Bone Marrow-derived Mononuclear Phagocytes Autoregulate Mannose Receptor Expression*

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This study extends our previous observation that surface mannose receptor expression by pure populations of CSF-1-dependent bone marrow-derived macrophages increases with time (Clohisy, D. R., Bar-Shavit, Z., Chappel, J. C., and Teitelbaum, S. L. (1987) J. Biol. Chem. 262, 15922–15928). We presently find, however, that the progressive enhancement of 125I-mannose-BSA, 125I-Mannose-bovine serum albumin (125I-Man-BSA) binding per cell reflects cell number rather than duration of culture. In fact, macrophages plated at high density bind 8-fold more 125I-Man-BSA than do their low density counterparts, with no difference in receptor-ligand affinity. Furthermore, cells cultured at high density are ultimately subjected to lower levels of exogenously provided macrophage growth factor, and fewer are in interphase. By obtaining synchronous populations of quiescent bone marrow macrophages, however, we demonstrate that neither cell cycling nor attendant levels of colony stimulating factor-1 influence mannose receptor expression.

Our next series of experiments established that density-related mannose receptor expression reflects removal, by marrow macrophages, of a “down-regulating” factor contained in culture medium. To this end, we treated mononuclear phagocytes with either macrophage- or control-conditioned medium and found that, via a fetal calf serum-residing protein(s), only control medium is capable of noncompetitively reducing 125I-Man-BSA binding in a dose-dependent manner. Moreover, reconstituted 20–40% (NH4)2SO4-precipitable fractions derived from either sham-conditioned medium or fetal calf serum are capable of down-regulating mannose receptor expression. Alternatively, the same fraction obtained from macrophage-conditioned medium contains no such activity. Finally, initial characterization of the down-regulating factor reveals it to be acid-activatable and trypsin-sensitive, yet resistant to heating to at least 80 °C, ribonuclease A, or freezing and thawing. We conclude that bone marrow macrophages up-regulate expression of their own plasma membrane mannose receptor by inactivating a noncompetitive, serum-residing inhibitory protein(s).

The mannose receptor is a 175-kDa plasma membrane component, which in the marrow resides exclusively in cells of the monocyte/macrophage family (1). The protein is known to be involved in recognition and endocytosis of particles and other substances displaying terminal mannose residues. Thus, it is likely that the mannose receptor is pivotal to an activity which characterizes the macrophage phenotype, namely phagocytosis (2).

Recently, the mannose receptor has also been found to serve as a marker of macrophage differentiation. 1,25-Dihydroxyvitamin D, an agent known to promote monocyte differentiation of leukemic cells (3), also accelerates mannose receptor expression (4). Taken with evidence from others that the mannose receptor is differentiation-dependent (5), our findings indicate that this membrane-residing protein may be used as a hallmark of macrophage maturation. Furthermore, the apparent association of mannose-receptor expression and macrophage differentiation raises the possibility that both events may be functionally related. Hence, an understanding of the means by which developing mononuclear phagocytes express this protein may yield important clues into the fundamentals of macrophage differentiation.

We found, during our prior studies, that appearance of the mannose receptor on bone marrow macrophage precursors increases with time in culture (4). While this finding may reflect the differentiation-associated properties of the receptor, they also raise the possibility that its expression is regulated by extracellular (medium-contained) components which are progressively modified by the developing cell. In fact, we demonstrate herein that a serum-residing protein factor “down-regulates” plasma membrane expression of the mannose receptor and that bone marrow macrophages progressively inactivate this inhibitory agent, thereby leading to enhanced binding of mannosylated radioligand.

MATERIALS AND METHODS

Unless otherwise specified, all chemicals were obtained from Sigma. Horse and fetal calf sera were purchased from Hazelto Dutchland Research Products (Denver, PA). Murine L929 cells were a gift of H. S. Lin (Washington University Medical School, St. Louis, MO), mannosylated BSA (42 mol of sugar/mol of protein) was purchased from E-Y Laboratories (San Mateo, CA), and ribonuclease A was from Boehringer Mannheim.

