FACTOR B, THE COMPLEMENT ALTERNATIVE PATHWAY SERINE PROTEINASE, IS A MAJOR CONSTITUTIVE PROTEIN SYNTHESIZED AND SECRETED BY RESIDENT AND ELICITED MOUSE MACROPHAGES

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Mononuclear phagocytes have been increasingly recognized as a source of many of the complement proteins (1, 2). Activities constituting the intact complement alternative pathway in serum (factor B, factor D, C3, and properdin) (3–8), as well as the regulatory proteins, factors H and I (5, 8), are produced by mouse peritoneal macrophages and human peripheral blood monocytes. Factors C2 and C4 are also synthesized by mononuclear phagocytes (1, 2, 5, 9–11).

Factor B, a glycoprotein of M_r ~93,000 that plays a central role in the alternative pathway of complement activation (12, 13), is closely associated with the immune response as a class III gene product of the major histocompatibility complex in mice (14, 15), guinea pigs (16), and humans (17). Activated factor B (Bb, M_r ~60,000) serves as a migration inhibiting factor (18), inducing macrophage and monocyte spreading (19, 20) and possibly stimulating cytotoxic (21, 22) and bacteriocidal activities (23, 24) of monocytes in vitro.

The hemolytic activity of factor B produced by resident mouse peritoneal macrophages (3, 6) increases linearly during 72–96 h in culture, and its synthesis is regulated by lipopolysaccharide (LPS)[1] (24). In contrast, a 48–72 h lag has been observed (5, 7) before synthesis of factor B is initiated by human peripheral blood monocytes in culture, and there are differences in synthesis between monocytes and tissue macrophages (9, 10) correlating with the recognized maturation of human peripheral blood monocytes into macrophages (25). The molecular sizes and chain composition of the cellular and secreted forms of

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[1] *Abbreviations used in this paper:* ApoE, apolipoprotein E; BCG, bacillus Calmette-Guerin; BSA, bovine serum albumin; CM, conditioned medium; CVF, cobra venom factor; DFP, diisopropylfluorophosphate; FBS, fetal bovine serum; LPS, lipopolysaccharide; PMSF, phenylmethylsulfonyl fluoride; RIA, radioimmunoassay; SAC, phosphate-buffered saline supplemented with Nonidet P-40, methionine, and sodium azide; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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human factor B produced by monocytes and hepatoma cells are similar to those of factor B purified from serum (7, 25–27). The high molecular weight forms of cellular factor B antigens that have been described (27) may be artifactual.

In response to developmental and environmental signals, mononuclear phagocytes display a variety of functional states characterized by differences in morphology, surface antigens, and protein secretion. Whereas some proteins, such as lysozyme, are secreted constitutively, others, such as apolipoprotein E (ApoE), vary according to the inflammatory state of the macrophage (28).

In this report we show that the major constitutive M_r 90,000–93,000 glycoprotein synthesized and secreted by mouse peritoneal macrophages is factor B, and that this protein is active in the complement system. In addition, we have compared the conditions under which ApoE and factor B are synthesized by macrophages.

Materials and Methods

Human Complement Proteins and Antibody to Human Complement Factor B. We prepared human complement proteins factor B (29), factor Bb (20, 29), factor D (30), and cobra venom factor (CVF) (31) as described previously. Factor B was radiolabeled with ^{125}I by the method of Bolton and Hunter (32), and factor Bb was labeled by the Iodogen (Pierce Chemical Co., Rockford, IL) method of Fraker and Speck (33). Specific rabbit antibody directed against factor B was prepared by affinity chromatography on Sepharose (CNBr-Sepharose; Pharmacia Fine Chemicals, Piscataway, NJ) containing covalently bound factor B antigen. Adsorbed Ig were eluted with 0.1 M acetate-buffered, 0.14 M saline, pH 2.5, containing 0.02 M EDTA, into 0.2 M Tris-buffered 0.14 M saline, pH 8.0.

Preparation of Mouse Plasma. We collected normal mouse plasma from ICR mice by retroorbital puncture into citrate phosphate dextrose at a final citrate concentration of 20 mM. Phenylmethylsulfonyl fluoride (PMSF) was added as a proteinase inhibitor at a final concentration of 5 mM. This normal mouse plasma was stored at −70°C until used. Alternatively, normal mouse plasma frozen in acid citrate dextrose was purchased from Pel-Freez Biologicals, Rogers, AR, and PMSF was added to a final concentration of 5 mM when the sample was thawed. We also collected fresh normal mouse plasma from BALB/c mice by cardiac puncture.

Purification of Mouse Factor Bb. We purified mouse factor Bb from normal mouse plasma by affinity chromatography on a 2 ml CNBr-Sepharose column containing 1 mg of affinity-purified anti–human factor B Ig. Mouse factor B was eluted with glycine-HCl buffer, pH 2.5, into Tris-HCl, pH 8; 106 ± 16 μg (mean ± SD; $n = 4$) of factor B antigen was isolated per milliliter of normal mouse plasma. Assuming a factor B concentration of 160 μg/ml in this plasma (as measured by radioimmunoassay [RIA]), the apparent recovery of protein in this step was 62 ± 3% ($n = 4$) by this column chromatographic method. We analyzed mouse factor B by nonreducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (34).

