Integrin αvβ5 Participates in the Binding of Photoreceptor Rod Outer Segments during Phagocytosis by Cultured Human Retinal Pigment Epithelium

Hai Lin and Dennis O. Clegg

PURPOSE. To examine the expression of integrin vitronectin receptors (integrins αvβ3 and αvβ5) in native human fetal retinal pigment epithelium (RPE) and cultured human fetal RPE and to examine the role of RPE ViNRS in the phagocytosis of photoreceptor rod outer segments (ROS).

METHODS. Monoclonal antibodies against human integrin subunit αv and heterodimers αvβ3 (LM609) and αvβ5 (P1F6) were used to label freshly isolated human fetal RPE explant and cultured human fetal RPE and to immunoprecipitate membrane proteins from cultured human RPE. Effects of antibodies and peptides that inhibit integrin vitronectin receptors on phagocytosis of ROS by RPE were determined using cultured human fetal RPE and fluorescein-labeled bovine outer segments.

RESULTS. Antibodies against integrin subunit αv and against αvβ5 (P1F6) labeled the RPE apical membrane in both native tissue and cultured RPE, while anti-αvβ3 antibody (LM609) labeled less than 1% of cultured RPE and did not label native RPE. Antibodies against αv and αvβ5 P1F6 also immunoprecipitated 2 protein bands corresponding to integrin subunits αv and β5 from a membrane extract of cultured human RPE. The peptide Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP, 1 mM) inhibited the total ROS uptake (externally bound and ingested) by 48% (P < 0.001) and ingestion of ROS by 37% (P < 0.001). Antibody P1F6 (50 µg/ml) inhibited the total ROS uptake and the ingestion of ROS by 63% (P < 0.001) and 43% (P < 0.01), respectively.

CONCLUSIONS. The integrin αvβ5 vitronectin receptor is expressed on the apical membrane of human RPE and participates in the binding of photoreceptor ROS during phagocytosis by cultured human RPE. (Invest Ophthalmol Vis Sci. 1998;39:1703-1712)
licated in mediating adhesion of RPE to the basal lamina. Several studies also reported β1 integrin immunoreactivity on the RPE apical membrane. Using tissue culture, Rizzolo et al. demonstrated that the polarized expression of α6 and β1 integrin subunits in chick RPE is determined, at least in part, by the neural retina. In the absence of retina, expression of α6 and β1 integrins is restricted to the RPE basolateral membrane. However, when RPE is apposed to the photoreceptor side of neural retina in tissue culture, α6 and β1 subunits localize to the apical membrane. This finding suggests that integrins might have an important role in the interactions between the apical membrane of RPE and the outer segments of photoreceptors in the retina.

The integrin αv subunit has been shown to be expressed in the developing chick RPE, and immunoreactivity to vitronectin receptors (αvβ3 and αvβ5) has been detected on monkey and human RPE. Anderson et al., using a polyclonal antibody that recognized αvβ3 and αvβ5, stained the interface of the RPE apical membrane, the outer segments of rods and cones from sections of rhesus monkey eyes, and the apical membrane of cultured human RPE. This study also found mRNAs of integrins αv and β5 subunits but not β3 subunit in monkey RPE. Another recent study reported that vitronectin is responsible for the serum-stimulated uptake of ROS by adult human RPE, and this stimulation was blocked by anti-αvβ5. In the present study, we further examined the expression of vitronectin receptors in freshly dissected human fetal RPE tissue and cultured human RPE cells. We also investigated the possible role or roles of vitronectin receptors in mediating phagocytosis of ROS by the RPE.

**MATERIALS AND METHODS**

**Retinal Pigment Epithelial Cell Culture**

Human fetal eyes (nominal gestation age 17–23 weeks) were obtained from Advanced Biological Resources (Alameda, CA). All procedures adhered to the protocol approved by the University of California, Santa Barbara Committee for Protection of Human Subjects and the tenets of the Declaration of Helsinki. Human fetal eyes were enucleated immediately after elective abortion, put in cold (4°C) Dulbecco’s modified Eagle’s medium ( Gibco-BRL, Grand Island, NY) containing 10% fetal calf serum. The dissociated RPE cells were pigmented, had developed apical membrane microvilli, and were closely packed in a hexagonal pattern. After the initial 10 to 14 days, cells were dissociated with trypsin and passed, at a density of 1:5, onto either laminin-coated tissue culture plates or glass coverslips. The passaged cells were grown for at least 14 days before use, when they were closely packed and morphologically differentiated. Although a recent study reported a lower rate of phagocytosis for differentiated versus undifferentiated RPE cells, we chose to use differentiated cells because they are closer in phenotype to RPE cells in vivo. Only cells from this first passage were used in this study. A photomicrograph of a first passage RPE cell culture is shown in Figure 1.

