Expression of Mannose Receptors For Pinocytosis and Phagocytosis on Rat Retinal Pigment Epithelium

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We report here the presence of a mannose-specific receptor on apical membranes of rat retinal pigment epithelial (RPE) cells. For pinocytic studies, 125I-Mannose-BSA (125I-Man-BSA) was incubated with RPE explants from normal (Long Evans) and dystrophic (pigmented RCS) rat retinas. Normal RPE bound 36.1 ng of ligand and, in the presence of mannan competitor, the amount bound was 18.3 ng. In a similar assay, total ligand uptake by dystrophic RPE was 25.9 ng with 9.8 ng specific for mannose recognition. Comparing the amounts of ligand bound, dystrophic RPE recognized 55% of the amount recognized by normal RPE. The presence of the mannose receptor was localized on both normal and dystrophic RPE apical plasma membranes by autoradiographic techniques using 125I-Man-BSA. Normal RPE showed a greater number of silver grains present at the apical cell membrane as compared to dystrophic RPE. Silver grains were significantly reduced when incubation with the ligand was carried out in the presence of a mannan competitor. Further, in phagocytic studies, latex beads coated with mannan were used as phagocytic particles. Normal RPE phagocytilized 4.52 mannan-beads per cell profile by a mannose-specific mechanism, whereas dystrophic RPE did not recognize mannan-beads. Our data suggest that RPE cells express surface receptors which recognize both soluble and particulate mannose ligands. The pinocytic and autoradiographic studies suggest that normal RPE binds more soluble ligand than does dystrophic RPE. If the mannose receptors mediate both pinocytosis and phagocytosis, a possible reduction in number of soluble mannose binding sites on the dystrophic RPE may be related to the diminished phagocytic recognition of particulate ligand by the dystrophic RPE. The difference in phagocytic recognition of mannose ligand between normal and dystrophic RPE may be related to the phagocytic defect in the dystrophic retinas. Invest Ophthalmol Vis Sci 29:742-748, 1988

Cell surface receptors which recognize glycoproteins via mannose residues have been described on macrophages, and appear to mediate both pinocytosis1,2 and phagocytosis.3,4 Carbohydrate residues of glycoconjugates have been implicated in the phagocytic interaction of photoreceptor outer segment (OS) tips and the retinal pigment epithelium (RPE).5-14 Previous studies have shown that uptake of OS tips by RPE was significantly reduced in the presence of D-mannose, methyl-alpha-D-mannoside, L-fucose and N-acetylglucosamine, while D-glucose, D-fucose or D-galactose had little effect.12-14 Morphological studies have demonstrated that OS plasma membranes bind concanavalin A (binds high mannose-type and glucose-containing glycoproteins15), suggesting that mannose residues are exposed and accessible on the OS surface for phagocytic uptake. Further, binding sites for the lectins, concanavalin A and Lens culinaris agglutinin (binds mannose-containing glycoconjugates which have a core-linked fucose to the glycopeptide15) are present on RPE.6,8,10,14 Additional evidence that further implicates the role of RPE cell surface glycoproteins in the process of ligand recognition and binding are the findings that the proteolytic digestion of RPE-inhibited phagocytosis of OS.16,17 Despite these numerous studies, a specific sugar or sugar binding receptor which mediates OS and RPE interactions remains to be identified. Since RPE and macrophages are both phagocytic cells and macrophages are known to have cell surface mannose receptors, we have explored the possibility that RPE cells may also express mannose receptors. The RPE recognizes, binds and phagocytizes OS tips...
were washed and processed for electron microscopy. In dystrophic RCS rat retinas, OS phagocytosis is greatly diminished due to a defect in the RPE which may involve an alteration in the recognition of oligosaccharide determinants. The dystrophic RCS rat retina offers an animal model for a phagocytic defect to test this hypothesis.

