Isolation and Characterization of a Mannose-specific Endocytosis Receptor from Human Placenta*

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Michelle R. Lennartz†, F. Sessions Colef, Virginia L. Shepherd§, Thomas E. Willemann, and Philip D. Stahl**
From the Department of Cell Biology and Physiology, Washington University School of Medicine and ¶Department of Pediatrics, Children’s Hospital, St. Louis, Missouri 63110

A receptor which recognizes glycoproteins bearing terminal mannose residues has been isolated from human placental membranes. Washed membranes were solubilized with buffer containing 1% Triton X-100 and applied to a mannose-Sepharose affinity column. The column was eluted with buffer containing 200 mM mannose and 1% cholate. The major protein eluted exhibited a molecular weight of 175 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The protein binds 125I-labeled mannosylated bovine serum albumin in a saturable fashion with a dissociation constant of 4 nM. Ligand binding is pH-dependent with maximal binding above pH 6.5. This binding can be inhibited with EDTA, mannose, fucose, mannan, glucuronidase, and bovine serum albumin conjugated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Polyclonal antibodies generated against the mannose binding protein immunoprecipitate a single 175-kDa protein species from both surface-iodinated and biosynthetically labeled human monocyte-derived macrophages.

Macrophages express a cell surface receptor that recognizes and assimilates mannosylated glycoproteins. Extracellular ligands bind to the receptor and enter the cell by receptor-mediated endocytosis (1). The receptor-ligand complex dissociates in an acidic endosomal compartment and the receptor recycles to the cell surface (2). In addition to its endocytic function, the mannose receptor may also be involved in phagocytosis of mannose-coated particles such as zymosan (3). The pathway followed by the receptor during phagocytosis has not been elucidated. The expression of the mannose receptor by macrophages appears to be closely regulated. It is absent from the surface of circulating monocytes (4) but is expressed upon monocyte-macrophage differentiation. The expression of receptor in differentiated macrophages can be increased by anti-inflammatory steroids (5) and reduced by lymphokines (6). The mechanism(s) underlying this regulation are unknown although anti-inflammatory drugs appear to act by increasing receptor biosynthesis.

In order to obtain sufficient quantities of the receptor for structural and biochemical studies, we have purified the human mannose receptor from placenta. This paper describes the purification of a placentally derived protein with properties identical to those of the macrophage endocytosis receptor.

EXPERIMENTAL PROCEDURES

Materials—Human term placenta was obtained from Jewish Hospital, St. Louis, MO. New Zealand White rabbits were purchased from Boswell’s Bunny Farm, Pacific, MO. Mannosylated bovine serum albumin was purchased from E-Y Laboratories, San Mateo, CA. Fucosylated bovine serum albumin was prepared by the method of Lee et al. (7). Glutaraldehyde-activated silica, leupeptin, and chymostatin were obtained from Boehringer Mannheim. Freund’s complete and incomplete adjuvants were obtained from ICN Immunobiologicals, Lisle, IL. Mannose-Sepharose was prepared as previously described (8). All other reagents were purchased from Sigma unless otherwise specified.

Buffers—Loading buffer was 10 mM Tris-Cl, pH 7.4, 1.25 mM NaCl, 15 mM CaCl2, 0.02% NaN3; Tris-buffered saline (TBS) was 10 mM Tris-Cl, pH 7.4, 150 mM NaCl; Phosphate-buffered saline (PBS) was 10 mM phosphate, pH 7.4, 150 mM NaCl; cell lysis buffer was PBS containing 10 mM iodoacetic acid, 1 mM phenylmethylsulfonyl fluoride and EDTA, 1 µg/ml leupeptin and chymostatin, and 1% Triton X-100; and immunoprecipitation buffer was PBS containing 1% Triton X-100.

Preparation of Placental Membranes—The syncytial trophoblast layer was scraped from fresh human term placenta and stored at ~80 °C. 450-600 g of frozen tissue were used for receptor isolation. The membranes were prepared for extraction as previously described (9).

Ligand Binding Studies—The binding assay of Lennartz et al. (10) was used to determine the dissociation constant for mannosylated bovine serum albumin (Man-BSA), the effect of pH on ligand binding, and the effect of monosaccharides and glycoproteins on the inhibition of Man-BSA binding. Briefly, 125I-Man-BSA (1.5 µg/ml, 2 x 106 cpm/µl) was incubated with 1.8 µg of receptor in a total volume of 0.2 ml (30 °C, 1 h). Bovine γ-globulin was added to a final concentration of 0.1% and receptor-ligand complexes were precipitated with 12.5% polyethylene glycol (M, 4000). The precipitated complexes were recovered by filtration using 0.45-µm Metricel filters and the bound radioactivity was quantitated. Control assays contained excess (200 µg/ml) Man-BSA.

