the responsiveness of these cells to BSF-1, also apparently increases the number of receptors expressed on these cells 5- to 10-fold.

Kinetic characterization of BSF-1 binding to FDC-P2 and CTLL cells revealed no obvious differences between the receptors found on these cells. Preliminary structural information on the BSF-1 receptor, provided by affinity crosslinking experiments, substantiated the similarity. Both cell types exhibited a crosslinked species having an average M_r of 124,000, which would correspond to a receptor size of M_r 75,000. A similar size receptor protein was also found on the 32D, P815, and BCL-1 cell lines. The size of the BSF-1 receptor and the failure of an anti-LFA-1 antibody to inhibit binding of BSF-1 to cells suggest that the receptor is not directly related to LFA-1, as has been suggested (36). This does not rule out the possibility, however, that LFA-1 could be associated with a BSF-1 receptor binding protein in the plasma membrane. It was also clear from inhibition studies that BSF-1 does not share a common receptor with either IL-2 or IL-3 nor do these molecules appear to compete with each other in any direct way for specific receptor binding. This point is of interest due to the ability of BSF-1 to stimulate the proliferation of several IL-2- and IL-3-dependent cell lines, in a manner similar to IL-2 and IL-3 themselves.

These studies have shown that though BSF-1 is capable of binding to a wide range of cell types, the receptor protein with which this molecule interacts exhibits no overt differences between cells of different lineages. The level of receptor expression, however, can be modulated at least on primary B and T cells by activation with mitogenic agents. It is hoped that further study on the distribution of the BSF-1 receptor, the conditions under which its expression is altered, and its possible interaction with other lymphokine receptors will produce some clues to explain the diverse array of activities associated with BSF-1.

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