Preparation of Stage 1 CSF-1—Prepared by modification of method of E. R. Stanley (6). Serum-free conditioned medium from L929 cells was chromatographed by a batch calcium phosphate method. Specifically, 250 ml of calcium phosphate gel (300 ml of 0.4 M NaPO4 plus 3 liters of 0.057 M CaCl2) were added per liter of L929 conditioned supernatant.

1 The abbreviations used are: BSA, bovine serum albumin; 125I-Man-BSA, 125I-mannosylated bovine serum albumin; CSF-1, colony stimulating factor-1; MEM, minimum Eagle’s medium; PBS, phosphate-buffered saline: Heps, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid.

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media and stirred overnight. The supernatant was recovered and the gel rinsed twice with 3 mM NaPO4 (pH 6.5) and once with 94 mM NaPO4. Supernatants were collected from the high molarity phosphate rinses and dialyzed against deionized water. The Lowry assay was used for protein determination (7). Final specific activities were typically 10^6 units/mg protein.

**Marrow Cells**—a modification of Eagle's Medium (MEM) (Sybron, Washington, D. C.) with 500 units/ml Stage I CSF-1, 15% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). **Marrow Cells**—Nonadherent cells were obtained from bone marrow cultures of 9-12-week-old male A/J mice (Jackson Labs, Benton Harbor, MI) as prepared as described previously (4). The ends of freshly harvested femurs were excised, and the cells collected by flushing the medullary cavity with ice-cold α-MEM through a 25-gauge needle. The marrow plug was dispersed by several passages through an 18-gauge needle, and the cells were pelleted (800 × g for 5 min at 4°C). The pellet was resuspended in ice-cold α-MEM and the number of nucleated cells determined by counting an aliquot of the resuspended cells in 2% acetic acid. Cells (1 × 10^6 cells/ml) were then seeded into tissue culture dishes (Falcon Plastics) at a density of 3.4 × 10^4 cells/cm^2 in the presence of complete medium containing 900 units/ml of Stage I CSF-1. After 24-h incubation, nonadherent cells were collected and discarded. The adherent population was then pelleted (800 × g for 7 min at 4°C), resuspended in 1 ml of Pronase solution (0.02% w/v Pronase, B grade, Calbiochem, 1.5 mM EDTA in PBS/10^6 nucleated cells) and incubated for 15 min at 37°C. Pronase treatment was stopped by the addition of horse serum (0.2 ml) to the supernate. The suspension was then resuspended onto 15 ml of ice-cold horse serum and incubated on ice for 15 min. The cell suspension was then pelleted (1200 × g for 7 min at 4°C), resuspended in complete medium and nucleated cell number determined. This group of cells, which is 24 h post-bone marrow cell transplantation, is designated "marrow cells." When these cells are cultured in the presence of 500 units/ml CSF-1 for 7 days, all colony forming units are positive for the monococyte-specific enzyme, α-naphthyl acetate esterase.

**Adherent Bone Marrow-derived Mononuclear Phagocytes**—Marrow cells were cultured at the concentrations indicated in 24-well plates (NUNC) in 2 ml of complete medium/well. After designated periods, adherent cells were used for cell cycle analysis and binding studies.

**Adherent Cell Counts**—Adherent mononuclear phagocytes were washed with cold PBS, detached from the tissue culture plastic after a 5-min incubation at 20°C in 0.005% tritertigen (Calbiochem), and counted by hemocytometer.

