CHARACTERIZATION OF THE HUMAN B CELL STIMULATORY FACTOR 1 RECEPTOR

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B cell stimulatory factor 1 (BSF-1, interleukin 4) was originally characterized as a molecule that stimulates the proliferation of anti-IgM-activated B cells (1), and has subsequently been found to have a multiplicity of other biological effects, many on cell lineages outside the B lymphocyte compartment. In addition to inducing the expression of class II MHC molecules on resting B cells (2, 3) and enhancing the secretion of IgE and IgG1 by stimulated B cells (4, 5), BSF-1 also stimulates the proliferation of factor-dependent cell lines of both T cell and hematopoietic lineages (6–11), as well as stimulating proliferation of both activated Lyt-2+ (9, 10) and L3T4+ (9–11) subpopulations of normal T lymphocytes.

Using 125I-labeled recombinant murine BSF-1, we have recently reported the presence of receptors for this lymphokine on a broad spectrum of cell lineages (12). Ohara and Paul (13), using radiolabeled natural murine BSF-1, found a similarly broad distribution. While this observation serves to corroborate the demonstration of diverse activities mediated by murine BSF-1, it also points out the likelihood that this list of activities may expand.

The recent cloning of the gene encoding human BSF-1 (14) has now enabled us to extend our studies to the characterization and distribution of the BSF-1 receptor on human cells. Using yeast-derived recombinant human BSF-1 radiolabeled with 125I, we have shown that the human BSF-1 receptor is present not only on in vitro cell lines of B, T, and hematopoietic lineages, but is also found on fibroblasts and cells of epithelial and endothelial origin. In this paper we report the kinetic and structural characterization of the BSF-1 receptor on both Raji cells (a human B lymphoma) and on primary human gingival fibroblasts. The similarity we observe between the BSF-1 receptor on these two different human cell lineages further supports the notion that the ability of BSF-1 to mediate a spectrum of biological events is not due to overt differences in the receptor for this lymphokine on different cell types.

Materials and Methods

Cell Preparations. FDC-P2 (15) and CTLL-2 (16) were maintained as previously described (6). All other cell lines were grown in RPMI-1640 containing 5–20% FCS and antibiotics. Adherent cell lines were harvested by scraping, following a brief treatment

Abbreviations used in this paper: BS3, bis-(sulfosuccinimidyl)suberate; BSF-1, B cell stimulating factor 1; GM-CSF, granulocyte/macrophage colony stimulating factor.
with 0.5 mM EDTA when necessary to aid cell detachment. Human peripheral blood mononuclear cells were prepared from leukocyte layers (Portland Red Cross, Portland, OR) and stimulated with PHA (Gibco Laboratories, Grand Island, NY) for 48 h, as previously described (17). Human gingival fibroblasts (kindly provided by Dr. R. C. Page, University of Washington, Seattle, WA) were maintained in DMEM containing 10% FCS and generally used between passages 9 and 10. For radioreceptor assays, cells were harvested with 0.05% trypsin and 0.02% EDTA, seeded at \(10^5\) cells/well in 6-well plates, and used when the cells had reached confluency (1.5–2 \(\times\) 10^5 cells/well) after a minimum incubation time of 72 h.

Hormone Preparations. Human recombinant IL-2 and granulocyte colony-stimulating factor (G-CSF) were expressed in yeast under the control of the alcohol dehydrogenase 2 (ADH2) promoter and purified from the yeast media to homogeneity by reversed-phase high performance liquid chromatography according to methods previously described (18–20). Recombinant murine BSF-1 and human granulocyte/macrophage CSF (GM-CSF) were expressed in yeast and purified to homogeneity as previously described (12, 21). Recombinant human IL-1α and IL-1β were expressed in E. coli and purified to homogeneity as previously described (22). Nerve growth factor, fibroblast growth factor, and epidermal growth factor were obtained from Bethesda Research Labs (Gaithersburg, MD). Human follicle stimulating hormone, human luteinizing hormone, human thyroid stimulating hormone, and human growth hormone were from Calbiochem-Behring (La Jolla, CA).

