Isolation and Characterization of a Mannose Receptor From Human Pigment Epithelium

Virginia L. Shepherd,* Berry I. Tarnowski,t and Barbara J. McLaughlin^r

Recent work demonstrated that a mannose receptor is involved in the phagocytosis of rod outer segments by the rat retinal pigment epithelium (RPE). In this study the binding of soluble mannose-containing ligands by human RPE explants is described. In addition, the authors report the isolation of a mannose receptor from human RPE and describe its relationship to the macrophage mannose receptor. Epithelial explants bound the soluble ligand 125I-mannose bovine serum albumin (BSA) by a mannose-specific process. The protein involved in mannose recognition was extracted from human tissue and purified using ligand-affinity chromatography. The protein that bound to the affinity column had a molecular weight of 175 kD by sodium dodecyl sulfate gel electrophoresis and migrated with the same mobility as the human macrophage mannose receptor. Antibodies directed against the macrophage receptor crossreacted with the mannose receptor from human RPE by immunoblot analysis. Binding specificity studies demonstrated that mannose and mannan inhibited ligand binding to the purified receptor by 65% and 90%, respectively; galactose had no effect. Using immunogold labeling of human RPE cells in explant culture, antimacrophage mannose receptor was localized at the apical plasma membrane. These results suggest that human RPE expresses a mannose receptor on its apical surface (as does the rat RPE) and that this receptor is similar to the human macrophage mannose receptor.


The importance of carbohydrate groups in recognition of macromolecules involved in cell–cell adhesion, pinocytosis, and phagocytosis is well documented. Sugar residues commonly found in terminal positions of glycoproteins such as mannose,1 galactose,2 and mannose 6-phosphate3 play key roles in these processes. Phagocytic cells such as macrophages were used to examine the role of carbohydrate residues in endocytic and phagocytic processes. Both mannose and mannose 6-phosphate receptors were found in macrophages.4-5 Mannose recognition is involved in the clearance of glycoproteins from the extracellular fluid6,7 and in the clearance of particulate ligands such as bacteria,8 yeast,9 and parasites.10 The macrophage mannose receptor was purified from several species and sources and identified as a macrophage cell-surface protein.11-13

A highly specific phagocytic cell layer in the retina, the pigmented epithelium (RPE), mediates clearance of rod outer segments (ROS) shed by rod photoreceptor cells.14 The phagocytic process is similar to that described in macrophages,15-17 but the RPE shows specificity toward ROS.18 Because of these similarities, and since many of the phagocytic processes in macrophages are carbohydrate mediated, carbohydrate residues may play a role in ROS–RPE recognition.

Others19-21 found exposed mannose units on the surface of ROS. We reported previously that rat RPE recognizes soluble and particulate ligands by a mannose-sensitive mechanism,22 suggesting that mannose groups on the ROS surface may serve as recognition markers for phagocytic uptake by a mannose-specific receptor on the RPE plasma membrane. Recently, we obtained more convincing evidence of this by demonstrating that uptake of ROS by rat RPE could be blocked by preincubation of RPE cells with mannose-receptor antibodies or by preabsorption of ROS with excess purified mannose receptor.23 In this article, we demonstrate that human RPE binds soluble mannose-containing ligands and that a mannose receptor has been isolated from human RPE. We present evi-
dence that this receptor is biochemically and immunologically similar to the macrophage cell-surface mannosereceptor.

Materials and Methods

Materials

Human eyes were obtained from the local eye bank within 24 hr postmortem. Mannose bovine serum albumin (ManBSA) was purchased from E-Y Laboratories (San Mateo, CA). Mannan-2 (Mnn2) for ligand-binding studies was a gift from Dr. Clinton Ballou (Berkeley, CA). Mannose-Sepharose was prepared as previously described.24

RPE Explant Preparation

The eyes were dissected in 0.9% saline at 4°C. The corneas and lenses were removed, and the rest of the eye was dissected into quarters. The eye quarters were agitated gently in saline at 4°C until the neural retina detached. Tissue for binding studies was further dissected as follows: the RPE and choroid were gently separated from the sclera, carefully floated onto a nitrocellulose filter (Millipore, Bedford, MA), and transferred to RPMI-1640 with glutamine.