Factor B RIA. We developed a double-antibody equilibrium RIA specific for factor B. Human factor B (50 μg) was radiolabeled with 1 mCi ^{125}I-labeled Bolton-Hunter reagent (32) (Amersham Corp., Arlington Heights, IL) to a final specific activity of 6–9 × 10^5 cpm/μg. Over 90% of radiolabeled factor B was precipitated with 10% trichloroacetic acid; >95% of cpm were M_r 90,000, as assessed by SDS-PAGE (6% acrylamide) (35), and >90% of cpm had a sedimentation rate of 5–6 S, as assessed by sucrose density gradient ultracentrifugation. Dilutions of all reactants were made in 0.1 M Tris-HCl, 0.14 M NaCl, pH 7.2, containing 2% heat-inactivated normal rabbit serum. The assay consisted of three compartments: (*a*) 250 μl ^{125}I-labeled factor B (0.5 nM); (*b*) 250 μl affinity-purified rabbit anti–factor B Ig (diluted 1:1500); and (*c*) 250 μl of a competitor. After 18 h at 4°C, 250 μl of goat anti-rabbit Ig was added, and allowed to react for 5 h at 4°C. The immunopre-

cipitates formed were removed by centrifugation (1,200 g for 30 min), and an aliquot of the supernatant (750 μl) was removed and counted to determine (by difference) the radioactivity bound in the immunoprecipitate.

Mouse Peritoneal Macrophages. We collected macrophages by lavage from the peritoneal cavities of CD-1, C3H/HeN (Charles River Breeding Laboratories, Inc., Wilmington, MA), and C3H/HeJ (The Jackson Laboratory, Bar Harbor, ME) strains of mice injected 12 d previously with 2–6 × 10^6 viable mycobacteria of strain bacillus Calmette-Guerin (BCG), or 4 d previously with 1 ml of Brewer's thioglycollate broth (Difco Laboratories, Detroit, MI), 25 mg/kg of pyran copolymer (a gift of Hercules Inc., Wilmington, DE), 0.5 ml of 5 mM $NaIO_4$, 10–30 μg of detoxified LPS from *Salmonella typhimurium*, or 1 mg *Corynebacterium parvum* (RIBI Immunochem, Hamilton, MT) (36, 37). We plated macrophages at a density of 5 × 10^5 cells in 2 cm^2 in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated (56°C, 30 min) fetal bovine serum (FBS) for 2 h at 37°C in an atmosphere of 95% air and 5% CO_2. Nonadherent cells were removed by washing, and adherent cells were placed in serum-free medium containing 0.2% lactalbumin hydrolysate. We prepared macrophage cellular extracts by washing the cell monolayer three times with 0.15 M NaCl and then scraping, in the presence of 0.1% Triton X-100, to remove cells. We stored lysates and medium samples at −20°C until used.

Mouse Bone Marrow–derived Macrophages. We flushed cells from mouse femurs with Hanks' balanced salt solution, then grew them in medium containing 10% FBS, 10% horse serum, and 10% L cell–conditioned medium (38, 39). The mouse macrophage line P388D1 was cultured as described previously (39).

Radiolabeling of Proteins. We incubated macrophages with [^{35}S]methionine, and analyzed secreted and cellular proteins by SDS-PAGE using the Laemmli buffer system (34, 36, 40). For radiolabeling of secreted macrophage serine esterases, we treated conditioned medium (CM) from thioglycollate-elicited macrophages with [^{3}H]diisopropylfluorophosphate (DFP) (New England Nuclear, Boston, MA), essentially as described previously (41).

Immunoprecipitation of Factor B from Mouse Macrophages. Radiolabeled proteins in macrophage-conditioned medium or cell lysates were immunoprecipitated according to a modification (36, 41) of the Jones procedure (42). Briefly, we prepared cell lysates by scraping cells into phosphate-buffered saline containing 0.5% Nonidet P-40, 2 mM methionine, and 0.02% NaN_3 (SAC buffer). CM was centrifuged to remove any cellular debris before preadsorption for 15 min on ice with 60 μg of rabbit anti–bovine serum albumin (anti-BSA) IgG (Cappel Laboratories, Cochranville, PA) per milliliter of sample and 150–200 μl of 10% *Staphylococcus aureus* suspension (Zysorbin; Zymed Labs, Burlingame, CA). The Zysorbin had been washed three times in SAC buffer, and resuspended in SAC buffer containing 1 mg/ml of ovalbumin. We then immunoprecipitated the preadsorbed samples for 30 min at 25°C with 10 μg rabbit anti–human factor B. Nonimmune IgG was used as a control. After immunoprecipitation, we added 200 μl of *S. aureus* suspension and allowed it to react for 20 min on ice. We centrifuged the samples and washed the pellets twice with SAC-ovalbumin buffer, once with SAC buffer, then resuspended them in Laemmli sample buffer, and boiled them in a water bath before electrophoresis. ApoE was identified by immunoprecipitation, and fibronectin was identified by binding to *S. aureus* (36). Factor B in macrophage secretions was also identified by immunoblotting onto nitrocellulose filters (43) essentially as described previously for ApoE (36).

Conditions for Activation of Macrophage Factor B. We studied the functional properties in the complement system of mouse macrophage factor B by testing for the ability of the protein to form a complex with CVF, in which factor B is cleaved by factor D. We incubated samples of media or cell extracts (100 μl) for 60 min at 37°C with 10 μg CVF, 16 μg of factor D, 4 mM $MgCl_2$, and 0.02 mM $CaCl_2$. After this incubation, we precipitated activated factor B antigens from the incubation mixture by rabbit anti–human factor B, and analyzed them by SDS-PAGE.

Results

Purification of Mouse Plasma Factor Bb. Mouse plasma factor B exhibited two or three polypeptides with apparent M_r of 60,000–65,000 under nonreducing conditions in SDS-PAGE; these polypeptides comigrated with human factor Bb produced by activation of purified factor B with C3b and factor D (Fig. 1). Under reducing conditions, with dithiothreitol, both mouse and human factor Bb migrated in SDS-PAGE as a single polypeptide species, suggesting that the three bands observed under nonreducing conditions may be due to slight heterogeneity in either the detergent binding or polysaccharide composition of Bb. The size of the polypeptides indicates that mouse factor Bb, and not native factor B, is the primary product of this purification procedure.