**Preparation of Bovine Rod Outer Segments**

Bovine ROS were isolated according to the methods developed by Molday et al. About 20 to 30 fresh bovine eyes were obtained from a local slaughterhouse. Under dim red light, retinas were placed in 10 ml homogenization solution containing 20% (wt/vol) sucrose, 20 mM Tris acetate, pH 7.4, 10 mM glucose, 5 mM taurine, and 2 mM MgCl2. The retinas were shaken gently and filtered two to three times through a 300-μm nylon filter to remove larger tissue fragments. The retina suspension was then layered onto a five-step sucrose gradient (27%–60% wt/vol) containing 20 mM Tris acetate, 10 mM glucose, and 10 mM taurine, and then centrifuged at 25,000 rpm for 1 hour at 4°C with a Beckman SW-28 rotor (Beckman Instruments, Palo Alto, CA). A pink band of ROS was collected, washed, and stored in homogenization solution. The integrity of the purified ROS was confirmed by microscopy. The ROS not immediately used were stored at a −80°C freezer. Experiments were carried out using both fresh and frozen bovine ROS, and similar results were obtained (see Results section). Previous studies have shown that frozen ROS maintained their morphology and were phagocytosed normally. All phagocytosis experiments reported have been reproduced using both fresh and frozen ROS, and data presented are averaged from experiments in which both sources of ROS were used. This investigation adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Immunofluorescence Labeling of Native and Cultured Human Fetal Retinal Pigment Epithelial Cells**

To label native human fetal RPE, freshly dissected RPE/choroid sheets were carefully placed on a poly-l-lysine–coated glass slide with the apical membrane facing up. The cell sheets were gently rinsed with PBS and then washed with PBS containing 3% bovine serum albumin. The tissues were incubated for 1 hour with 10 μg/ml monoclonal antibodies against αv (Chem-
FIGURE 1. A bright field image of cultured human fetal retinal pigment epithelium, first passage.

Immunoprecipitation of Integrins

Immunoprecipitation of cultured human fetal RPE and MG-63 membrane protein was based on methods described previously. Human fetal RPE and MG-63 cells grown in 10-cm-diameter tissue culture plates were scraped off, washed with an immunoprecipitation buffer containing 150 mM NaCl, 10 mM NaH₂PO₄, 1 mM MgCl₂, 1 mM CaCl₂, 1 mM iodoacetamide, and 2 mM phenylmethyl sulfonfluoride, at pH 7.4. Cells were labeled with 0.3 mg/ml sulfo-N-hydroxysuccinimidobiotin (Pierce Chemical, Rockford, IL) in immunoprecipitation buffer for 1 hour at 4°C. The biotinylation was stopped by adding 10 mM Tris–Cl, pH 7.4, to the buffer. To prepare membrane extracts, biotinylated and unbiotinylated cells were each washed twice with the immunoprecipitation buffer and lysed by three cycles of freeze/thaw and 1 hour incubation in immunoprecipitation buffer plus 0.5% Nonidet P-40 (vol/vol). Supernatants of the lysates were saved for immunoprecipitation.

To reduce nonspecific binding, the biotinylated lysate was incubated with Sepharose gel coupled to goat anti-mouse IgG (Organon Teknika) for 1 hour at room temperature. The Sepharose gel was removed by brief centrifugation. The preabsorbed lysate was divided into fractions containing approximately 0.15 mg to 0.2 mg protein, and each fraction was incubated with 2.5 µg specific antibody for 5 to 12 hours at 4°C. Normal mouse IgG was used as a negative control. The lysate-antibody mixture was then incubated for 1 hour at room temperature with approximately 20 µl Sepharose gel coupled to goat anti-mouse IgG, which had been preadsorbed with unbiotinylated RPE or MG-63 cell membrane lysate (1 hour, room temperature). The Sepharose gel was pelleted and extensively washed. Finally, the gel was mixed with 50 µl electrophoresis sample buffer and heated for 5 minutes at 90°C, and briefly centrifuged to remove the Sepharose.