In this study we report that mannose-specific receptors are present on retinal PE cells, and that these receptors mediate pinocytosis of soluble mannose ligands by both normal and dystrophic RPE. Further, in the normal RPE, the mannose receptor participates in phagocytosis of particulate ligands. It is possible that the decreased phagocytic recognition of particulate mannose ligands by the dystrophic RPE may be related to the phagocytic defect in these retinas.

**Materials and Methods**

**Animals**

Explants were prepared from 10–13 day postnatal Long Evans rats (normal retinas) and Royal College of Surgeons rats (dystrophic retinas). Animals were anesthetized, then the eyes were enucleated and placed in 0.4 M sucrose containing 30 μM Ca²⁺ for 1 hr at 4°C. The cornea and lens were removed, and the eyes were quartered and soaked in fresh 0.4 M sucrose until the neural retina detached (about 30 min). The explants (RPE, choroid capillaris and sclera) were rinsed in RPMI 1640/glutamine medium at 37°C in a 5% CO₂ atmosphere for 1 hr. Fresh medium was added and mannannan-beads were added four times at 30 min intervals at a concentration of 5 X 10⁹/2 ml/well in the absence or presence of excess soluble mannan (1 mg/ml). Following a 3 hr incubation, the explants were fixed in 2% glutaraldehyde, 2% paraformaldehyde and 0.5% acrolein in a 0.1 M cacodylate buffer, osmicated for 1 hr, and resuspended in 1 ml RPMI 1640/glutamine medium.

**Pinocytosis**

Mannose (30)-BSA (Man-BSA), prepared by the method of Lee et al, was iodinated by the chloramine T method. Three to five explants were incubated with 4 μg/ml of ¹²⁵I-Man-BSA in 400 μl of Hank’s balanced salt solution containing 1% BSA in a 16 mm well for 90 min at 37°C, washed and counted for bound ¹²⁵I-Man-BSA. Companion wells received ¹²⁵I-Man-BSA plus excess mannan (1 mg/ml) to determine nonspecific uptake. Assays were done in triplicate and the results presented are representative of three separate experiments.

**Autoradiography**

Explants from either normal or dystrophic rat RPE were incubated with ¹²⁵I-Man-BSA for 90 min at 4°C, with and without mannan (1 mg/ml). The explants were washed and processed for electron microscopy. Semithin sections (approximately 0.5 μm) were placed on clean glass slides and coated with Kodak NT2B emulsion (Eastman Kodak Co., Rochester, NY) (diluted 1:1, v/v with distilled water). The slides were exposed for 2 weeks in the dark at 4°C and developed with Kodak D-19 Developer (Eastman Kodak Co.) to visualize the activated silver grains.

Silver grains associated with the RPE cells were counted at the light microscopic level. Silver grain density in control areas which contained no tissue was determined using the alternating grid square method. Silver grain density was determined in a minimum of 25 cell profiles from at least three animals per condition. Statistical comparisons were made between experimental and mannan control tissue as well as between normal and dystrophic RPE.

**Phagocytosis**

The particulate ligand used for phagocytic uptake was mannan-coated, carboxylated, 1 μm diameter microspheres (1 ml, 5 x 10⁹/ml) (Polysciences, War- rington, PA). The beads were incubated with 4 mg of N-ethyl-5-phenyl-isoxozolium 3' sulfonate (Sigma, St. Louis, MO) as previously described, rinsed with PBS and agitated for 1 hr with 4 mg of mannan (Sigma) in 2 ml PBS at 22°C. The beads were washed and resuspended in 1 ml RPMI 1640/glutamine medium.

For phagocytic experiments, five explants were placed with the RPE uppermost on a stainless steel support positioned in a 20 mm diameter well of an organ culture dish. The explants were incubated in RPMI 1640/glutamine medium at 37°C in a 5% CO₂ atmosphere for 1 hr. Fresh medium was added and mannan beads were added four times at 30 min intervals at a concentration of 5 x 10⁹/2 ml/well in the absence or presence of excess soluble mannan (1 mg/ml). Following a 3 hr incubation, the explants were washed and processed for electron microscopy.