**Analysis of DNA Content per Nuclei**—Adherent mononuclear phagocytes in 24-well plates were rinsed three times with PBS and 1.0 ml of Krishan's reagent (9). After the cells detached, they were harvested and rinsed twice with fresh Krishan's reagent. The resulting nuclei were placed on ice and DNA content per nuclei determined in a Coulter Counter (Model ZM, Beckman Instruments, Fullerton, CA). **[3H]Thymidine Incorporation Assay**—Adherent mononuclear phagocytes were pulsed with 50 μCi of [3H]thymidine (ICN) and incubated at 37°C. After a 60-min incubation, the cells were rinsed with PBS and incubated (37°C) for 30 min in 10% trichloroacetic acid. They were then re-rinsed with an ethanol-ether (3:1) solution and extracted for counting in 0.1 N NaOH.

**Preparation of Control- and Macrophage-conditioned Medium**—Marrow cells were plated into 24-well plates at 1.5 × 10^6 cells/ml and 2 ml/well in complete medium. After 48 h in culture, the macrophage-conditioned medium from each well was collected, concentrated 1:10 (Amicon-YM5), and stored at 4°C. Control-conditioned medium was generated in a similar but cell-free manner.

**Mannose-Bovine Serum Albumin Iodination (10)**—100 μg of mannose-bovine serum albumin (Man-BSA) was mixed with 1 μCi of Na_2^125I (Amersham Corp.) and 300 μg of chloramine T in 80 μl of 0.1 M NaPO4 buffer (pH 7.6). The reaction was terminated after 10 min on ice by addition of 190 μl of sodium metabisulfite (2.4 mg/ml) and 190 μl of potassium iodide (10 mg/ml). The sample was then run on a Sephadex G-50 column (1 × 20 cm) buffered in 10 mM Tris-HCL (pH 7.5). 0.4-ml samples were collected and active fractions identified by their radioactivity. Protein determination was performed by the Miller method (11) and specific activity was typically 5–8 × 10^6 cpm/μg Man-BSA with >95% of total counts trichloroacetic acid-precipitable. Ligand was used within 2 weeks of iodination.

**lZ5I-Man-BSA Binding Assay**—Binding determinations at 4°C of 125I-Man-BSA binding to bone marrow mononuclear phagocytes involved slight modifications of techniques previously described (10). Such procedures result in nonspecific binding representing 10–20% of total cell-associated counts.

The cells were washed three times (0.4 ml/well/wash) with HHBG ( Hank's Balanced Salt Solution, 10 mM Hepes, 10 mM Tris, 0.1% glucose, and 10 mg/ml BSA, pH 7.1) and incubated with 0.2 ml of various concentrations of 125I-Man-BSA in HHBG plus 0.2 ml of fetal calf serum. Equilibrium binding was achieved after a 48-h incubation, and the level of cell-associated ligand determined. After 48 h, the incubation medium was aspirated, and cell layers quickly rinsed six times with Hank's Balanced Salt Solution. Cells were dissolved in 1.0 N NaOH (0.5 ml/well) and cell-bound radioactivity of NaOH-solubilized material was determined for all binding points. Transformation of binding data to determine dissociation constants and estimate the number of available binding sites was performed by methods of Scatchard (12).

**Evaluation of the Influence of Conditioned Medium and Fetal Calf Serum on 125I-Man-BSA Binding**—Marrow cells were plated at 0.5 × 10^6 cells/ml in 24-well plates (1 ml/well). Twenty-four hours later, various aliquots of either macrophage- or sham (control)-conditioned medium or fetal calf serum were added to appropriate wells. After the indicated additional culture period (12–36 h), cells were placed at 4°C and the specific binding of 125I-Man-BSA per mg of cell protein determined by incubation with 2 μg/ml 125I-Man-BSA ± 2 mg/ml Man-BSA. Cell-associated binding was assessed by the method of Lowry (7) and standardized per unit of protein.

**CSF-1 Levels**—Radioimmunoassay determinations of CSF-1 levels were kindly performed by E. R. Stanley (Albert Einstein Medical Center, Bronx, NY).

**Ammonium Sulfate Precipitation**—All ammonium sulfate samples were precipitated in a percent-to-percent methodology described by Green and Hughes (13). The precipitated samples were dissolved in PBS, which had been diluted 1:10 at a concentration 10 times that of the original sample. The solutions were dialyzed against α-MEM prior to use.