Recombinant Human BSF-1 Purification and Radiolabeling. Human BSF-1 cDNA was cloned from a cDNA library made from RNA extracted from peripheral blood T lymphocytes (purified by E-rosetting) activated for 18 h with PHA and PMA. The BSF-1 cDNA clone was isolated by hybridization with a synthetic oligonucleotide based on the published cDNA sequence (14). The full-length cDNA was subcloned into a yeast expression vector that included pBR322 sequences, the Trp1 gene of yeast for tryptophan selection, the yeast 2 μ origin of replication, the yeast alcohol dehydrogenase 2 (ADH2) promoter and the α factor leader sequences to direct synthesis and secretion. The expression plasmid was transformed into yeast strain 79 (α trp1-1, leu2-2) selecting the Trp⁺ transformants. Cultures were grown in rich medium (1% yeast extract, 2% peptone, 1% glucose) at 30°C until stationary phase, and cells were removed by centrifugation and filtration. Recombinant BSF-1 was purified from the yeast supernatant after 10-fold concentration on an Amicon hollow-fiber device followed by addition of four parts acetone (–20°C) to six parts yeast supernatant on ice. The acetone precipitate was resuspended in 50 mM Hepes, pH 7.4, at 1 mg/ml and applied to S-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) at 20°C. Recombinant BSF-1 was eluted using a linear gradient of LiCl (0–1 M) in 50 mM Hepes, pH 7.4. Fractions containing BSF-1 were applied directly to a PROPC column (Pharmacia Fine Chemicals) before elution with a linear gradient of acetonitrile (0–100%) in 0.1% trifluoroacetic acid. In some cases, S-Sepharose-purified BSF-1 (0.5 mg in 50 mM Hepes, pH 7.4, 0.3 M LiCl) was treated with 0.25 U of N-glycanase (Genzyme Corp., Boston, MA) for 50 d at 4°C before purification by reverse-phase fast protein liquid chromatography. After purification, rBSF-1 concentrations were determined by amino acid analysis, and activity was measured in a B cell proliferation assay. The specific activity of purified recombinant human BSF-1 was 10^4 U/μg. For the B cell assay of rBSF-1, human tonsillar B cells were purified by depletion of T cells by E-rosetting followed by depletion of granulocytes and monocytes by Sephadex G10 filtration. The resting B cells (>95% pure) were assayed for their proliferative response to rBSF-1 in the presence of submaximal concentrations of goat F(ab')2 anti-human IgM, in a 72-h assay with 10^5 cells cultured in 200 μl RPMI and 10% FCS.

Both untreated and glycanase-treated human recombinant BSF-1 were radiolabeled using the enzymobead radiiodination reagent (BioRad Laboratories, Richmond, CA), essentially as previously described for murine recombinant GM-CSF (23). The specific activities of both radiolabeled preparations were estimated to be 2–6 \(\times\) 10^15 cpm/mmol, based on determination of an initial protein concentration by amino acid analysis and on a recovery of 40% from control experiments in which an aliquot of BSF-1 was spiked with
125I-labeled BSF-1 and put through the iodination protocol with omission of 125I. Bioactivity of 125I-BSF-1 was determined in the human B cell proliferation assay described above.

Binding Assays and Data Analysis. Binding assays were performed by a phthalate oil separation method (24) essentially as described previously for murine 125I-GM-CSF (23). Sodium azide (0.2%) was included in all binding assays to inhibit internalization and degradation of 125I-BSF-1 by cells at 37°C. Adherent human gingival fibroblasts were assayed by incubating confluent monolayers in six-well plates (prepared as described above) with 125I-BSF-1 in 1 ml of RPMI-1640 containing 2% BSA, 20 mM Hepes buffer, and 0.2% sodium azide, pH 7.2 (binding medium). Plates were incubated on a gyratory shaker for either 1 h at 37°C or 2 h at 4°C, after which an aliquot of the supernatant in each well was removed and counted in a gamma counter, and the monolayers were washed rapidly once with 5 ml of binding medium and three times with 5 ml of PBS. After incubation in 1 ml of trypsin-EDTA at 37°C for 15 min, cells were harvested and the entire aliquot was counted in a gamma counter. The number of cells per well was determined by harvesting and counting cells from control wells that had undergone the same incubation and wash conditions in the absence of 125I-BSF-1.