The number of phagocytized beads was analyzed by transmission electron microscopy. Blocks were randomly selected and sectioned so that beads were counted in an average of 25 cell profiles per animal. Also, the number of cell profiles which contained beads was quantitated and expressed as a percentage of the total number of cell profiles counted. The Mann-Whitney U Test was used to compare bead uptake in the two strains of rats.

**Electron Microscopy**

Tissue from autoradiography and phagocytosis experiments was fixed in 2% glutaraldehyde, 2% formaldehyde and 0.5% acrolein in a 0.1 M cacodylate buffer (pH 7.3). The tissue was washed three times in 0.1 M cacodylate buffer, osmicated for 1 hr,
Table 1. Amounts of $^{125}$I-mannose-BSA pinocytized by RPE explants

<table>
<thead>
<tr>
<th>RPE</th>
<th>(−) Mannan</th>
<th>(+) Mannan</th>
<th>Specific binding</th>
<th>% of normal</th>
</tr>
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<tbody>
<tr>
<td>Normal*</td>
<td>36.1 (5.2)</td>
<td>18.3 (3.5)</td>
<td>17.8 (49%)</td>
<td>100</td>
</tr>
<tr>
<td>Dystrophic†</td>
<td>25.9 (0.8)</td>
<td>16.1 (0.3)</td>
<td>9.8 (38%)</td>
<td>55</td>
</tr>
</tbody>
</table>

* Long Evans rat RPE.
† Pigmented RCS rat RPE.
Values are expressed as ng $^{125}$I-Man-BSA/hr/well, plus or minus the standard deviation of the mean of triplicate determinations.

Results

Pinocytosis

The presence of a mannose receptor on the cell surface of RPE cells was detected by the binding of a soluble mannose ligand, $^{125}$I-Man-BSA. In all assays, mannose-specific recognition was measured by adding yeast mannan, a competitive inhibitor of ligand uptake, to companion cultures. The binding and uptake of Man-BSA by normal and dystrophic RPE is expressed as nanograms per hour per well, plus or minus the standard deviation of the mean (Table 1), and is representative of three separate experiments. Normal RPE pinocytized 36.1 ng (5.2) of the ligand added. In the presence of mannan (1 mg/ml) uptake was reduced by 49%, resulting in 17.8 ng being taken up by a mannose specific process. In contrast to normal RPE, dystrophic RPE took up 25.9 ng (0.8) of ligand when no competitor was present. Uptake was reduced to 16.1 ng (a 38% decrease) in the presence of the mannan competitor thus resulting in 9.8 ng being taken up via a mannose-specific mechanism. The amount of soluble ligand taken up by dystrophic RPE is approximately 55% of the ligand recognized by normal RPE. Nonspecific binding (background levels) was consistently high in all experiments, possibly due to the use of tissue explants.

Autoradiography

In autoradiographic studies, silver grains were present at the apical membrane of RPE explants from both normal and dystrophic retinas. A minimum of 37 cell profiles was counted from each of three animals. The quantitation of silver grains is expressed as the number per cell profile, plus or minus the standard error of the mean. Normal RPE had 1.2 (0.08) silver grains per cell profile (Fig. 1). The silver grains associated with the sclera and cut edges of the explants were similar in number to those grains seen where no tissue was present. The addition of yeast mannan to the incubation mixture uniformly reduced the number of silver grains associated with the RPE cell surface to 0.35 (0.13) silver grains per cell profile. Dystrophic RPE had significantly fewer silver grains than did normal RPE at the apical cell surface. In the dystrophic RPE, silver grains seen associated with the plasma membrane were 0.73 (0.14) per cell profile (Fig. 1). The specific silver grains associated with dystrophic RPE represent approximately 68% of those associated with normal RPE. These data are similar to the pinocytic data where dystrophic RPE uptake was approximately 55% of the normal RPE.