**Acid Activation**—The 20–40% (NH_4)_2SO_4-precipitable fraction of fetal calf serum was redissovled and concentrated 10 times in PBS and dialyzed against α-MEM. The solution was then titrated to pH 2.0 with 1 N HCl for 5 min at 4°C, during which time a precipitate formed which was removed by microfiltration (12,000 × g for 5 min). The supernatant was aspirated and left at 4°C for 1 week after which it was dialyzed against α-MEM overnight. Control material was treated identically except for acidification. A suboptimal down-regulating volume (10 μl) of material was tested for its effects on 125I-Man-BSA binding.

## RESULTS

### Effect of Cell Number on the Binding of 125I-Man-BSA by Bone Marrow Mononuclear Phagocytes—We recently observed that 125I-Man-BSA binding by bone marrow mononuclear phagocytes increases with time in culture (4). Since these cells are actively dividing, we explored the possibility that such binding actually relates to cell number and not culture duration. To this end, we plated various concentrations of marrow cells (1.5, 7.5, and 15 × 10^6 cells/ml) and after 2 days assessed 125I-Man-BSA binding at 4°C.

As seen in Fig. 1A, specific binding of 125I-Man-BSA per 10^6 cells increases progressively with cell density. These differences are reflected by maximal specific bindings of 0.3, 0.8, and 2.6 ng of radioligand/10^6 cells at cell densities of 5.0, 18.1, and 42.5 × 10^6 cells/well, respectively. Accompanying Scatchard plots (Fig. 1B) demonstrate that this progressive enhancement of binding reflects increased available sites per cell, as receptor affinities are similar. Specifically, low, intermediate, and high density cells display approximately 2.5, 6.0, and 20 × 10^6 receptors/cell, respectively. These data demonstrate that mannose receptor expression is influenced by cell number and not duration of culture.

### Effect of Cell Density on CSF-1 Levels and Cell Cycling—Having determined that 125I-Man-BSA binding per cell increases with cell number, we next sought to identify distinct characteristics of high and low density populations which may alter radioligand binding. Two parameters which we consid—
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**Table I**

**Characterization of high and low density cell populations**

| Marrow Cells Plated | Adherent Cell Number per Well | CSF-1 pM | DNA Content per Nucleus |
|---------------------|-------------------------------|---------|-------------------------|
| LOW                 | (0.15 x 10^6 cells/ml)       | 4.6 x 10^4 | 344                     |
| HIGH                | (1.5 x 10^6 cells/ml)        | 4.2 x 10^5 | 106                     |

**Fig. 1.** Effect of cell number on 125I-mannose BSA binding by bone marrow-derived mononuclear phagocytes. Marrow cells were plated at various densities and 125I-Man-BSA binding determined per 10^6 cells after 48 h. **A**, saturation binding isotherms; **B**, Scatchard analysis of binding data. All samples shown are the means of duplicate determinations. □, 4.2 x 10^6 cells/well; △, 1.8 x 10^6 cells/well; ○, 0.5 x 10^6 cells/well.

**Fig. 2.** Regulation of mononuclear phagocyte mitogenesis. Asynchronously dividing bone marrow macrophages received 50 units/ml CSF-1 for 24 h and were then reexposed (A) to either a low (50 units/ml) or mitogenic dose (1000 units/ml) of CSF-1. Tyramine incorporation per well was determined at designated times, and DNA content per nucleus examined by flow cytometry analysis at points A, C, and D (see respective inserts). ○, asynchronous cells; △, 50 units/ml CSF-1; ◊, 1000 units/ml.