Association and dissociation kinetic experiments were conducted as previously described (23). Binding data was also analyzed, as described elsewhere (23).

Affinity Crosslinking. Crosslinking experiments with human 125I-BSF-1 to Raji cells were performed as previously described for murine BSF-1 (12). For human gingival fibroblasts, confluent monolayers of cells in 9-cm dishes were incubated with 125I-BSF-1 (1.3 X 10^-9 M) at 4°C in 3 ml of binding medium both in the presence and absence of a 100-fold or greater molar excess of unlabeled BSF-1. After 2 h the plates were washed three times in ice-cold PBS, and 4 ml of 0.1 mg/ml bis-(sulfosuccinimidyl) suberate (BS3) in PBS was added to each. After a 30-min incubation at 25°C, plates were again washed three times with PBS and cells were harvested by scraping and centrifugation, after a 15-min incubation at 37°C in 4 ml of 5 mM EDTA. Cells were then incubated in 50 μl PBS/1% Triton containing 2 mM phenylmethylsulfonylfluoride, 10 μM pepstatin, 10 μM leupeptin, 2 mM phenylmethylsulfonylfluoride, 10 μM pepstatin, 10 μM leupeptin, 2 mM o-phenanthroline, and 2 mM EGTA for 30 min at 25°C, centrifuged at 12,000 g for 10 min, and the supernatants were retained.

SDS-PAGE. Samples were boiled for 3 min in sample buffer (0.06 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-ME) and analyzed on 8, 10, or 10-20% gradient gels according to the stacking gel procedure of Laemmli (25). Methyl [35S]labeled molecular markers: cytochrome C (M, 12,500), lactoglobulin A (M, 18,367), carbonic anhydrase (M, 31,000), ovalbumin (M, 46,000), BSA (M, 69,000), phosphorylase B (M, 97,400), and myosin (M, 200,000) were from New England Nuclear (Boston, MA). After electrophoresis, gels were stained with Coomassie blue (0.25% in 25% isopropanol, 10% acetic acid), dried and then exposed to Kodak X-omat AR film at -70°C.

Results

Purification and Radiolabeling of Human BSF-1. Recombinant human BSF-1 was expressed in yeast, purified to apparent homogeneity as described in Materials and Methods, and iodinated to high specific activity with the enzyme-bond radioiodination reagent. Fig. 1, lane a, shows an autoradiograph of an iodinated recombinant human BSF-1 preparation, which exhibits a broad band of ~60,000 M, (average). Radiolabeled preparations of BSF-1 were calculated to have specific activities in the range of 2-6 × 10^15 cpm/mmol, based on protein concentrations determined by amino acid analysis. Such preparations retained >50% of their biological activity (as measured in a human B cell proliferation assay), and were stable for at least 1 mo when stored at 4°C in medium containing 2% BSA and 0.2% sodium azide. Treatment of recombinant human BSF-1 with N-glycanase resulted in the appearance of a major species after iodination (sp act, 2.5 × 10^15
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FIGURE 1. Characterization of human $^{125}$I-BSF-1 by SDS-PAGE. Lane a, $^{125}$I-labeled recombinant BSF-1, sp act 3.2 $\times$ 10$^{15}$ cpm/mnmole; and (b) $^{125}$I-labeled N-glycanase-treated BSF-1, sp act 2.5 $\times$ 10$^{15}$ cpm/mnmole were boiled in sample buffer containing 2% SDS and 5% 2-ME, and 5,000 cpm were applied to a linear 10-20% gradient gel. Electrophoresis and autoradiography were conducted as described under Materials and Methods.

cpm/mmol) of $\sim$15,000 $M_r$ (Fig. 1, lane b). The relative molecular mass of the digested protein is similar to the molecular weight of the nonglycosylated protein predicted from its cDNA sequence, and suggests that recombinant human BSF-1 is extensively glycosylated by the yeast host, similar to that previously observed with yeast-derived recombinant murine BSF-1 (12).