Immunoprecipitated proteins were separated by electrophoresis on 6% sodium dodecyl sulfate-polyacrylamide gels under nonreducing conditions. Prestained molecular weight markers (Bio-Rad, Hercules, CA) were run parallel to the samples. Proteins were then electrophoretically transferred to a 0.45-µm nitrocellulose membrane (Micron Separations, Westborough, MA) using a semiPhor dry blotter (Hoeffer-PharMacia, South San Francisco, CA). The blot was washed with a Tris–Tween 20–buffered solution containing 150 mM NaCl, 10 mM Tris–HCl, pH 8.0, 0.05% Tween 20 (vol/vol), and blocked with the same solution containing 5% nonfat dry milk. The blot was then incubated for 1 hour at room temperature with
horseradish peroxidase-conjugated streptavidin (1:1000 dilution in Tris-Tween 20–buffered solution plus 5% nonfat dry milk; Amersham, Arlington Heights, IL). The immunoprecipitated bands were detected with an Enhanced Chemi-Luminescence western blot detection reagent (Enhanced Chemi-Luminescence; Amersham) on Kodak X-OMAT film (Eastman Kodak, Rochester, NY).

**Phagocytosis Assay of Rod Outer Segments by Cultured Human Fetal Retinal Pigment Epithelial Cells**

In most experiments, phagocytosis of ROS was assayed according to the method of Lutz et al. Briefly, bovine ROS were first labeled with 10 to 20 μM fluorescein isothiocyanate (FITC) in Hanks’ balanced salt solution (HBSS) overnight in the dark at 4°C. The FITC-labeled ROS were washed with HBSS, resuspended in cell culture medium, and incubated with RPE cells at 37°C in 5% CO₂. Preliminary experiments showed that phagocytosis increased over time but then began to plateau after 2 hours, so we used a 2-hour incubation time for our experiments. After the incubation, the wells were gently washed four to five times with warm (approximately 37°C) HBSS to remove unbound ROS, and 0.1 ml (for 96-well plate) or 0.5 ml (for 24-well plate) HBSS was added to each well. The total number of fluorescent ROS (termed Total ROS Uptake, which includes externally bound and ingested ROS) was determined from photomicrographs obtained using an Olympus IMT-2 microscope equipped to detect epifluorescence (Olympus, Lake Success, NY) (Fig. 5A). To obtain the number of ROS ingested by RPE, an equal volume of HBSS containing 0.4% trypan blue was added to each well for 10 minutes to quench fluorescein outside the RPE cells, and the number of internalized ROS was determined (Fig. 5B). Each condition was duplicated, and for each well either 3 (96-well plate) or 5 (24-well plate) randomly selected fields (0.33 mm²) were photographed and counted. Results are presented as the mean ± SEM. Statistical significance was calculated using an independent Student’s t-test.

To compare our results with previous studies, the extent of phagocytosis was also measured using the double label immunofluorescence protocol developed by Mayerson and Hall, except that images were obtained using a Bio-Rad 1024 laser scanning confocal microscope.

**Peptide Inhibition Assay**

RPE cells were cultured in laminin-coated 24-well plates. Cell culture media containing different concentrations (0, 0.02, 0.2, and 2 mM) of Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP) and Gly-Arg-Gly-Glu-Ser-Pro (GRGESP) (synthesized by the UCSB Advanced Instrumentation Center) were kept at 37°C in 5% CO₂ for at least 1 hour before they were added to cells. The pH of the media was checked to ensure that there was no significant pH variation. To each well, 0.5 ml peptide-containing medium was added, followed by the addition of approximately 5×10⁶ fluorescence-labeled ROS in 0.5 ml cell culture medium. The RPE cells were incubated with ROS for 2 hours at 37°C in 5% CO₂, and the phagocytosis was analyzed. As a control, ROS were also added to wells containing no peptide. The final concentrations of the peptides were 0.01 mM, 0.1 mM, and 1 mM.

**Antibody Inhibition Assay**

RPE cells were cultured in laminin-coated 96-well plates. To each well, 50 μl cell culture media, containing 5 μg of specific antibody, was added. The two antibodies used were specific monoclonal antibodies against human integrin αvβ3 (LM609) or αvβ5 (P1F6). As controls, medium containing either no antibody or 5 μg normal mouse IgG was added. The RPE cells were incubated with antibody for 10 minutes at room temperature, and then approximately 10⁵ FITC-labeled ROS were added, in 50 μl cell culture medium, to each well. The RPE cells were then incubated with ROS for 2 hours at 37°C in 5% CO₂.