In the factor B RIA, purified factor B and normal human serum competed with a typical dose response (Fig. 2*a*). Purified factor D, C3, C5, human albumin, or serum immunochemically depleted of factor B did not compete, indicating the specificity of the assay (data not shown). The cross-reaction of mouse factor B with antibody to human factor B is shown in Fig. 2*b*. At a dilution of 1:96, normal mouse plasma inhibited the binding of anti–human factor B to ^{125}I-labeled factor B to a maximum of 10–15% (Fig. 2*b*). We calculated the apparent concentration of factor B antigen in normal mouse plasma to be 120–180 μg/ml (in human factor B antigenic equivalents). FBS, calf serum, goat serum, and normal rabbit serum containing hemolytically active factor B did not significantly compete with human factor B at dilutions of 1:50 to 1:500 (<4% inhibition).

Identification of Factor B as a Major Protein Secreted by Mouse Macrophages. The CM from BCG-elicited macrophages incubated with [^{35}S]methionine contained two prominent, newly synthesized polypeptides migrating as a doublet of M_r 90,000 and 93,000 in SDS-PAGE. They were identified as complement factor B by their comigration with purified human serum factor B, immunoprecipitation with affinity-purified antibodies from antisera raised to human factor B, Western immunoblotting, and mapping of the products of partial digestion with chymotrypsin (Fig. 3). In addition, factor B is a serine proteinase that is weakly labeled after incubation with [^{3}H]DFP (44). When macrophage-conditioned culture medium was incubated with [^{3}H]DFP and then analyzed by SDS-PAGE, the prominent M_r 90,000 macrophage polypeptide was weakly labeled with [^{3}H]-DFP, although there was strong labeling of many other polypeptides (data not

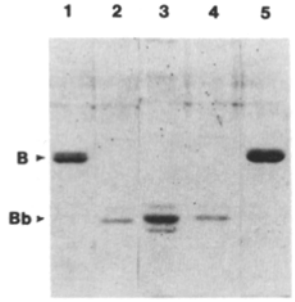

FIGURE 1. Comparison of 6% SDS-PAGE of purified mouse factor Bb (*3* and *4*) with purified human factor B (*1* and *5*) and factor Bb (*2*).

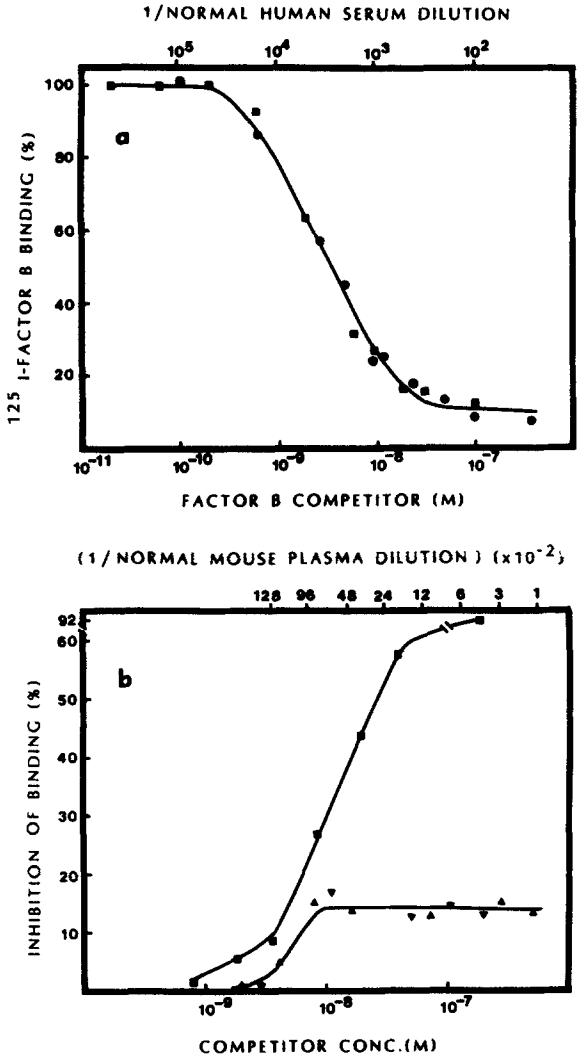

FIGURE 2. (*a*) Characteristics of a competitive equilibrium double-antibody RIA for factor B using 0.5 nM ^{125}I-labeled human factor B, rabbit anti-human factor B, and goat anti-rabbit Ig. (■) Factor B competitor; (●) normal human serum competitor. (*b*) Antigenic cross-reactions between (▼) purified mouse factor Bb, (▲) factor B in normal mouse plasma, and (■) human factor B using the competitive equilibrium RIA for human factor B.

shown). Thioglycollate-elicited macrophages were collected by lavage of the peritoneal cavity, and immediately placed in suspension with [^{35}S]methionine. Synthesis and secretion of factor B were detectable by immunoprecipitation, remaining at the same rate for the first 6 h in culture (data not shown). Factor B was first detected in the medium of thioglycollate-elicited macrophages after 30–60 min of continuous radiolabeling, reaching a constant rate of secretion after 2 h, remaining linear for ~6 h, and still increasing after 24 h (Fig. 4). Factor B represented ~0.5% of total cellular protein synthesized at 0.5 h of labeling. Intracellular factor B reached a plateau after 3–4 h of labeling and then remained constant at about the level of factor B secreted in a 2 h period. In