Binding Characteristics of BSF-1 with Cell Surface Receptors. Binding studies undertaken with the glycosylated form of human BSF-1 showed that it exhibited specific binding to the human B cell line, Raji (26). Preliminary experiments showed that binding was saturable and extremely rapid, requiring <30 min to reach equilibrium at either 37 or 4°C. Fig. 2A illustrates the association kinetics at 37°C of $^{125}$I-BSF-1 with Raji cells, showing that both the final equilibrium amount of ligand bound to cells and the approach to equilibrium are dependent on the initial concentration of $^{125}$I-BSF-1 in the medium. Although the initial rate of binding is so rapid that it makes collection of data at early times difficult, the best-fit single exponential time-dependency curves passing through the data (27) are consistent with the presence of a single population of receptors on these cells. The inset (Fig. 2B) shows a plot of the pseudo-first-order forward rate constants determined from the curves in Fig. 2A relative to the initial concentration of $^{125}$I-BSF-1 in the medium. The data shown fit a straight line, as expected for a biomolecular reaction, and generate values of $3.36 \pm 0.43 \times 10^9$/M-min for the forward rate constant (the slope) and $0.22 \pm 0.03$/min for the reverse rate constant (the intercept on the ordinate).

Fig. 2B shows the dissociation of $^{125}$I-BSF-1 from Raji cells at 37°C, both in medium alone, where a fraction of the receptors are occupied, and in the presence of 1.37 $\times$ 10$^{-7}$ M unlabeled BSF-1, where almost all the receptors are occupied. Under both conditions, a biphasic kinetic pattern was observed, very similar to that shown previously for murine BSF-1 (12). The dissociation rate constants of
the fast component were $7.6 \pm 0.9 \times 10^{-2}$/min in medium alone and $6.1 \pm 0.8 \times 10^{-2}$/min in the presence of excess unlabeled BSF-1, while in both cases the rate constant of the slow component was $<10^{-2}$/min. Comparison of these rates revealed no significant sensitivity to receptor occupancy, suggesting that BSF-1 receptors on Raji cells are noncooperative (28). Using the average value for the fast component of $6.9 \pm 1.6 \times 10^{-2}$/min, a ratio of the forward and reverse rate constants gives a range of values for the affinity constant of radiolabeled BSF-1 for its receptor of $2.6-7.2 \times 10^9$/M. This range is likely to be somewhat low, both because it does not take into account the slow component of the dissociation kinetics and because the difficulties in accurately measuring the extremely rapid initial rate of $^{125}$I-BSF-1 binding may in fact underestimate the forward rate constant. This latter problem may also contribute to the somewhat higher value for the reverse rate constant determined from the association kinetics experiments.

Fig. 3 illustrates typical equilibrium binding data for $^{125}$I-BSF-1 to Raji cells at $37^\circ$C (Fig. 3A), as well as to adherent human gingival fibroblasts at $4^\circ$C (Fig. 3B). Scatchard analysis of the data yielded a straight line, indicative of a single class of binding sites for BSF-1 on both cell types. Nonspecific binding was extremely low ($<0.1\%$ of the total cpm added) and increased linearly with increasing concentration. From the average of eight binding experiments, the calculated apparent $K_a$ of BSF-1 binding to Raji cells was $6.10 \pm 2.15 \times 10^9$/M with $2,160 \pm 530$ specific binding sites per cell. This $K_a$ was in the same range
FIGURE 3. Equilibrium binding of $^{125}$I-BSF-1 to human gingival fibroblasts and Raji cells. Raji cells, $1.35 \times 10^7$ cells/ml (A) and human gingival fibroblasts, $2.4 \times 10^5$ cells/well (B) were incubated with various concentrations of $^{125}$I-BSF-1 (sp act $4.2 \times 10^{14}$ cpm/mmol) for 1 h at 37°C and for 2 h at 4°C, respectively. Binding was assayed as described in Materials and Methods. Data are corrected for nonspecific binding ($2.1 \times 10^{-12}$ and $1.9 \times 10^{-12}$ molecules/cell/M for A and B, respectively), measured in the presence of a 200-fold molar excess of unlabeled BSF-1. The insets show Scatchard representations of specific binding replotted from A and B. Curve fitting was done as described in Materials and Methods.