Binding of 125I-ManBSA to RPE Explants

Binding was assayed using ManBSA labeled with Na125I using chloramine-T4. One eye quarter on a nitrocellulose filter (Millipore, Bedford, MA), and transferred to RPMI-1640 with glutamine. for 30 min, and the resulting pellet was suspended in 50 ml of 10 mM Tris, pH 7.4, containing 1.25 M NaCl, 15 mM CaCl2, 0.1 mM PMSF, and 1% Triton X-100 (extraction buffer). The mixture was incubated overnight in the cold and then centrifuged at 16,500 × g for 30 min. The supernatant was incubated with 10 ml of a slurry of mannoase-Sepharose with shaking overnight. The mixture was poured into a 1 × 30-cm column at room temperature and washed with 200 ml of extraction buffer containing 0.1% Triton. Bound proteins were eluted with the same buffer minus CaCl2 and with added EDTA (10 mM). The protein content of each fraction was measured using the BioRad protein reagent (BioRad Laboratories, Richmond, CA). The Mnn2 binding activity was measured as described by Townsend and Stahl.24

Sugar Specificity of the Isolated Human RPE Mannose Receptor

Isolated RPE mannose receptor (approximately 0.1 μg in 25 μl) was mixed with 3 × 105 cpm/0.3 μg of 125I-Mnn2 in Tris buffer, pH 7.8 containing BSA and CaCl2 in a total volume of 500 μl. The reaction mixture contained no inhibitors (control) or the concentration of inhibitors as indicated in Table 2. The mixture was incubated for 5 min at 25°C. Receptor-Mnn2 complexes were precipitated with 50% ammonium sulfate, collected by filtration, and quantified by gamma counting.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of the RPE Mannose Receptor

The molecular weight and purity of the isolated receptor was analyzed by SDS-PAGE using a minigel apparatus ( Hoefer, San Francisco, CA). The protein was electrophoresed under reducing conditions on 7.5% gels, and the bands were visualized by Coomassie blue or silver staining. Standard protein markers were myosin (205 kD), beta-galactosidase (116 kD), phosphorylase b (97.4 kD), BSA (66 kD), ovalbumin (45 kD), and carbonic anhydrase (29 kD).

Immunoblot Analysis

For immunoblot analysis of the isolated human mannose receptor, the samples were electrophoresed under reducing conditions on SDS gels. The proteins were transferred electrophoretically to nitrocellulose in transfer buffer (9.7 g Tris base, 45 g glycine, and 800 ml methanol in 4000 ml total volume). The transfer was complete after 1 hr at 60 V. The nitrocellulose paper was washed in Tris buffered saline (TBS) and incubated for 1 hr with TBS containing 3% BSA. The nitrocellulose was then incubated overnight.
at 4°C with either a 1:100 dilution of rabbit antiserum raised against the purified human alveolar macrophage mannose receptor13 or preimmune rabbit serum. After extensive washing with TBS and TBS containing 0.05% NP-40, horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin (Ig) G was added at a 1:2000 dilution, and the paper incubated for 2 hr at room temperature. The paper was washed, and the reactive bands were visualized by the HRP-catalyzed oxidation of 4-chloronaphthol.

Immunocytochemistry

All procedures were done at 4°C unless otherwise indicated. Eye quarters were rinsed in 0.1 M phosphate buffer (pH 7.2), then fixed for 1 hr in 0.1 M phosphate buffer containing 3% paraformaldehyde and 0.1% glutaraldehyde. The tissue was rinsed with TBS containing 5% sucrose and stored overnight in TBS with 10% sucrose. The tissue was frozen on dry ice in cryostat holders with Tissue-Tek O.C.T. compound (Miles, Naperville, IL). Cryostat sections (15 μm) were rinsed in TBS containing 5% normal goat serum (Miles). Either rabbit anti-human mannose receptor13 or preimmune rabbit serum was added to the sections at concentrations of 1:100 in TBS/1% normal goat serum and incubated at 4°C overnight. The tissue was washed extensively in TBS (pH 8.2)/1% normal goat serum, and goat anti-rabbit IgG conjugated to 15-nm gold (Janssen, Piscataway, NJ) (1:20 dilution) was added for incubation overnight. The tissue was rinsed in TBS and then 0.1 M cacodylate buffer (pH 7.2), fixed for 1 hr in 4% paraformaldehyde with 2% glutaraldehyde in 0.1 M cacodylate buffer, and dehydrated and embedded for electron microscopy.

Results

Binding of 125I-ManBSA to Human RPE Explants

Human RPE explants bound the ligand 125I-ManBSA by a mannose-inhibitable process (Table 1). Approximately 35% of the ligand bound could be competed for by the addition of excess cold ligand.