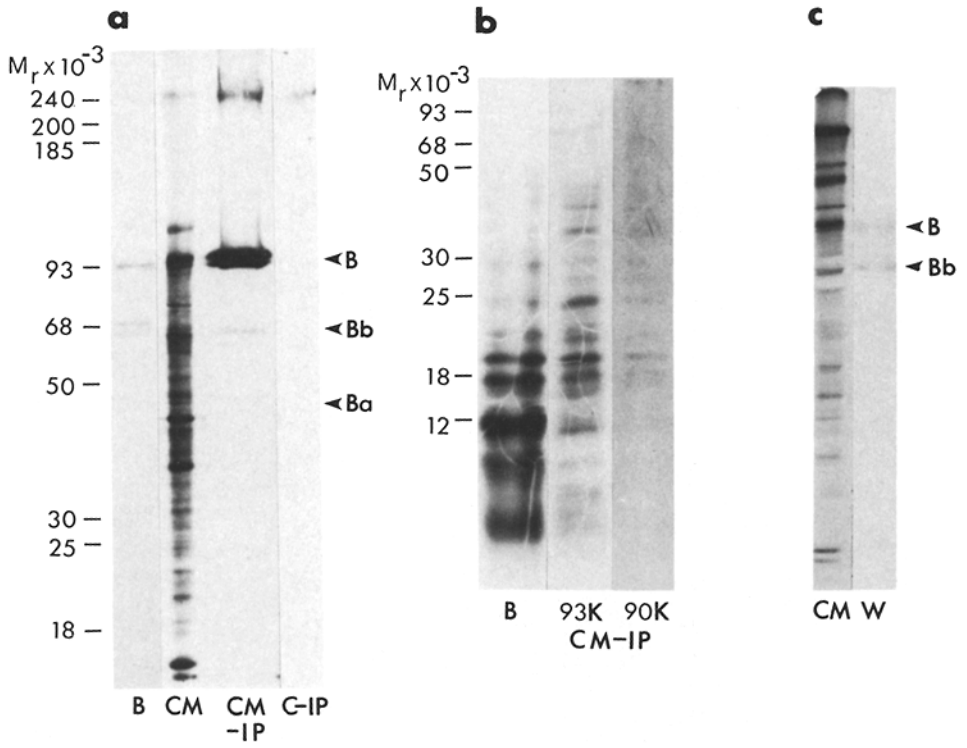

FIGURE 3. (*a*) Immunoprecipitation of factor B from CM of BCG-elicited macrophages. Cells (10^6) were incubated with 25 μCi/ml [^{35}S]methionine for 19 h, and secreted proteins were analyzed by SDS-PAGE. *B*, ^{125}I-labeled factor B standard, showing B, Bb, and Ba components; *CM*, trichloroacetic acid precipitate of 50 μl of CM; *CM-IP*, immunoprecipitate of factor B from 500 μl of CM by rabbit anti-human factor B; *C-IP*, control immunoprecipitate of CM by rabbit anti-BSA IgG. Molecular weight markers are indicated at the left. (*b*) Chymotryptic peptide maps of factor B from the M_r 93,000 band from factor B standard (*B*) and the M_r 93,000 and 90,000 bands from the immunoprecipitate from macrophage secretions (*CM-IP*). (*c*) Western immunoblot of factor B from resident macrophages. *CM*, fluorogram of trichloroacetic acid precipitate of 100 μl of CM from [^{35}S]methionine-labeled cells; *W*, immunoblot of factor B from the same CM.

pulse-chase experiments, after 30 min of incubation with [^{35}S]methionine, factor B appeared in the medium with a half-time of 60 min.

Effect of Inflammatory Stimuli on Cellular and Secreted Factor B. Peritoneal macrophages elicited by various inflammatory agents differ in their metabolic and functional activities (36, 37, 45–47). Nonspecifically elicited and immunologically activated peritoneal macrophages display distinct patterns of protein secretion immediately after explanting, and some differences are maintained after culture for several days (36). However, newly synthesized factor B was a constant proportion of the total secreted proteins from 2 to 72 h in culture, and did not significantly differ in thioglycollate-, $NaIO_4$-, BCG-, or pyran copolymer-elicited macrophages (Table I). The amount of factor B in the cell extracts and culture medium of mouse macrophages elicited by a variety of stimuli was quantified by equilibrium RIA (Tables II and III). The cell lysates contained 2–4 nmol factor B per 5×10^5 cells (Table II), and medium samples contained 2–

