Phagocytic activity of the pigmented retinal epithelium. III. Interaction between lysosomes and ingested polystyrene spheres*

Joe G. Hollyfield and Adriana Ward

Outer segment fragments discarded by rod photoreceptors are phagocytized by the pigmented retinal epithelium (PE). Within the PE, lysosomes interact with the phagosomes and then lysosomal enzymes degrade the outer segment debris. The ability of the pigmented retinal epithelium to phagocytize polystyrene spheres was exploited as a means of altering the composition of the phagosomes. A suspension of polystyrene spheres was injected between the retina and its epithelium in Rana pipiens tadpoles. At 2, 4, and 8 days after injection, the retinal epithelium was prepared for acid phosphatase cytochemistry. Around some of the phagocytized beads, acid phosphatase reaction product was observed indicating an interaction between the experimental phagosome and lysosomes. Lysosomes were not observed in the PE cells which contained ingested polystyrene spheres. Our findings suggest that the component of the phagosome necessary for lysosomal interaction is the membrane surrounding the phagosome and not the contents of the phagocytic vesicle.

Phagocytosis is one of the indispensable functions of the pigmented retinal epithelium (PE) in photoreceptor maintenance. During the process of outer segment renewal in rods, the continued addition of new discs at the outer segment base is balanced by the intermittent sloughing of packets of discs from the outer segment distal tip.1-2 The closely apposed PE cells phagocytize these discarded outer segment fragments.3-5 When the PE fails to remove this material, as is the case in rats with inherited retinal dystrophy, outer segment debris accumulates between the retina and PE, the photoreceptors progressively degenerate, and blindness ensues.6-7

In order to better understand the normal phagocytic capabilities of the PE our previous studies have tested the ability of this tissue to engulf or exclude material not normally encountered.8,9 We have found that polystyrene spheres are readily ingested by the normal PE, whereas pasteurized micrococci of similar dimensions are not. These results clearly indicate that the uptake of particulate material by the PE is not strictly limited to the material this tissue normally engulfs but that the nature of the material presented to the PE...
Phagocytic activity of PE 1017

Phagocytic activity of PE can determine whether or not that material will be phagocytized.

Within the PE, lysosomal enzymes are responsible for degrading the ingested outer segment debris. Following fusion of the lysosomal membrane with the membrane of the phagocytic vesicle (phagosome), hydrolytic enzymes are dumped into the phagosome and the outer segment fragments are degraded. It is not known how the phagosome is distinguished from the other cellular organelles by the lysosome prior to this interaction.

The phagosome consists of two distinct components which have strikingly different origins. The outer segment debris is a product of the photoreceptor but the membrane surrounding this material was, before phagocytosis, a portion of the plasma membrane on the apical surface of the PE cell. The site of recognition by the lysosome could reside in either of these two components. This study exploits the ability of the PE to phagocytize polystyrene spheres as a means of altering the composition of the phagosome. With a cytochemical method specific for the localization of lysosome enzymes, we have determined whether PE lysosomes interact with the experimental phagosome.

Materials and methods

Polystyrene spheres, 1.1 μ in diameter (Dow Chemical), were prepared for injection between the retina and PE following the procedures described in detail previously. Rana pipiens larvae used in this study were purchased from the Amphibian Facility, University of Michigan, Ann Arbor, Mich., or were collected locally. Using midlarval tadpoles (stages XII to XVIII) a small puncture was made in the superior quadrant of the globe as near the fundus as possible. A fine glass syringe filled with the polystyrene bead suspension was inserted between the PE and retina and the suspension was expelled between these tissues.

Seventeen tadpoles were maintained at room temperature and were killed two (five animals), four (eight animals), and eight (four animals) days following injection. Small areas of the PE immediately surrounding the injection site were removed and fixed. Portions of the PE of three noninjected tadpoles were also fixed.