FIGURE 4. Inhibition of $^{125}$I-BSF-1 binding to Raji cells by unlabeled BSF-1. Raji cells ($1.35 \times 10^7$ cells/ml) were incubated with $^{125}$I-BSF-1 ($3.04 \times 10^{-10}$ M) and varying concentrations of unlabeled BSF-1. Incubation was for 45 min at 37°C and binding was assayed as described in Materials and Methods. The continuous curve passing through the data was calculated from a one-site competitive inhibition equation using a $K_i$ value for $^{125}$I-BSF-1 of $8.2 \times 10^{-9}$ M. All data were corrected for nonspecific binding measured in the presence of $1.9 \times 10^{-8}$ M unlabeled BSF-1.

as that obtained by analysis of association and dissociation kinetics ($4.9 \pm 2.3 \times 10^9$/M). Similar experiments carried out at 4°C showed an apparent $K_s$ of $6.12 \pm 1.12 \times 10^9$/M with $1,670 \pm 300$ specific sites per cell, values that are not significantly different than those obtained at 37°C. Binding of BSF-1 to human gingival fibroblasts also yielded similar values, with a calculated $K_s$ of $1.1 \pm 0.1 \times 10^{10}$/M and $1,950 \pm 70$ specific sites per cell.

Fig. 4 shows the inhibition of binding of $^{125}$I-BSF-1 to Raji cells at 37°C by unlabeled BSF-1. The curve passing through the data was calculated with a single-site competitive inhibition equation consistent with the presence of a single class of BSF-1 receptors (29), and yielded an inhibition constant of $1.17 \pm 0.10 \times 10^{10}$/M. This value is only slightly higher than the $K_s$ of the radiolabeled material as measured by equilibrium binding to Raji cells ($6.10 \pm 2.15 \times 10^9$/M), supporting the use of iodinated BSF-1 as a valid probe for characterization of receptor binding properties.
FIGURE 5. Specificity of $^{125}$I-BSF-1 binding to Raji cells. Raji cells ($1.33 \times 10^7$ cells/ml) were incubated with $^{125}$I-BSF-1 ($3.5 \times 10^{-10}$ M) and the following unlabeled proteins at the concentrations indicated: a, none; b, recombinant human BSF-1, $2.6 \times 10^{-7}$ M; c, recombinant murine BSF-1, $5 \times 10^{-8}$ M; d, human IL-1α, $5 \times 10^{-7}$ M; e, human IL-1β, $5 \times 10^{-7}$ M; f, human IL-2, $5 \times 10^{-7}$ M; g, recombinant human GM-CSF, $7.5 \times 10^{-7}$ M; h, recombinant human G-CSF, $1.8 \times 10^{-7}$ M; i, epidermal growth factor, $8 \mu$g/ml; j, fibroblast growth factor, $1 \mu$g/ml; k, nerve growth factor, $2 \mu$g/ml; l, luteinizing hormone (human), $1 \mu$g/ml; m, growth hormone (human), $1.7 \times 10^{-7}$ M; n, thyroid-stimulating hormone, $1 \mu$g/ml; o, follicle-stimulating hormone, $1 \mu$g/ml. Concentrations of partially pure hormone preparations are given in micrograms per milliliter total protein. Incubation was for 1 h at 37°C and binding was assayed as described under Materials and Methods.

In addition, inhibition experiments with nonglycosylated human BSF-1 (N-glycanase treated) yielded an inhibition constant indistinguishable from that of the glycosylated form (data not shown). Equilibrium binding experiments also showed the iodinated nonglycosylated BSF-1 to have a similar $K_a$ ($3.50 \pm 0.55 \times 10^9$/M) to that of the glycosylated form. Consequently, this nonglycosylated material was judged to be a suitable analogue to glycosylated BSF-1 for affinity crosslinking experiments, in which its smaller, more homogeneous size is a distinct advantage (see below).