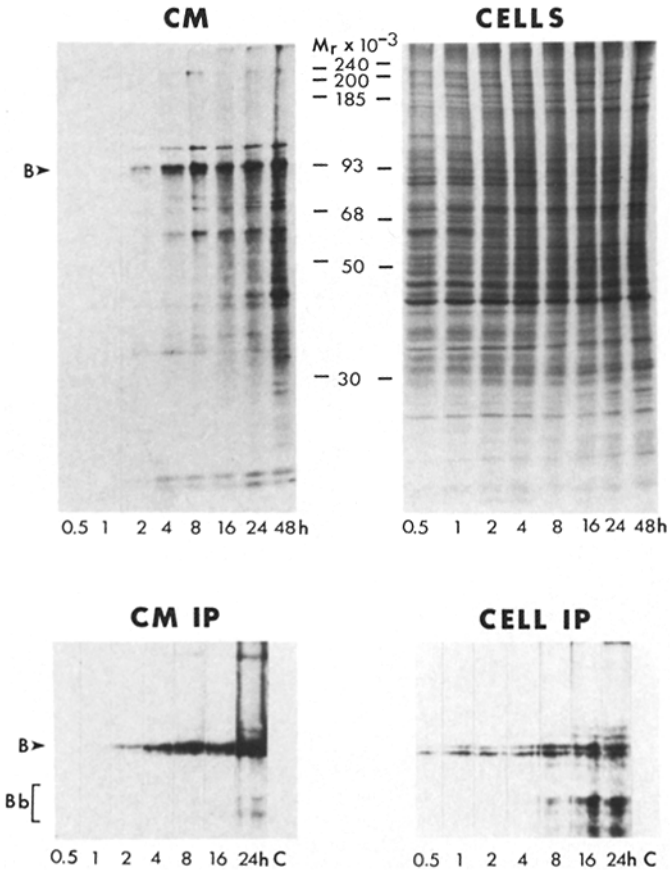

FIGURE 4. Appearance of factor B in cell extracts and medium of thioglycollate-elicited macrophages during continuous labeling. Cells were plated for 2 h, washed, and incubated with 25 μCi/ml [^{35}S]methionine for the times indicated. Medium was either acid-precipitated (*CM*) or immunoprecipitated (*CM-IP*) by rabbit anti-human factor B or by rabbit anti-BSA IgG. Cell lysates were treated in the same manner. The factor B band and molecular weight markers are indicated.

10 nM factor B (Table III). Medium obtained from P388D1, a continuous mouse macrophage cell line, contained similar amounts of factor B antigen; however, medium from L cells (a continuous fibroblast cell line) contained <0.01 nM factor B (Table III).

Activation of Macrophage Factor B to Factors Bb and Ba. The complement system activity of the prominent biosynthesized factor B (M_r 90,000 and 93,000) polypeptides in CM was investigated by studying conversion of factor B to factor Bb (M_r 60,000–65,000) and Ba (M_r 30,000–38,000) activation fragments. [^{35}S]-Methionine-labeled secretion products of thioglycollate-elicited or BCG-activated peritoneal macrophages were incubated with CVF, factor D, and Mg^{++}; and factor B was immunoprecipitated by affinity-purified antibody against human factor B. SDS-PAGE showed biosynthetically labeled molecular species of apparent M_r 93,000, 90,000, 65,000, 63,000, 60,000, 38,000, 36,000, and 30,000 (Fig. 5). These polypeptides were not precipitated by nonimmune serum or by

TABLE I

Effect of Inflammatory Stimuli on the Rate of Incorporation of [^{35}S]-Methionine Into Secreted Factor B of Macrophages after Various Times in Culture

Eliciting agent	Time in culture	Secreted factor B (percent of total secreted protein)
	h	
Thioglycollate	2	6.7
	48	4.5
	72	4.3
$NaIO_4$	2	4.4
	48	6.5
	72	4.2
BCG	2	6.8
	48	6.7
	72	6.8
Pyran copolymer	2	5.4
	48	5.3
	72	5.7

Elicited macrophages (5×10^5 cells/well) were cultured for 2–72 h, and secreted factor B was determined by densitometry of fluorograms of SDS-PAGE of secreted proteins from macrophages incubated with [^{35}S]methionine for 2 h.

TABLE II

Concentration of Factor B in Lysates of Elicited Peritoneal Macrophages as Measured by RIA

Eliciting agent	Time in culture	Factor B concentration per 5×10^5 cells	
	h	*nmol*	*ng*
Thioglycollate	2	2.8 ± 0.8	176
	24	2.5 ± 1.1	158
BCG	2	3.6 ± 1.5	227
	24	4.1 ± 1.8	258

Elicited macrophages (5×10^5 cells/well) were cultured for 2 or 24 h, and lysates were prepared. Five dilutions of each of duplicate samples were assayed in the factor B RIA. Data are expressed in equivalents of human factor B (mean ± SD).

an unrelated antigen-antibody system, BSA/anti-BSA. The prominent M_r 90,000–93,000 immunoprecipitable radiolabeled polypeptides were significantly reduced after incubation with CVF, factor D, and Mg^{++}, with concomitant increases in the labeling of M_r 60,000–65,000 and M_r 30,000–38,000 polypep-

TABLE III

Accumulation of Factor B in the CM of Macrophages, as Measured by RIA

Macrophage source	CM collection time	Factor B concentration	
	h	*nM*	*ng/ml*
Resident	48	2.4 ± 2.3	151
BCG-elicited	48	1.1 ± 0.6	69
LPS-elicited	24	10.8 ± 1.1	680
C. parvum–elicited	48	10.0 ± 0.0	610
P388D1	24	2.9 ± 2.5	183
L cell fibroblasts	24	<0.01	<0.6

Macrophages ($5–10 \times 10^5$ cells/well) were cultured for 24 or 48 h in 1 ml of serum-free medium; for the peritoneal macrophages, this began 2 h after explanting into culture. Macrophages were obtained from either CD-1 or C3H/HeN mice. Each type of culture, in duplicate or triplicate, was assayed at three dilutions in the factor B RIA. Data are expressed in equivalents of human factor B (mean ± SD).