All tissues remained for three hours at 4°C in a mixture of 2 per cent paraformaldehyde, 2.5 per cent glutaraldehyde, and 0.025 per cent CaCl₂ in 0.09 M cacodylate buffer at pH 7.4. After three rinses with 0.1 M cacodylate buffer with 0.2 M sucrose at pH 7.4, tissues were stored overnight at 4°C in the sucrose-buffer mixture. Prior to incubation for acid phosphatase, tissues were rinsed twice in 7 per cent sucrose. Tissues were then incubated for 30 minutes to 3 hours at 20°C in 0.05 M acetate buffer containing 0.003 M lead nitrate at pH 5.0 with Na-β-glycerophosphate (0.1 M) as a substrate for acid phosphatase. Control tissues were incubated for identical times in the buffer-lead nitrate solution which lacked the substrate. All tissues were postfixed with 1 per cent OsO₄ in 0.9 M cacodylate buffer at pH 7.4. After dehydration in a graded ethanol series, the material was passed through propylene oxide and embedded in Epon. Thin sections, cut on a Porter-Blum MT-2-B ultramicrotome, were picked up on single hole, parlodion coated grids. After staining with uranyl acetate and lead citrate, sections were examined in a Siemens Elmiskope 1A.

Results

The localization of acid phosphatases, one of the classes of hydrolytic enzymes present in lysosomes, is based on the ability of these enzymes to liberate phosphate from the substrate provided in the incubation medium. In the presence of lead nitrate the free phosphate forms an insoluble salt, lead phosphate, which precipitates at the reaction site. The presence of this electron dense precipitate within the tissue is evidence for the presence of acid phosphatase activity at that location.

The appearance of the lead phosphate precipitate present in the PE was dependent on the time the tissues were incubated in the incubation medium. In the presence of lead nitrate the free phosphate forms an insoluble salt, lead phosphate, which precipitates at the reaction site. The presence of this electron dense precipitate within the tissue is evidence for the presence of acid phosphatase activity at that location.

The appearance of the lead phosphate precipitate present in the PE was dependent on the time the tissues were incubated in the incubation mixture. In tissues incubated for 30 minutes to 1 hour, the precipitate had a fine granular appearance (Figs. 2, 3, and 5). In the tissues incubated for longer periods the precipitate formed larger particles and had a clumped appearance (Figs. 1 and 5).

In the PE from noninjected eyes, acid phosphatase activity was found associated with membrane-bound bodies approximately 0.2 μ in diameter (Fig. 1). Similar structures have been described as lys-
Fig. 1. Circular profile of a membrane-bound lysosome present near the basal region of the PE. Note the intense acid phosphatase reaction product which obscures most of the profile (arrows). Bruch’s membrane is at upper right. Bar represents 0.25 μ.

Fig. 2. PE melanosome with acid phosphatase reaction product around the pigment granule periphery (arrows). Bar represents 1 μ.

Fig. 3. Portion of a large phagosome in the PE. Acid phosphatase reaction product is present in association with the outer segment debris near the center of the micrograph (arrows). Bar represents 0.25 μ.
Fig. 4. Portion of the PE from a tadpole injected with polystyrene spheres four days before the tissue was fixed. Five profiles of ingested polystyrene spheres (P) are present in the area shown. One of the profiles (P-L) has an intense reaction product localized at the periphery (enlarged in inset). Some reaction product is also present around the melanosomes (M). BM, basement membrane. Bar in upper right represents 0.5 μ.
Fig. 5. Portion of a PE cell from a tadpole injected with polystyrene spheres eight days before
the tissue was fixed. Several profiles of phagocytized beads (P) are present. The periphery of
one of these profiles (P-L) shows acid phosphatase activity. Also note the reaction product
surrounding the melanosomes (M). Bar in lower left represents 0.5 μ.

The localization of the lead phosphate precipitate over these structures fulfills the
criteria necessary for morphologic identification of lysosomes.\textsuperscript{10} Lysosomes were
numerous in the PE cells and were more frequently encountered near the apical
side than in the basal region. Acid phosphatase activity was also present around
the periphery of most of the melanosomes examined (Fig. 2).

When phagocytized outer segment fragments were encountered, varying degrees
of acid phosphatase activity were observed. This probably reflects differences
in the amounts of acid hydrolases present

in the phagosome resulting from variation
in the length of time these structures had
been present in the PE at the time the
tissues were fixed. Acid phosphatase was
also observed associated with the Golgi
apparatus though this was not a consistent
finding.

No acid phosphatase activity was noted
over the rough or smooth endoplasmic
reticulum, myeloid bodies, mitochondria,
or nuclei. In the control tissues incubated
in the absence of the substrate, no precipi-
tate was present.