The specificity of human BSF-1 binding to Raji cells was assessed by testing a number of purified lymphokines and other polypeptide hormones for their ability to compete with $^{125}$I-BSF-1 binding to its receptor (Fig. 5). When present in concentrations that were 100–1,000-fold greater (on a molar basis) than that of $^{125}$I-BSF-1, human BSF-1 eliminated >95% of $^{125}$I-BSF-1 binding, while none of the other molecules tested, including human IL-1, IL-2, GM-CSF, and G-CSF, exhibited any ability to compete.

Cellular Distribution of Human BSF-1 Receptors. Cell lines of human, monkey, and mouse origin were examined for their ability to bind human $^{125}$I-BSF-1 (Table I). 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. Reminiscent of the broad cellular distribution of murine BSF-1 receptors, human BSF-1 was found to bind to cells of all human lineages examined, including fibroblasts and epithelial cells. Human BSF-1 also bound to both monkey lines tested, but not to any murine lines. Although receptor levels varied over a 25-fold range, expression on most cells was extremely low. Two human T lymphoma lines, RPMI-8402 and JMB12, did not exhibit detectable levels of BSF-1 receptor. Peripheral blood mononuclear cells also express low receptor levels when resting, but appear to increase their receptor numbers by about fivefold upon stimulation with PHA.

Affinity Crosslinking. Further characterization of the BSF-1 receptor on Raji cells and human gingival fibroblasts, both of which express ~2,000 receptors/cell,
Table 1

Cellular Distribution of Human BSF-1 Receptors

| Designation          | Characteristics | BSF-1 bound* | molecules/cell |
|----------------------|-----------------|--------------|----------------|
| Peripheral blood mononuclear cells | Resting         | 140 ± 110    |                |
|                       | PHA-activated   | 710 ± 260    |                |
| Gingival fibroblasts |                 | 1,950 ± 70   |                |

Cell lines

- Raji: B lymphoma, 2,280 ± 210
- Daudi: B lymphoma, 2,585 ± 55
- Cess: B lymphoma, 525 ± 25
- BMB: B lymphoma, 190 ± 30
- ARH 77: B lymphoma, 200 ± 30
- RPMI-1788: B lymphoma, 220 ± 50
- NALM 6: Pre-B lymphoma, 115 ± 15
- Peer: T leukemia, 210 ± 50
- HSB2: T lymphoma, 210 ± 10
- Jurkat: T lymphoma, 115 ± 15
- RPMI-8402: T lymphoma, <10
- JMB 12: T lymphoma, <10
- HL-60: Promyelocytic leukemia, 125 ± 45
- U937: Myelogenous leukemia, 640 ± 50
- SK HEP: Hepatoma, 570 ± 270
- HPT: Pancreatic tumor, 610 ± 60
- HBT: Bladder carcinoma, 495 ± 55
- Calu-6: Epithelial, 565 ± 105
- HeLa: Epithelial, 445 ± 25
- MLA: T leukemia (simian), 1,820 ± 120
- CVI: Kidney fibroblast (simian), 2,190
- CTLL: T cell (murine), <10
- FDC-P2: Bone marrow-derived (murine), <10
- L cells: Fibroblast (murine), <10

* Binding experiments were conducted as described in Fig. 3. 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 human origin unless otherwise indicated.