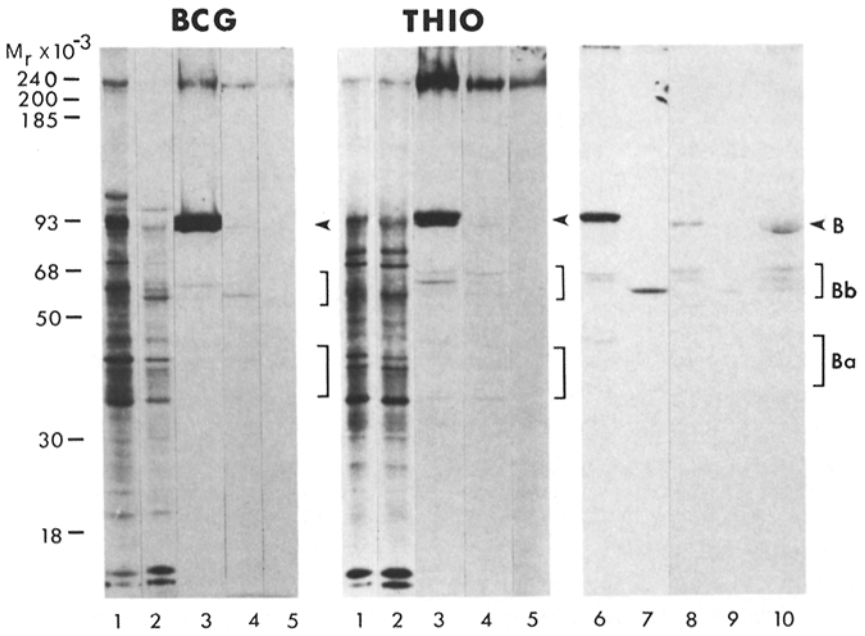

FIGURE 5. Activation of factor B from CM of BCG-activated (*BCG*) or thioglycollate-elicited (*THIO*) macrophages. Cells were incubated with [^{35}S]methionine, and secreted proteins were acid-precipitated, immunoprecipitated by rabbit anti–human factor B, or activated by incubating 100-μl samples as described in Materials and Methods. Samples were then analyzed by SDS-PAGE. Molecular weight markers are indicated at the left. (*1*) Trichloroacetic acid precipitate of 50 μl of CM; (*2*) trichloroacetic acid precipitate of 280 μl of activated CM; (*3*) immunoprecipitate of factor B from 500 μl of CM; (*4*) immunoprecipitate of factor B from 280 μl of activated CM; (*5*) control immunoprecipitate of 500 μl of CM by rabbit anti–BSA IgG; (*6*, *7*) immunoprecipitate of ^{125}I-labeled factor B and Bb standards; (*8*, *9*) ^{125}I-labeled factor B and Bb standards; (10) activated ^{125}I-labeled factor B.

tides. These molecular sizes correspond to the sizes of purified factor Bb (Fig. 1) and Ba activation fragments.

Distinct Phenotypes for the Synthesis and Secretion of Factor B, ApoE, and Fibronectin by Macrophages in Culture. Thioglycollate-elicited macrophages synthesized and secreted detectable factor B, ApoE, and fibronectin at 2 and 72 h of culture (Fig. 6). In contrast, macrophages elicited with pyran copolymer synthesized only factor B at 2 h, and synthesized ApoE after culture for 72 h, while their secretion of factor B decreased somewhat. They did not synthesize fibronectin (Fig. 6).

During the culture of bone marrow–derived cells in the presence of L cell–conditioned medium, mononuclear phagocyte precursors serially express the differentiated properties of monocytes and macrophages, including protein secretion (39). Secretion of factor B was detectable by day 5, and remained at approximately the same relative rate through day 14 of culture (Fig. 7). In contrast, ApoE secretion did not commence until day 9 (Fig. 7) (39). Thus, factor B expression appears relatively early during the development of these cells from bone marrow precursors, and is constitutive by the monocyte and immature macrophage stages.

Modulation of Macrophage Factor B Secretion by LPS. There is evidence, based on hemolytic assays, that macrophage factor B activity is increased after treatment

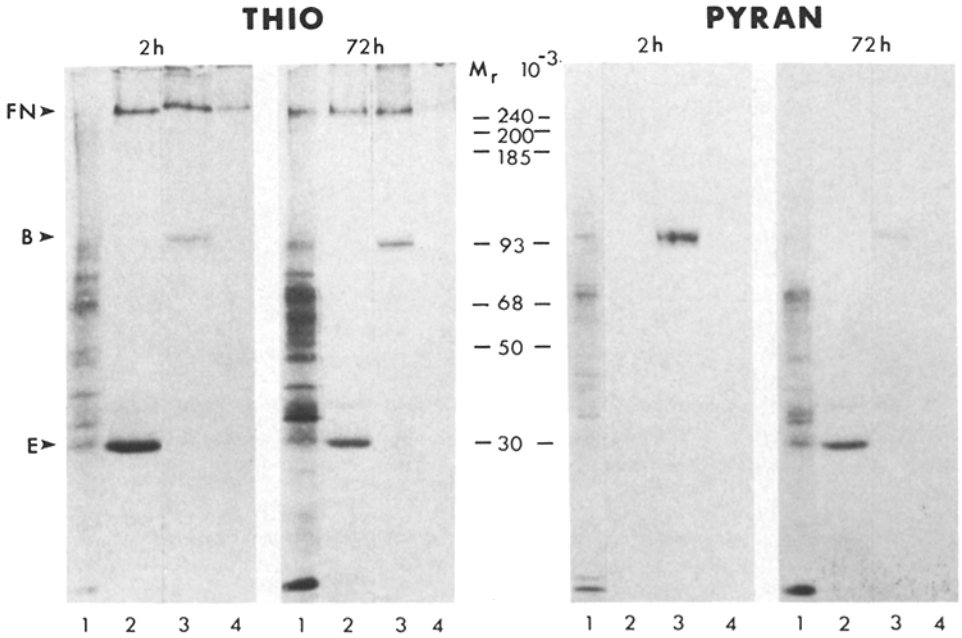

FIGURE 6. Comparison of factor B (*B*), ApoE (*E*), and fibronectin (*FN*) synthesis by thioglycollate-elicited (*THIO*) and pyran copolymer–elicited (*PYRAN*) macrophages in culture for 2 or 72 h. Fibronectin was identified as the M_r 240,000 band binding to *S. aureus* precipitates. (*1*) Trichloroacetic acid precipitate of CM from 1.25×10^5 macrophages; (*2*) immunoprecipitate of fibronectin plus ApoE from CM of 2×10^5 macrophages by rabbit anti–rat ApoE IgG; (*3*) immunoprecipitate of factor B from CM of 2×10^5 macrophages; (*4*) control immunoprecipitate with rabbit anti-BSA IgG of CM.