In the eyes recovered two, four, or eight
days after injection, the PE cells were dis-
tended with numerous membrane-bound
circular profiles which corresponded in size to the dimensions of the polystyrene spheres. The number of beads present in a typical section varied from 15 to 20 to over 200 per PE cell. Many of the bead profiles were electron transparent resulting from the complete extraction of the polystyrene by propylene oxide as was previously reported.\(^8\) In others, varying amounts of the electron translucent polystyrene remained.

Acid phosphatase activity was not detected in association with the majority of phagocytized polystyrene beads. However, when each bead present in the tissue section was systematically examined, usually one to three phagocytized beads per PE cell were found on which a halo of lead phosphate precipitate was present at the bead periphery (Figs. 4 and 5). Lead phosphate precipitate was also present around the periphery of the melanosomes (Figs. 4 and 5). No lysosomes were observed in the PE cells which contained ingested polystyrene spheres.

Acid phosphatase reaction product was present to a limited extent in the Golgi apparatus but was not noted in the mitochondria, rough or smooth endoplasmic reticulum, myeloid bodies, or nuclei. In the control PE injected with polystyrene spheres, but incubated in the absence of substrate, no precipitate was noted.

Discussion

Though the majority of the polystyrene spheres ingested by the PE were free of acid phosphatase activity, the localization of acid phosphatase reaction product around the periphery of some of the phagocytized spheres indicates that lysosomes are able to interact with the experimental phagosomes. This finding indicates that the component of the phagosome recognized by the lysosome is the membrane surrounding the phagosome and not the contents of the phagocytic vesicle.

The small number of polystyrene beads around which reaction product was observed may reflect a limited store of lysosomes in the PE cell. It is likely that PE lysosomes interact with the initial phagosomes taken into the cell following introduction of the polystyrene beads and the lysosomal population is quickly depleted. As phagocytosis continues and additional beads are ingested, these remain free of acid phosphatase activity because lysosomes are no longer available for interaction with the later arriving phagosomes. The finding that lysosomes were numerous in the normal PE but were absent in the PE cells which also contained ingested polystyrene spheres supports this view.

The high level of acid phosphatase activity at the periphery of the melanosomes in normal and experimental PE was an unexpected finding. Reaction product was associated not only with PE melanosomes but was also present around these organelles in the melanocytes of the choroid as well. The significance of this observation is not readily apparent. Acid phosphatase activity has been observed around melanosomes in the PE of the chick embryo and it was suggested that this enzyme may function to control tyrosinase activity.\(^{20}\) In the albino rat, acid phosphatase activity was not observed around the amelanotic melanosomes in the PE.\(^{10}\)

In the mammalian epidermis, melanosomes extruded from the melanocytes located near the basal layer are phagocytized by adjacent keratinocytes.\(^{21,22}\) Melanosomes in the epidermal melanocytes are not acid phosphatase positive,\(^{23}\) whereas the phagocytized melanosomes in the keratinocytes show intense localization of acid phosphatase reaction product.\(^{23,24}\) It was suggested that the melanosomes in the epidermal cells interact with lysosomes and that the lysosomal enzymes degrade the melanosome within the keratinocyte.\(^{24}\) Our observations of acid phosphatase activity associated with melanosomes in the PE and melanocytes of the choroid may indicate that melanosomes undergo degradation within these cells.

In the embryos of *Rana pipiens*, *Xenopus laevis*, and *Notophthalmus viridescens*,
ococyte melanosomes eliminated from the developing retina are phagocytosed by the subjacent PE. Within the PE, newly synthesized melanin is deposited around the oocyte melanosomes. It might be argued that some of the phagocytized spheres in our experiments served as nuclei around which the PE cells deposited melanin and that the acid phosphatase reaction product we observed was associated with melanin similar to that observed in association with the melanosomes. We do not believe this to be the case since the halo of material surrounding the acid phosphatase-positive beads is not as electron dense as the PE melanosomes. Furthermore, the granular appearance of this material is similar to the texture of the lysosomes observed in the normal PE. The absence of lysosomes in the PE cells containing the phagocytized beads also implies that the acid phosphatase-positive material surrounding the ingested beads is of lysosomal origin.

REFERENCES