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**Characterizing a Model to Isolate Cycling and Noncycling Populations of Bone Marrow-derived Mononuclear Phagocytes**—With the intent of ultimately defining the role of cell cycling in regulating 125I-Man-BSA binding, we adopted previously described techniques designed to yield populations of bone marrow-derived macrophages enriched with quiescent (G_0/G_1 phase) or cycling (S-phase) cells (14). Such methods are predicated on the finding that cell survival and proliferation are dependent on the macrophage growth factor, CSF-1 (8). To obtain quiescent cells, we determined that 50 units of CSF-1/ml is sufficient to maintain marrow macrophage survival without stimulating mitogenesis (data not shown). More specifically, 24 h after treating asynchronously dividing cells with 50 units of CSF-1/ml, we found a greater than 90% decrease in [3H]thymidine incorporation and more than 90% of the cells in G_0/G_1 (i.e., pre-DNA synthetic) phase by flow cytometry analysis (Fig. 2, insert A). These data characterize such cells as a population of noncycling, bone marrow-derived macrophages.

With the capacity to produce quiescent cells in hand, we turned to the isolation of their cycling counterparts. To this end, we treated quiescent cells with increasing quantities of CSF-1 and determined that 1000 units/ml induces maximal mitogenesis (data not shown). Further, as shown in Fig. 2, when quiescent cells (point A) are exposed to 1000 units of
CSF-1, they exhibit a time-dependent increase in [3H]thymidine incorporation. Most notably, after addition of the mitogen (Fig. 2, point A), [3H]Tdr incorporation, and thus entry into S-phase, commences after 12–16 h with the peak in DNA synthesis occurring at 36 h. Accompanying insets of analysis of DNA content per nucleus estimate that after a 36-h exposure to 1000 units/ml CSF-1, 45% of bone marrow macrophages are in S-phase (Fig. 2, insert D) as compared to less than 5% exposed for the same time period to 50 units/ml phages are in S-phase (Fig. 2, insert D) as compared to less of DNA content per nucleus estimate that after a 36-h exposure to 1000 units/ml CSF-1, 45% of bone marrow macrophages are in S-phase (Fig. 2, insert D) as compared to less than 5% exposed for the same time period to 50 units/ml CSF-1 (Fig. 2, insert C).

Comparison of 125I-Man-BSA Binding by Cycling versus Noncycling Mononuclear Phagocytes—This set of experiments was designed to determine if cell cycling influences 125I-Man-BSA binding. Culture methods described in the previous section were instituted to obtain simultaneously separate populations of noncycling (Fig. 2, point C) and cycling (Fig. 2, point D) cells and their respective capacities to bind 125I-Man-BSA are compared in Fig. 3. The data demonstrate that regardless of cell cycling dissociation constants as well as estimated number of available binding sites are similar.

Influence of CSF-1 on 125I-Man-BSA Binding by Bone Marrow Macrophages—We next sought to determine if CSF-1 influences expression of the 125I-Man-BSA binding site. Hence, we cultured quiescent cells (Fig. 2, point A) with either 1000 units of CSF-1/ml or 50 units of CSF-1/ml, and after 8 h (Fig. 2, point B) assessed their binding of 125I-Man-BSA.

Fig. 4 demonstrates that both binding affinity and capacity are nearly indistinguishable regardless of CSF-1 concentration. It should be noted that since the cells in this particular experiment bound relatively high levels of 125I-Man-BSA (1 ng/10^6 cells), we also examined the influence of CSF-1 on those expressing fewer receptors (i.e. low density cultures) and found that in this circumstance CSF-1 also fails to impact on 125I-Man-BSA binding (data not shown).

The Influence of Medium-contained Factors on 125I-Man-BSA Binding—To evaluate the feasibility that bone marrow macrophages may alter marrow binding by modifying their environment, we incubated complete culture medium for 48 h in the presence of bone marrow macrophages (macrophage-conditioned medium) or in their absence (control-conditioned medium) concentrated 10-fold. One hundred μl of either macrophage- or control-conditioned medium was then added to bone marrow macrophages, and after 12, 24, or 36 h, 125I-Man-BSA bound per mg cell protein was determined at a saturating dose of the ligand (2 μg/ml).