A one-way analysis of variance (ANOVA) with preplanned contrast showed that the number of silver grains on normal RPE was significantly greater than on dystrophic RPE ($P < 0.015$). Additionally, in the presence of the mannan competitor, silver grain numbers were reduced on both normal RPE ($P < 0.0005$) and dystrophic RPE ($P < 0.005$). Nonspecific binding (background) was not significantly different between normal and dystrophic RPE.

Phagocytosis

Phagocytosis of mannan-coated beads by normal and dystrophic RPE explants was visualized and stained en bloc for 1 hr with 2% uranyl acetate, dehydrated in ethanol and embedded in Epon/Araldite.
Figs. 2 and 3. Electron micrographs of RPE explants incubated with mannan-coated beads. In both figures, arrowheads denote beads; MV, microvilli; BR, Bruch's membrane. Fig. 2: Normal RPE avidly phagocytized mannan-coated beads as numerous beads are seen in the cytoplasm (×8,835). Fig. 3: Dystrophic RPE explants, incubated with mannan-coated beads, phagocytized very few beads (×8,835).

Figure 2 shows a typical RPE cell from a normal retina following a 5 hr incubation with mannan-beads. Numerous beads have been phagocytized and are seen within the cell cytoplasm. Following a similar 5 hr incubation with mannan-coated beads, phagocytosis by dystrophic RPE cells was only rarely observed. Typically, beads were seen only outside the dystrophic cells near the apical microvillous processes (Fig. 3). In addition, normal RPE cells did not engulf any mannan-coated beads in the presence of excess soluble mannan (1 mg/ml) as mannan-beads were seen only in the external milieu (Fig. 4). As a control to rule out the possibility that soluble mannan exerted some toxic or nonspecific effect on the phagocytic ability of the RPE cells, normal RPE explants were incubated with uncoated beads (known to be phagocytized by normal RPE) in the absence or presence of 1 mg/ml mannan. Normal RPE phagocytized the uncoated beads in similar numbers when mannan was added to the incubation medium (Fig. 5) as compared to the numbers of uncoated beads taken up when mannan was not present (not shown).

The Mann-Whitney U Test was used to compare bead uptake in the two strains of rats. Values are expressed as the number of beads ingested per cell profile, plus or minus the standard error of the mean. Normal RPE phagocytized 4.52 (2.59) beads per cell.

Fig. 4. Specificity of mannan-coated bead uptake was shown by the addition of soluble mannan (1 mg/ml) to the incubation medium of normal RPE explants. This electron micrograph shows that mannan-coated beads are not ingested in the presence of mannan as a bead (arrowhead) is present at the apical membrane but none are seen in the cytoplasm. MV, microvilli; PG, pigment granules (×9,675).
profile as compared to dystrophic RPE which phagocytized very few, 0.67 (0.67), mannan-beads per cell profile (Table 2). Additionally, bead uptake by normal RPE was reduced to 0 when mannan was added to the medium (Table 2).

**Discussion**

Consistent differences in mannose ligand recognition were detected between RPE from normal and dystrophic rat retinas. The amount of soluble ligand bound was greater in normal RPE than in dystrophic RPE as assayed by both pinocytosis and autoradiography. Another difference was in the phagocytic ability of normal and dystrophic RPE to recognize and engulf a particulate mannose ligand. Normal RPE avidly phagocytized mannan-beads while dystrophic RPE took up none of these beads. The difference in the interactions of mannose ligands between normal as compared to dystrophic RPE, as shown by the three different assays, suggests that mannose recognition may be related to the phagocytic defect in the dystrophic retinas.