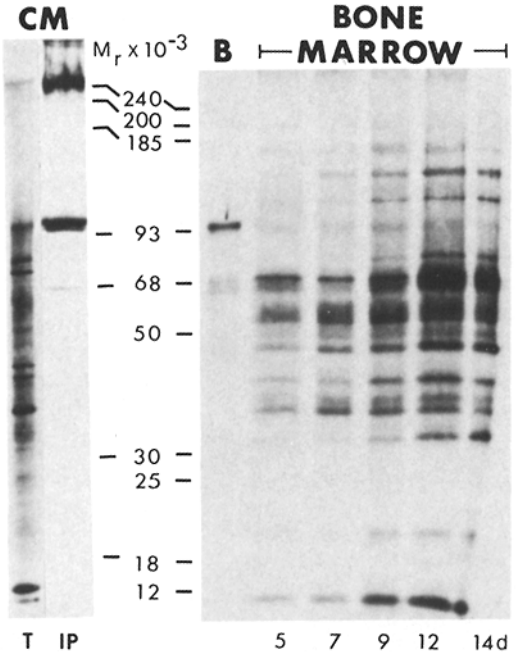

FIGURE 7. Secretion of factor B during culture of bone marrow–derived macrophages. Mouse bone marrow macrophages were cultured in Dulbecco's modified Eagle's medium plus 10% FBS supplemented with 10% horse serum and 10% L cell–conditioned medium for 5, 7, 9, 12, or 14 d. The media were then acid-precipitated (*BONE MARROW*) and analyzed on SDS-PAGE. CM from thioglycollate-elicited macrophages (*CM*) was also acid-precipitated (*T*) and factor B immunoprecipitated (*IP*) for comparison. Factor B standard (*B*) shows both M_r 93,000 and 68,000 bands. ApoE is the secreted protein seen at M_r 33,000 (arrow). Molecular weight markers are indicated.

TABLE IV

Effect of LPS on Accumulation of Factor B in the CM of Resident Peritoneal Macrophages

LPS concentration	Factor B concentration		Ratio of treated/control
ng/ml	*nM*	*ng/ml*	
0	0.6 ± 0.1	38	1
1	1.2 ± 0.6	75	2
10	1.2 ± 0.0	75	2
100	6.5 ± 0.7	409	11

Resident peritoneal macrophages (5×10^5 cells/well) from C3H/HeN mice were placed in serum-free medium containing 0–100 ng/ml of LPS for 24 h, in duplicate to quadruplicate cultures. Each medium sample was assayed at three dilutions in the human factor B RIA. Data are expressed in equivalents of human factor B (mean ± SD).

of cells with LPS (24). In the present study, injection of mice with LPS yielded macrophages with increased secretion of factor B into the medium (Table III). In addition, LPS added directly to cultures of macrophages increased production of factor B by as much as 11-fold at 100 ng/ml, as measured by RIA (Table IV).

Treatment of macrophages from the endotoxin-resistant C3H/HeJ mouse strain (37) in culture with as much as 10 μg/ml of LPS increases factor B accumulation by less than twofold. In contrast, LPS decreases ApoE secretion (37).

Discussion

Our data indicate that the major M_r 90,000–93,000 polypeptides synthesized by mouse macrophages in culture are factor B of the complement system. This glycoprotein is recognized to be the serine proteinase active in the alternative pathway of complement activation. The Bb activation fragment is, as has recently been shown, biologically active in inducing macrophage spreading (19, 20), intracellular killing (22, 23), and lymphocyte blastogenesis (48). Macrophages may also bind factor B to their surfaces (49). The results presented here provide physical evidence and quantitative data that confirm and extend earlier observations, based largely on measurements of hemolytic activity, that factor B is synthesized by macrophages (4–6, 24, 27). Our findings also indicate that a large percentage of factor B synthesized by macrophages is hemolytically active in the complement system, because biosynthetically radiolabeled factor B was cleaved after the addition of purified CVF, factor D, and Mg^{++}.

Several lines of evidence support these conclusions. First, the radiolabeled polypeptides of M_r 60,000–65,000 and 30,000–38,000, sometimes detected in immunoprecipitates from macrophage extracts and culture medium, may be the endogenous factor B activation fragments, factor Bb and factor Ba, respectively. Antibody prepared to factor B specifically precipitated biosynthetically labeled polypeptides of M_r 90,000–95,000, 60,000–65,000, and 30,000–38,000 from the medium and cellular extracts of mouse macrophages. Second, biosynthetically labeled M_r 90,000 and 93,000 polypeptides in macrophage culture medium decreased after complement activation in the medium by the addition of CVF and purified factor D, with a concomitant increase in the smaller M_r 60,000–65,000 ("Bb-like") and M_r 30,000–38,000 ("Ba-like") activation fragments. Immunochemical comparison by RIA of macrophage-associated factor B antigens and purified factor B showed that these polypeptides share common epitopes, indistinguishable in RIA analyses. The finding that the P388D1 macrophage cell line synthesized factor B while cultured in serum-free medium adds additional support to the interpretation that factor B is a biosynthetic product of macrophages and not of some other possible contaminating cell type. Finally, factor B accumulated in the CM of mouse macrophages to 10 nM (600 ng/ml) and was present in cell extracts at 4–8 nmol/10^6 cells, as determined by RIA.