was carried out by affinity crosslinking. Radiolabeled nonglycosylated BSF-1 (N-glycanase treated) was bound to cells in the presence or absence of unlabeled BSF-1 and cells subsequently exposed to the bifunctional crosslinking reagent BS², extracted with PBS/1% Triton containing a cocktail of protease inhibitors, and the soluble fractions were analyzed by SDS-PAGE. Fig. 6 shows a SDS-PAGE analysis, run under reducing conditions, of ¹²⁵I-BSF-1 crosslinked to Raji cells and human gingival fibroblasts. One major crosslinked band was observed on both cell types (Fig. 6, lanes b and d). Controls showed that no crosslinked species were found in the absence of crosslinker (lane a) or in a sample containing excess unlabeled BSF-1 (Fig. 6, lane c). From crosslinking experiments run on both 8
FIGURE 6. Characterization of the human BSF-1 receptor by affinity crosslinking. Lanes a–c: Raji cells (2.0 × 10^6) were incubated with ^125^I-BSF-1 (3 × 10^-9 M) both in the absence (a and b) and presence (c) of unlabeled BSF-1 (1.3 × 10^-7 M). Incubation was for 1.5 h at 37°C, and cells were then harvested, washed, and crosslinked and extracted as described in Materials and Methods. Lane a shows a control with no crosslinker added. Lane d: human gingival fibroblasts (5 × 10^5 cells/plate) were incubated with ^125^I-BSF-1 (1.2 × 10^-9 M) for 2 h at 4°C. Plates were washed and cells were crosslinked, harvested, and extracted as described in Materials and Methods. Aliquots corresponding to 10^6 Raji cells or 7.5 × 10^5 gingival fibroblasts were boiled for 3 min in sample buffer containing 2% SDS and 5% 2-ME, and subjected to electrophoresis on a 10% gel.

and 10% polyacrylamide gels, an average relative molecular mass for the crosslinked species on both Raji cells and gingival fibroblasts was calculated to be 154,000 ± 5,000. After subtraction of BSF-1 (15,000 M_r), the crosslinked species would be a membrane protein of M_r 139,000 ± 5,000.

Discussion

^125^I-labeled recombinant human BSF-1 was used to examine the cellular distribution of receptors for this lymphokine, as well as to further characterize these receptors on both Raji cells, a human B lymphoma, and on primary human gingival fibroblasts. Reminiscent of the broad cellular distribution previously shown for BSF-1 receptors on murine cells (12, 13), BSF-1 receptors were found on in vitro human cell lines of B, T, and hematopoietic lineages. This distribution has now been further extended by the detection of BSF-1 receptors on a number of adherent human cell lines of both epithelial and endothelial character, as well as on primary gingival fibroblasts. Using ^125^I-labeled murine BSF-1, we have also observed BSF-1 receptors on similar murine cell lines (L. Park, unpublished data). The low level of expression of BSF-1 receptors on human cells was comparable to that seen on mouse cells, and stimulation of human peripheral blood mononuclear cells with PHA resulted in an approximate fivefold increase in BSF-1 receptor expression, similar to the increased expression of BSF-1 receptors reported after activation of primary murine B and T cells (12, 13).

Comparison of the binding characteristics of the yeast hyperglycosylated form of BSF-1 (M_r ~60,000) and the N-glycanase–treated, sugar-free form on Raji cells showed no significant differences in kinetic parameters. This provides evidence that the addition of carbohydrate to the BSF-1 molecule at natural glycosylation sites does not alter the binding characteristics of human BSF-1 for its receptor. This result is in agreement with results with murine BSF-1, where
very similar binding characteristics were obtained by us (12) using a recombinant yeast-derived hyperglycosylated species \( (M, 49,000) \), and by Ohara and Paul (13) using natural BSF-1 \( (M, 20,000) \).