Results displayed in Table II document that the specific binding of 125I-Man-BSA by cells treated with either control- or macrophage-conditioned medium increases in a stepwise fashion with time in culture. More importantly, however, those cells exposed to macrophage-conditioned medium bind more radioligand at each time point than do their sham medium-treated counterparts. Fig. 5 documents that the enhanced binding capacity of the macrophage-conditioned medium-exposed cells reflects a greater than 2-fold increase in the number of sites per cell with unaltered affinity.

These differences in 125I-Man-BSA binding capacity could, however, represent either "up-regulation" of available binding sites by macrophage-conditioned medium or "down-regulation" by control-conditioned medium. To resolve this issue, cells were exposed to increasing aliquots of either form of medium for 36 h. As before, 125I-Man-BSA specific binding was determined at a saturating concentration of the ligand (2 μg/ml). The results of this experiment demonstrate that macrophages incubated with control-conditioned medium bind progressively less 125I-Man-BSA (Fig. 6). Specifically, untreated cells bind 103 ± 11 ng of 125I-Man-BSA/mg protein, those exposed to 150 μl of control-conditioned medium, only 23 ± 8 ng/mg protein, and macrophages incubated with up to 150-μl aliquots of macrophage-conditioned medium bind as much radioligand (96 ± 15 ng/mg protein) as do virgin cells.

Influence of Fetal Calf Serum on 125I-Man-BSA Binding—We next turned to identifying the inhibitor of 125I-Man-BSA binding present in control-conditioned culture medium, which conceivably could include components of modified Eagle's Medium, Stage I CSF-1, or fetal calf serum. Data not shown.
demonstrate that the inhibitory factor is maintained within $M_r$ 14,000 exclusion dialysis tubing, precluding the possibility that the factor of interest is modified Eagle's Medium. In addition, data presented under "Influence of CSF-1 on $^{125}$I-Mannose-BSA Binding by Bone Marrow Macrophages," excludes the possibility that CSF-1 or any other element of our Stage I CSF-1 preparation is responsible for regulation of $^{125}$I-Mannose-BSA binding.

Consequently, we explored the effect of fetal calf serum on $^{125}$I-Mannose-BSA binding. Thus, marrow cells were cultured for 24 h in complete medium followed by addition of various volumes of 10-fold concentrated fetal calf serum (0–100 μl). After an additional 36 h, $^{125}$I-Mannose-BSA binding was determined at a saturating quantity of the ligand (2 μg/ml).

As shown in Fig. 7, $^{125}$I-Mannose-BSA binding by bone marrow macrophages falls with increasing volumes of serum. In fact, whereas nonsupplemented cells bind 43 ± 3 ng of $^{125}$I-Mannose-BSA/mg of protein, those treated with 100 μl of concentrated calf serum bind only 27 ± 6 ng/mg protein.

These observations raised the possibility that fetal calf serum simply competes for available $^{125}$I-Mannose-BSA binding sites and is, in effect, acting as cold ligand. This concern was investigated by simply preincubating bone marrow macrophages at 4 °C in 1 ml of binding buffer (HBBG) plus 0–100 μl of fetal calf serum for 6 h, rinsing the wells, and then measuring $^{125}$I-Mannose-BSA binding per mg cell protein by our standard assay.

Data shown in Fig. 8 document that fetal calf serum does not compete with $^{125}$I-Mannose-BSA for available binding sites. Taken together, these findings demonstrate that a component of fetal calf serum truly "down-regulates" $^{125}$I-Mannose-BSA binding sites.

Influence of Fetal Calf Serum Ammonium Sulfate-precipitable Fractions on $^{125}$I-Mannose-BSA Binding—Initial characterizations of element responsible for down-regulating mannose receptor expression was undertaken by assessing the capacity of (NH$_4$)$_2$SO$_4$-precipitable fractions of fetal calf serum to...
regulate \(^{125}\text{I}-\text{Man-BSA}\) binding. Thus, marrow cells incubated in complete medium for 24 h were supplemented with 100 \(\mu\)l of each \((\text{NH}_4)_2\text{SO}_4\)-precipitable fraction (0, 0–20, 20–40, 40–60, and 60–80\%). Thirty-six hours later, \(^{125}\text{I}-\text{Man-BSA}\) (2 \(\mu\)g/ml) was added at 4 °C and binding per mg cell protein determined. All values shown represent the means ± S.E. of quadruplicate determinations.