Table 1. Binding of 125I-ManBSA by human RPE explants

<table>
<thead>
<tr>
<th>Ligand bound (cpm)</th>
<th>-Mnn</th>
<th>+Mnn Specific</th>
<th>% as Specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>15,422 ± 1492</td>
<td>9717 ± 1001</td>
<td>5705</td>
<td>37</td>
</tr>
</tbody>
</table>

RPE explants on nitrocellulose filters were incubated with 125I-ManBSA (10 μg/ml; 5 x 10^5 cpm/μg) for 90 min at 37°C in HBSS assay medium (HBSS containing 1% BSA). Companion explants were incubated as above with added excess mannan (1 mg/ml). The explants were washed three times, and the bound ligand quantitated by gamma counting. Results are the averages of triplicate determinations ± SD.

These results agree with previous work with rat RPE explants where approximately 49% of the added ManBSA was bound specifically.

Isolation of a Mannose Receptor From Human RPE Tissue

The RPE mannose receptor was isolated from detergent-solubilized human RPE tissue by affinity chromatography on mannose-Sepharose as described earlier for isolation of the human lung mannose receptor.13 Figure 1 shows the elution profile for the human RPE receptor. The protein was localized using the protein dye reagent (Bio-Rad), and Mnn2 binding activity was measured as previously described.24 The SDS-PAGE analysis of the human RPE receptor is shown in Figure 2. The predominant Coomassie-staining band from human RPE migrated with a relative molecular weight of 175 kD (Fig. 2, Lane 2), identical to the mobility of purified human lung mannose receptor (Fig. 2, Lane 1).

Immunoblot Analysis of the Isolated Human RPE Mannose Receptor

The relationship of the human RPE mannose receptor to the human alveolar macrophage mannose receptor was investigated using rabbit antiserum raised against the purified human alveolar macrophage receptor. Figure 2 shows the result of immunoblot analysis after electrophoretic transfer of proteins from SDS gels. Lane 3 shows the reaction of antirabbit IgG conjugated to 15-nm gold (Janssen) with the purified human lung mannose receptor (relative molecular weight, 175 kD). Lane 4 is the reaction of antimacrophage receptor with the purified macrophage receptor (relative molecular weight, 175 kD).
Fig. 2. SDS–PAGE and immunoblot analysis of the human RPE mannose receptor. An aliquot of purified receptor containing approximately 2 μg of protein was electrophoresed under reducing conditions of 7.5% SDS gels. The gels were either stained with Coomassie blue or processed for immunoblot analyses. An approximate molecular weight of 175 kD was calculated using the standard proteins indicated. Lanes 1 and 2: human alveolar macrophage mannose receptor and human RPE mannose receptor stained with Coomassie blue. Lanes 3 and 4: immunoblot of purified alveolar and RPE mannose receptor following incubation with rabbit anti-human macrophage mannose receptor.

tivity of the macrophage antibody with the purified 175-kD RPE mannose receptor.

Sugar Specificity of the Isolated Human RPE Mannose Receptor

The sugar specificity of the isolated RPE receptor was studied by testing the ability of galactose, mannose, and mannan to compete with 125I-Mnn2 for binding. As shown in Table 2, galactose at 200 mM did not inhibit. Mannose (200 mM) inhibited binding by 65%, and mannan (40 μg/ml) inhibited binding by 90%. These results demonstrate that the predominant sugar recognized by this receptor is mannose, in agreement with the results reported for the isolated macrophage receptor.13

Immunolocalization of the Human RPE Mannose Receptor

Antimacrophage mannose receptor reactivity was localized using en bloc immunogold labeling of human RPE cells in explant culture at the apical plasma membrane (Figs. 3, 4). As shown in Figure 3, gold labeling was localized on various regions of the microvilli. In Figure 4 little or no labeling was present on explants incubated with preimmune serum as a control.

Discussion

In our study, mannose-specific binding was found in human RPE explants. In addition, a mannose receptor from human RPE was isolated and partially characterized using mannose-Sepharose. The receptor was biochemically similar to the human macrophage mannose receptor. Each had a relative molecular weight on SDS gels of 175 kD, both bound mannose in a Ca2+-dependent manner, and inhibition studies suggested that the macrophage and RPE proteins had the same sugar-binding specificity. Antibodies raised against the purified macrophage receptor reacted with the RPE 175-kD receptor on immunoblot analysis. Using immunogold-labeling techniques, the same antimacrophage receptor antibody was localized on the apical membrane of the human RPE. These results not only demonstrate the existence of a mannose receptor on an epithelial cell, but also raise the possibility that the RPE and macrophage mannose receptors are identical.