Generation of Rabbit Antibodies—Antibodies were generated in New Zealand White rabbits by intradermal injection of purified placental receptor. Immunization was initiated with 50 µg of receptor emulsified in Freund’s complete adjuvant. The rabbit was subsequently boosted 26 days later with 50 µg of antigen in Freund’s incomplete adjuvant. The animal was bled at 33 and 40 days and the serum was tested by Ouchterlony double diffusion analysis (11). The antiserum was affinity-purified on a receptor affinity column and used for immunoprecipitation studies.

Receptor Affinity Column—Purified receptor (500 µg) was coupled to 1 ml of glutaraldehyde-activated silica according to manufacturer’s instructions. Isolation of affinity-purified antibodies was accomplished by incubation of the antiserum with the affinity matrix overnight at 4 °C. The column was washed with 20 ml of PBS and the antibody was eluted with 1 M propionic acid. The eluate was

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† National Arthritis Foundation Postdoctoral Fellow.
§ Present address: Depa. of Biochemistry and Medicine, Center for Health Sciences, University of Tennessee, Memphis, TN 38163.
¶ Parker B. Francis Foundation Fellow.
** To whom correspondence should be addressed: Washington University School of Medicine, Dept. of Cell Biology and Physiology, 660 S. Euclid Ave., Box 8101, St. Louis, MO 63110.

1 The abbreviations used are: Man-BSA, mannosylated bovine serum albumin; HEPBS, N-2-hydroxethylpipеразине-N’-2-этилэтилениамид; MES, 2-(N-morpholino)ethanesulfonic acid; SDS, sodium dodecyl sulfate.
Isolation of Human Mannose Receptor

RESULTS

Isolation of the Mannose Receptor—Placental membranes were extracted in loading buffer containing 1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride (overnight, 4°C) and the unextracted debris was removed by centrifugation (19,000 × g, 30 min). Solubilized membranes were subjected to affinity chromatography on mannose-Sepharose. The column was washed with 200 µl of buffer containing 30 mM acetoacetate (O), MES (Δ), or HEPES (□). Nonspecific binding at each pH was determined by the addition of excess Man-BSA (400 µg/ml) to companion assays.

Effect of pH on binding of Man-BSA to the mannose receptor. The assay mixture contained 1.8 µg of receptor and 1.5 µg/ml of [125I]-Man-BSA (2 × 10⁶ cpm/µg) in buffers containing 30 mM acetate (O), MES (Δ), or HEPES (□). Nonspecific binding at each pH was determined by the addition of excess Man-BSA (400 µg/ml) to companion assays.
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The assay involved the precipitation of ligand-receptor complexes with polyethylene glycol (10). The assay was developed to study the properties of the purified placental protein. The assay involved the precipitation of a single protein (Fig. 4A). Immunoprecipitation experiments using an antibody raised against the placental protein and monocyte-derived macrophages show that human macrophages not only make an immunologically related protein of the same molecular weight, but also insert this protein into their plasma membrane (Fig. 4). We take the above observations as strong evidence that the placental mannose binding protein is the macrophage endocytosis receptor. The placenta is known to contain a significant number of macrophages. It is not clear whether the protein isolated in this study derives from such mononuclear phagocytes or from the trophoblastic cells of the placenta. Trophoblastic cells are highly endocytic and could transport mannosylated proteins. The availability of the receptor antibody will allow us to identify the cell types within the placenta that contain the mannose receptor.

Identification of Receptor on Human Macrophages—To determine whether the placental protein was related to the macrophage mannose receptor, two immunoprecipitation approaches were taken. Each required antibody to the placental protein; this was generated in rabbits and affinity-purified as described (9). Immunoprecipitates were subjected to SDS-polyacrylamide gel electrophoresis. Radiolabeled proteins were visualized by radioautography. The positions of standard molecular weight markers are indicated.

The techniques described above have allowed the purification from human placenta of a protein that binds mannosylated and fucosylated ligands. The protein has the same properties as those reported for the mannos-specific endocytosis receptor on human monocyte-derived macrophages (4) and rabbit and rat alveolar macrophages (9, 10, 18, 19). The placental protein has a molecular weight of 175 kDa, binds specifically, and with high affinity, to glycoproteins containing mannose and fucose, and requires Ca2+ and a neutral pH. Immunoprecipitation experiments using an antibody raised against the placental protein and monocyte-derived macrophages show that human macrophages not only make an immunologically related protein of the same molecular weight, but also insert this protein into their plasma membrane (Fig. 4). We take the above observations as strong evidence that the placental mannose binding protein is the macrophage endocytosis receptor. The placenta is known to contain a significant number of macrophages. It is not clear whether the protein isolated in this study derives from such mononuclear phagocytes or from the trophoblastic cells of the placenta. Trophoblastic cells are highly endocytic and could transport mannosylated proteins. The availability of the receptor antibody will allow us to identify the cell types within the placenta that contain the mannose receptor.

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