**Fig. 8. Effect of fetal calf serum on the assay for \(^{125}\text{I}-\text{Man-BSA}\) binding.** Cells were incubated for 6 h at 4 °C in 1 ml of binding buffer (HBBG) plus 0–100 \(\mu\)l of fetal calf serum. The wells were then rinsed, incubated with \(^{125}\text{I}-\text{Man-BSA}\) (2 \(\mu\)g/ml) at 4 °C and binding per mg cell protein was determined. All values represent the mean ± S.E. of quadruplicate determinations.

**Fig. 9. Effect of treatment with reconstituted \((\text{NH}_4)_2\text{SO}_4\)-precipitable fractions of fetal calf serum on \(^{125}\text{I}-\text{Man-BSA}\) binding.** After 24 h in culture, cells were supplemented with 100 \(\mu\)l of reconstituted 20–40\% \((\text{NH}_4)_2\text{SO}_4\)-precipitable fractions from control and bone marrow-derived macrophage-conditioned media. Thirty-six hours later, \(^{125}\text{I}-\text{Man-BSA}\) (2 \(\mu\)g/ml) was added at 4 °C and binding per mg cell protein determined. All values shown represent the mean ± S.E. of quadruplicate determinations. ---

**Table III**

| Positive control | Effect of trypsin treatment of 20–40\% \((\text{NH}_4)_2\text{SO}_4\)-precipitable serum fraction on \(^{125}\text{I}-\text{Man-BSA}\) binding |
|-----------------|---------------------------------------------------------------------|
| 20-40% (NH\(_4\)_2SO\(_4\)) | 64,271 |
| 20-40% (NH\(_4\)_2SO\(_4\)) | 17,207 |
| 20-40% (NH\(_4\)_2SO\(_4\)) | 22,770 |
| 20-40% (NH\(_4\)_2SO\(_4\)) | 41,647 |
| 20-40% (NH\(_4\)_2SO\(_4\)) | 49,997 |
| 20-40% (NH\(_4\)_2SO\(_4\)) | 64,727 |

**Table IV**

| Positive control | Effect of ribonuclease A treatment of 20–40\% \((\text{NH}_4)_2\text{SO}_4\)-precipitable serum fraction on \(^{125}\text{I}-\text{Man-BSA}\) binding |
|-----------------|---------------------------------------------------------------------|
| 20-40% (NH\(_4\)_2SO\(_4\)) | 27,936 |
| 20-40% (NH\(_4\)_2SO\(_4\)) | 3,239 |
| 20-40% (NH\(_4\)_2SO\(_4\)) | 1,765 |

**Fig. 10. Effect of 20–40\% \((\text{NH}_4)_2\text{SO}_4\)-precipitable fractions of control- and macrophage-conditioned medium on \(^{125}\text{I}-\text{Man-BSA}\) binding.** After 24 h in culture, cells were supplemented with 100 \(\mu\)l of reconstituted 20–40\% \((\text{NH}_4)_2\text{SO}_4\)-precipitable fractions from control and bone marrow-derived macrophage-conditioned media. Thirty-six hours later, \(^{125}\text{I}-\text{Man-BSA}\) (2 \(\mu\)g/ml) was added at 4 °C and binding per mg cell protein determined. All values shown represent the mean ± S.E. of quadruplicate determinations.