The RPE cell has a number of functions in common with macrophages including phagocytosis, expression of specific cell-surface receptors, secretion of lysosomal enzymes, antigen presentation, and cytokine production. The macrophage recognizes and ingests many particles, some of which are specifically cleared by the mannose receptor. For example, the mannose receptor is involved in phagocytosis of certain bacteria, yeast, and parasites. The

Table 2. Sugar specificity of the isolated human RPE mannose receptor

<table>
<thead>
<tr>
<th>Addition</th>
<th>125I-Mnn2 bound</th>
<th>cpm</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8998</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Galactose (200 mM)</td>
<td>8632</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Mannose (200 mM)</td>
<td>3136</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Mannan (40 μg/ml)</td>
<td>901</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Isolated RPE mannose receptor (approximately 0.1 μg in 25 μl) was mixed with 1 × 105 cpm/0.3 μg of 125I-Mnn2 in Tris buffer, pH 7.8 containing BSA and CaCl2 in a total volume of 500 μl. The reaction mixture contained no inhibitors (control) or the concentration of inhibitors. The mixture was incubated for 5 min at 23°C. Receptor-Mnn2 complexes were precipitated with 50% ammonium sulfate, collected by filtration, and quantitated by gamma counting. Results are the average of duplicate determinations.
RPE cell, on the other hand, can also phagocytize bacteria and yeast, but it is highly specific in its phagocytosis of shed ROS. Since the RPE cell expresses the phagocytic macrophage mannose receptor, it is possible that this receptor participates in ROS clearance by the RPE cell, similar to its participation in pathogen clearance by the macrophage.

The involvement of carbohydrate in ROS recognition has been a controversial issue in recent years. Early studies from several groups suggest that mannosone or other sugars might be involved in ROS binding to RPE cells. On the other hand, Lentrichia et al. reported that several simple sugars, including mannose, had no effect on ROS binding to chick RPE cells. Furthermore, others suggest that a mannose recognition system does not operate in ROS phagocytosis based on the finding that mannose glycoconjugates are not effective inhibitors. A support of a role for mannose recognition, Tarnowski et al. found that rat RPE cells ingest mannan-coated beads in a mannan-inhibitable process. Recently, others have shown that ROS phagocytosis by rat RPE can be inhibited by mannose receptor antibodies and by preabsorbing ROS with purified macrophage mannose receptor.

These discrepancies might be explained by several observations. First, we know from studies with the macrophage mannose receptor that the monosaccharide mannose is a poor inhibitor of mannose-bearing ligand binding unless present in high concentrations. Second, a particulate mannose-bearing ligand, rather than a soluble one, could act more effectively as a competitive inhibitor of ROS phagocytosis. The mannose receptor in macrophages is known to function not only in phagocytosis but also in pinocytosis of mannose-bearing ligands. Since mannan and ManBSA are both soluble and not particulate ligands, they may be effective only as inhibitors of pinocytic uptake of soluble ligands (as shown here) and may not be effective as inhibitors of particulate ligands such as ROS.

The ligand on the surface of the ROS involved in recognition by the RPE has not yet been identified. Molday and Molday reported that there are three major proteins on the ROS plasma membrane with molecular weights of 36 kD (rhodopsin), 38 kD, and 52 kD. Rhodopsin is a glycoprotein and contains terminal mannose and N-acetylglucosamine residues. As early as 1976, it was suggested that this protein might play a role in ROS phagocytosis. Kean et al. reported that RPE cells bound rhodopsin, and Shirikawa et al. demonstrated that RPE cells ingested rhodopsin-containing liposomes. However, this interaction did not appear to involve the oligosaccharide side chains of rhodopsin. In addition, oligosaccharide-containing peptides from rhodopsin did not block ROS ingestion. A possible candidate for the ROS ligand might be the 52-kD protein. This protein has been reported to bind to concanavalin A, a plant lectin which has a similar binding specificity as the mannose receptor.

Our results and other recent work in our laboratory lead us to conclude that human RPE cells express a mannose receptor that is similar, if not identical, to the macrophage mannose receptor. This receptor is a likely candidate for participation in the binding and ingestion of shed photoreceptor outer segments in the human retina.

Key words: retinal pigment epithelium, mannose receptor, phagocytosis

References

3. Stoolman LM, Tenforde TS, and Rosen SD: Phosphomanno-
syl receptors may participate in the adhesive interaction between lymphocytes and high endothelial venules. J Cell Biol 99:1535, 1984.