Equilibrium binding of human BSF-1 to Raji cells and gingival fibroblasts revealed no obvious differences between the receptors found on these cells. Similarly, preliminary structural information on the BSF-1 receptor obtained by affinity crosslinking showed that both Raji cells and gingival fibroblasts exhibited a crosslinked species having an average \( M_r \) of 154,000, which would correspond to a receptor subunit size of 139,000 \( M_r \). The large size of the crosslinked species was unexpected from our previous crosslinking data, which showed a receptor size of \( \sim 75,000 \ M_r \) on a number of murine cell lines (12), and from similar experiments by Ohara and Paul which showed a receptor size on murine spleen cells of 60,000 \( M_r \) (13). Several explanations for this discrepancy can be suggested: (a) the BSF-1 receptor on human and mouse cells may differ by 60,000–80,000 \( M_r \). While human and murine BSF-1 exhibit considerable amino acid sequence homology (14), they show complete species specificity in receptor binding, suggesting that substantial differences might exist in receptor structure between the two species. (b) A large difference in apparent receptor size could be caused by species-specific differences in the posttranslational modification of a similar protein core. (c) The BSF-1 receptor may be composed of a complex of two or more proteins, and human BSF-1 may crosslink to a different component of the complex than murine BSF-1. (d) Finally, the receptor size we observed on murine cells may represent a proteolytically cleaved fragment of the native receptor. In previous work with the murine GM-CSF receptor (23), we showed that this receptor was acutely protease sensitive, and if extracted from cells in the absence of a cocktail of protease inhibitors, it would be degraded from a 130,000 to a 70,000 \( M_r \) form. Depending on the cell type involved, some degradation could not be prevented even in the presence of the protease inhibitor cocktail. We have used the same protease inhibitor cocktail for extractions during crosslinking studies with both mouse and human BSF-1, however the murine receptor may be more protease sensitive than the human receptor. We are currently investigating the possibility that the native receptor on murine cells is actually comparable in size to that on Raji cells and human gingival fibroblasts.

Although the diversity of biological activities attributable to BSF-1 is substantial, they have thus far been limited to effects on cells of lymphoid or myeloid origin. With the observation that BSF-1 is also capable of binding to fibroblasts, this lymphokine may soon join the ranks of other regulatory molecules such as interleukin 1 (30) and transforming growth factor \( \beta \) (31), the receptors for which are expressed on a wide array of different tissues and which appear to be involved in a complex network of biological activities. Molecules such as interleukin 1, \( \beta \) interferon, transforming growth factor \( \beta \), and tumor necrosis factor are known to stimulate fibroblast growth (32, 33) and secretion of prostaglandins, collagenase, and collagen (34–36), and have recently been implicated in regulating the release of cytokines from fibroblasts as well (37). Future studies on BSF-1 and its receptor should clarify the role that this molecule may play in a complex
regulatory system of cytokines that bridge hematopoietic, connective tissue, and lymphoid compartments.

Summary

$^{125}$I-labeled recombinant human B cell stimulatory factor 1 (BSF-1) was used to characterize receptors specific for this lymphokine on in vitro cell lines representing human B, T, and hematopoietic lineages, as well as on adherent cell lines of epithelial and endothelial origin, and on primary human gingival fibroblasts. BSF-1 binding was extremely rapid and saturable at both 4 and $37^\circ$C, with a slow dissociation rate. On all human cell types examined, BSF-1 bound to a single class of high-affinity receptor (<3,000 receptors per cell) with a $K_a$ of $0.5-1.0 \times 10^{10}$/M. Human BSF-1 also bound to cell lines of simian but not murine origin. Comparison of kinetic characteristics obtained with a yeast-derived hyperglycosylated form of BSF-1 ($M_r$, 60,000) and N-glycanase–treated, sugar-free BSF-1 ($M_r$, 15,000) showed no significant differences. Among a panel of lymphokines and growth hormones, only unlabeled human BSF-1 was able to compete for the binding of $^{125}$I-labeled human BSF-1. Affinity crosslinking experiments resulted in the identification on both Raji cells and on primary human gingival fibroblasts of a receptor subunit with an average $M_r$ of 139,000. These studies show that the BSF-1 receptor on human cells has an extremely broad cellular distribution, while further supporting the notion that the ability of BSF-1 to mediate a spectrum of biological activities cannot be accounted for by overt differences in the receptor for this lymphokine on different cell lineages.

We thank Drs. Robert Tushinski, Diane Mochizuki, Bruce Acres, Ken Grabstein, Virginia Price, and Ms. Carol Ramthun for many helpful discussions and other contributions to this work. We also thank Betsy McGrath, Ralph Klinke, Kurt Shanebeck, and Susan Call for excellent technical assistance, and Linda Troup for her help in the preparation of this manuscript.

Received for publication 24 April 1987.

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