**Fig. 11. Effect of trypsin treatment of 20–40\% \((\text{NH}_4)_2\text{SO}_4\)-precipitable serum fraction on \(^{125}\text{I}-\text{Man-BSA}\) binding.** After 24 h in culture, cells were supplemented with 100 \(\mu\)l of reconstituted 20–40\% \((\text{NH}_4)_2\text{SO}_4\)-precipitable fractions from control and bone marrow-derived macrophage-conditioned media. Thirty-six hours later, \(^{125}\text{I}-\text{Man-BSA}\) (2 \(\mu\)g/ml) was added at 4 °C and binding per mg cell protein determined. All values shown represent the mean ± S.E. of quadruplicate determinations. ---

similarly treated macrophage-conditioned medium bind 28 ± 6 ng (p < 0.05) (Fig. 10). These data demonstrate that bone marrow-derived mononuclear phagocytes inactivate a medium-residing protein factor contained in the 20–40\% \((\text{NH}_4)_2\text{SO}_4\)-precipitable fraction of fetal calf serum responsible
TABLE V
Effect of freezing and thawing of 20–40% (NH₄)₂SO₄-precipitable serum fraction on ¹²⁵I-Man-BSA binding

| ¹²⁵I-Man-BSA | cpm bound/well |
|-------------|----------------|
| Negative control | 64,271 |
| 20–40% (NH₄)₂SO₄ fractions | |
| Untreated | 17,207 |
| Freeze-thaw | 19,847 |
progressively inactivate the down-regulating factor. Failure of
pretreatment with fetal calf serum at 4 °C to reduce radioli-
gand binding indicates that our observations do not merely
reflect competition for the mannose receptor by a nonradioac-
tive moiety.

Finally, we have begun to characterize the down-regulating
factor. It is a protease-sensitive, acid-activable moiety with
what appears to be exceptionally stable characteristics. For
example, the activity resists freezing, thawing, and heating to
at least 80 °C.

Thus, our experiments document the presence of a serum-
residing protein factor capable of down-regulating membrane
expression of the mannose receptor and that macrophages are
capable of inactivating this regulatory protein(s). These find-
ings raise important issues regarding the phagocytic capacity
of macrophages and underscore the importance of carefully
defining culture conditions in studies involving expression of
this surface binding site.

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REFERENCES
1. Wileman, T. E., Lennartz, M. L., and Stahl, P. (1986) Proc. Natl.
   Acad. Sci. U. S. A. 83, 2501–2506
2. Stahl, P., Schlesinger, P. H., Sigardson, E., Rodman, J. S., and
   Lee, Y. C. (1980) Cell 19, 207–215
3. Bar-Shavit, Z., Teitelbaum, S. L., Reitsma, P., Hall, A., Pegg, L.
   E., Trial, J., and Kahn, A. J. (1983) Proc. Natl. Acad. Sci.
   U. S. A. 80, 5907–5911
4. Clohisy, D. R., Bar-Shavit, Z., Chappel, J. C., and Teitelbaum, S.
   L. (1987) J. Biol. Chem. 262, 15922–15929
5. Shepard, V. L., and Stahl, P. D. (1984) in Lysosomes (Dingle, J.
   T., Dean, R. T., and Sly, W., eds) pp. 83–98, Elsevier Scientific
   Publishing Co., Amsterdam
6. Stanley, E. R. (1985) Methods Enzymol. 116, 564–587
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.
   (1951) J. Biol. Chem. 193, 265–275
8. Tushinski, R. J., Oliver, J. T., Guilbert, L. J., Tynan, P. W.,
   Warner, J. R., and Stanley, E. R. (1982) Cell 28, 71–81
9. Krishan, A. (1975) J. Cell Biol. 66, 188–193
10. Konish, M., Sheperd, V., Holt, G., and Stahl, P. (1983) Methods
    Enzymol. 98, 301–304
11. Miller, G. L. (1959) Anal. Chem. 31, 964
12. Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 660–672
13. Green, A. A., and Hughes, W. L. (1955) Methods Enzymol. 1, 67–
    90
14. Gandour, D. M., and Walker, W. S. (1983) J. Immunol. 130,
    1108–1112