Resident macrophages and macrophages elicited with the nonspecific inflammatory agent, thioglycollate, synthesize ApoE, but those elicited with pyran copolymer do not, suggesting that synthesis of ApoE is repressed during cellular activation (36). In contrast, factor B was synthesized, secreted, and accumulated in the medium of resident peritoneal macrophages, macrophages elicited with thioglycollate, and macrophages activated with pyran copolymer or BCG. We found no significant difference in the concentration of factor B in the medium at 24 or 48 h when peritoneal macrophages were collected from mice injected with thioglycollate, BCG, LPS, or *C. parvum*. Factor B was also present in the medium of mouse bone marrow–derived mononuclear phagocytes at 5 d in

culture, an early monocytic stage in their maturation, whereas ApoE secretion began only after 9 d. These data suggest that factor B is a constitutive product of macrophages. However, the synthesis of factor B could be modulated by certain signals, because treatment of macrophages with LPS resulted in an increase both in synthesis rate and in the concentration of factor B in the medium. We also noted that, after 72 h in culture, macrophages activated in vivo by BCG and pyran copolymer decreased their rates of secretion of factor B as their activated state was lost (36). This is in keeping with a recent observation that γ-interferon stimulates factor B synthesis (Z. Werb, unpublished data).

Although these experiments have established that the proenzyme factor B is secreted in prodigious amounts by macrophages, the immunoprecipitation patterns suggest that conversion of factor B to the active form, Bb, occurs slowly, if at all, in culture, raising questions about the events that may be required to activate macrophage-derived factor B to Bb. The cellular proteinases mediating activation of factor B are, at present, unknown; however, trypsin (59), plasmin (30), kallikrein (51), and factor D (in the presence of C3b) (30) have been shown to cleave and activate factor B in an experimental system of purified proteins. Because factor D and C3 are synthesized by macrophages (1, 2, 4, 5), and C3b is associated with lymphocytes (52), it is possible that these proteins and cells may act cooperatively to activate macrophage-derived factor B.

To assess the possible biological significance of macrophage-derived factor B, the relative amount of factor B synthesized by macrophages was quantified. In a previous study (20), activated factor Bb induced macrophage spreading at concentrations of 1–30 nM in culture medium. In the present study, factor B constituted 4–6% of the total biosynthetically labeled protein secreted by macrophages. Cellular and secreted factor B concentrations, measured by RIA, were well within this range of potential biological activity; however, it is important to note that the extent of activation of factor B to Bb in these cultures was not determined. Although factor Bb is significantly less active in cleaving C3 and C5 after release from the C3b-Bb complex (53), free factor Bb also retains a limited ability to cleave C3 and C5 in the absence of C3b (54). These combined findings make it difficult, at present, to assess what biological role factor B may play in macrophage cultures.

It is unlikely that factor B plays a major role as a macrophage plasminogen activator (55), because the properties of macrophage plasminogen activator (40, 56) are not similar to those of factor B. Synthesis of plasminogen activator is induced when macrophages are elicited with thioglycollate or BCG (35, 40, 56), whereas synthesis of factor B was constitutive. Although factor Bb exhibits an apparent K_m for plasminogen cleavage of 20 nM, it may be a relatively ineffective plasminogen activator, cleaving <0.8 mol of substrate plasminogen per mol of factor Bb per minute under the best experimental conditions to date (J. Sundsmo, unpublished data.).

Many bacterial, cellular, viral, parasitic, and chemical agents may activate the alternative pathway of complement. The present findings raise the possibility that macrophage-derived factor B may participate locally in certain physiologic and pathologic immune responses. Whether several cell types are necessary to

activate and assemble complement proteinases of the leukocyte complement system (52) remains to be determined.

Summary

Factor B, the complement alternative pathway serine proteinase, a class III gene product of the major histocompatibility complex, is a major constitutive secretion product of mouse mononuclear phagocytes. This glycoprotein was synthesized and secreted by macrophages as a doublet of M_r 90,000 and 93,000 polypeptides that were immunoprecipitable with antibodies raised to human serum factor B, and that were indistinguishable from plasma factor B by immunoreactivity, peptide mapping, and molecular weight. Macrophage factor B was cleaved and activated to factor Bb- and Ba-like fragments by factor D and cobra venom factor. Some conversion of macrophage factor B to Bb-sized fragments occurred spontaneously in the conditioned culture medium after several hours. Factor B represented ~0.5% of newly synthesized protein and 4–6% of the secreted protein of resident peritoneal macrophages and macrophages elicited with thioglycollate broth, pyran copolymer, $NaIO_4$, bacillus Calmette-Guerin, or *Corynebacterium parvum*. We detected synthesis of factor B immediately upon explanting these macrophages in culture; synthesis continued for several days in culture. The rate of secretion of factor B, as a proportion of total protein secretion in culture, remained constant with time. By radioimmunoassay, factor B antigens accumulated in the 24-h macrophage-conditioned culture medium at 2–10 nM, and was present in cell lysates at 4–8 nmol per 10^6 cells. We detected synthesis of factor B in bone marrow–derived macrophages as early as 5 d of culture. The P388D1 macrophage line synthesized factor B, but mouse L cells did not. In contrast, apolipoprotein E, another secreted protein of macrophages, was secreted by resident and thioglycollate-elicited macrophages but not by freshly harvested pyran copolymer–activated macrophages. Its synthesis was initiated at day 9 in culture of bone marrow–derived macrophages. These data support the classification of factor B as a constitutive biosynthetic and secreted protein of immature and mature macrophages in various states of activation. Production of factor B was modulated by treatment of macrophages in vivo or in culture with bacterial lipopolysaccharide endotoxin, which increased the synthesis, secretion, and accumulation of factor B up to 11-fold.

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