The mannose receptor has been well characterized on macrophages, another phagocytic cell type, and is thought to play an important role in lysosomal enzyme recognition and clearance. The mannose receptor on the macrophage cell surface binds glycoproteins which contain mannose units, delivers the ligand to the lysosomal compartment, then recycles back to the cell surface for further rounds of pinocytosis.28 Ligands such as rat preputial beta-glucuronidase,2 neoglycoproteins29 and horseradish peroxidase30 have all been used to characterize the receptor function. A receptor with similar sugar-binding specificity on macrophages appears to participate in phagocytosis and ingestion as well. Sung et al31 have reported that a macrophage mannose receptor is active in yeast recognition and phagocytosis. Berton and Gordon4 have shown that a mannose receptor mediates attachment of zymosan particles. In addition, it has been suggested that mannose recognition may also play a role in attachment and ingestion of *Leishmania donovani* promastigotes. Finally, Perry and Ofek32 have shown that mannosse may be an important determinant in *E. coli* clearance.

Phagocytic RPE cells, like macrophages, can specifically recognize particulate ligands for uptake,33-35 but specific receptors which mediate phagocytosis of OS tips have yet to be identified. It is known that photoreceptor outer segment plasma membranes have mannose-containing glycoconjugates which could serve as ligands for interaction with a RPE mannose receptor during phagocytosis. A glycoprotein, rhodopsin, is a major protein of rod photoreceptor OS and contains high mannose oligosaccharide units.36 In accord with this, Heth and Bernstein37 have shown that a 33 kD protein isolated from bovine and rat RPE binds to a rhodopsin-Sepharose column in a mannose-specific manner. This 33 kD protein may be an endogenous lectin which has sugar specificities similar to Con A. Additionally, this notion is consistent with the findings of McLaughlin and Wood which showed that OS have accessible Con A-binding sites.8

In our study, we have demonstrated that a difference exists between normal and dystrophic RPE rec-

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**Table 2. Number of mannan-beads phagocytized by RPE explants**

<table>
<thead>
<tr>
<th>RPE</th>
<th>Mannan</th>
<th>Mannan (%)</th>
<th>% of normal</th>
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<tbody>
<tr>
<td>Normal*</td>
<td>4.52 (2.59)</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Dystrophic†</td>
<td>0.67 (0.67)</td>
<td>—</td>
<td>15</td>
</tr>
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</table>

* Long Evans rat RPE.
† Pigmented RCS rat RPE.

Beads were counted in three or four animals with an average of 21 cell profiles scored per animal. All values are expressed, plus or minus the standard error of the mean. The percent of cell profiles containing beads of the total number of cells counted was: normal RPE, 39.0% (7.9); dystrophic RPE, 1.03% (1.03).
ognition of particulate mannose ligands. The soluble ligand binding and autoradiographic studies establish the presence of mannose receptors on the RPE cell surface and suggest that dystrophic RPE binds less ligand than does normal RPE. It is possible that the reduced binding in dystrophic RPE may be due to fewer mannose binding sites. If the mannose receptors mediate both pinocytosis and phagocytosis, the decreased phagocytic recognition of particulate man-

nose beads by the dystrophic RPE may be related to the possibly fewer binding sites. Accordingly, the number of binding sites may be too small to facilitate the circumferential binding and zippering of particles as occurs in macrophage phagocytic ingestion.38 It is possible, however, that there are two classes of man-
nose receptors, one for phagocytosis and the other for pinocytic uptake. If this idea is correct, the amount of soluble ligand bound by pinocytic receptors is unrelated to the recognition of particulate ligand. Al-

though there is no evidence that carbohydrate-me-
diated phagocytosis involves a cytoskeletal interac-
tion with the receptors, perhaps RPE phagocytosis is characte-
rized by an interaction of these mannose recep-
tors with actin or with a molecule which links the recep-
tor to the cytoskeleton. Actin is known to be involved in the ingestion step of phagocytosis in RPE cells.40-42 A basket-like array of actin has been shown to underlie ROS bound to the RPE surface and an associa-
tion of microfilaments with RPE plasma membrane has been noted beneath attached parti-
cles.42 We speculate that if OS phagocytosis by RPE occurs by a mannose-sensitive event, our studies may provide a clue to identify an alteration involved in the phagocytic defect in the dystrophic RPE.

Key words: mannose receptor, pinocytosis, autoradiog-

raphy, phagocytosis, retinal pigment epithelium

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