**RESULTS**

Expression of Integrin αvβ5 in Human Retinal Pigment Epithelial Cells

Previous work identified immunoreactivity to vitronectin receptors on the apical surface of cultured human RPE, but the exact subunit composition of the vitronectin receptors was not clear. To address this question, expressions of integrins αvβ3 and αvβ5 were examined in both the native human fetal RPE explants and in cultured fetal human RPE by immunofluorescence staining. Monoclonal antibodies against human integrins αv, αvβ3 (LM609), and αvβ5 (P1F6) were used to stain freshly dissected sheets of fetal human RPE cells. Confocal images (apical x, y sections) showed that antibodies against αv and αvβ5 clearly labeled the RPE apical membrane microvilli (Figs. 2A and 2C), whereas the αvβ3 antibody did not show any significant labeling compared with normal mouse IgG (Figs. 2B and 2D).

In cultured fetal human RPE, αv and αvβ5 antibodies intensely labeled the RPE apical membrane microvilli in a uniform pattern (Figs. 3A and 3C). Cultured fetal human RPE did not exhibit much immunoreactivity to the αvβ3 antibody (Fig. 3B). However, in some but not all RPE cultures grown on glass coverslips, a very small percentage of cells (approximately 1%) in isolated clusters were clearly labeled by the αvβ3 antibody (data not shown). Immunoprecipitation experiments were also carried out to confirm the immunofluorescence staining results. Biotinylated cell membrane extracts of cultured fetal human RPE were immunoprecipitated with the same monoclonal antibodies against integrins αv, αvβ3, and αvβ5. Immunoprecipitated proteins were separated on a 6% sodium dodecyl sulfate-polyacrylamide gel under nonreducing conditions, transferred to nitrocellulose, and detected using streptavidin–horseradish peroxidase. The resulting autoradiogram is shown in Figure 4A. The antibodies against integrins αv (lane 1) and αvβ5 (lane 3) immunoprecipitated two protein bands with molecular weights of approximately 150 kDa and 98 kDa, which closely correspond to the known sizes of integrin subunits αv and β5, respectively. No clearly identifiable protein band was immunoprecipitated by the antibody against αvβ3 (lane 2). As a positive control, immunoprecipitation was carried out using biotinylated membrane proteins from the human osteosarcoma cell line MG63, which is known to contain both integrins (Fig. 4B).

Inhibition of Rod Outer Segment Binding and Phagocytosis by an RGD Peptide and a Function-Blocking Anti-αvβ5 Antibody

To investigate whether the αvβ5 integrin might play a role in the binding and/or phagocytosis of ROS, we exposed
cultured human RPE to fluorescein-labeled bovine ROS, and assayed phagocytosis by the method of Lutz et al. Photic micrographs of total ROS uptake (externally bound plus ingested ROS) were obtained (Fig. 5A), and then trypsin blue was added to quench the externally bound ROS (Fig. 5B). A subset of cells active in phagocytosis show bright unquenched fluorescence due to ingestion of multiple ROS. The localization of the internalized ROS was confirmed by confocal microscopy (data not shown).

The extent of phagocytosis measured by this method was 514 ± 38 ROS per mm², which is similar to that measured by Lutz et al., who used rat RPE and bovine ROS (290 ± 54 ROS per mm²). However, these numbers are considerably lower than those reported by Mayerson and Hall, who used a double label immunofluorescence assay to measure rat RPE phagocytosis of rat ROS (approximately 2500-9500 ROS per mm²). The latter assay may be more sensitive, however. Using the double-label assay, we measured 2045 ± 298 ROS (>1 μm in diameter) ingested per mm². Smaller ingested phagosomes containing small ROS fragments were visualized that could not be detected by the Lutz et al. assay. We chose to use their assay in this study, because it uses less stringent washes, allowing a more sensitive analysis of the binding of ROS to the RPE surface.

Several agents that block the function of the αvβ3 integrin were tested for their effects on phagocytosis. Figure 6 shows that the total ROS uptake and ingestion of ROS by RPE were significantly inhibited by the presence of 0.1 mM and 1 mM RGD-containing peptide GRGDSP, but were not affected by the control peptide GRGESP (1 mM). Peptide GRGDSP, at 0.1 mM, inhibited the total ROS uptake (externally bound and ingested) by 41% (P < 0.001) and the ROS ingestion by 36% (P < 0.01). At 1 mM, GRGDSP inhibited the total ROS uptake and ingestion by 48% (P < 0.001) and 37% (P < 0.001), respectively.

The phagocytosis of ROS by RPE was also inhibited by a function-blocking antibody against human integrin αvβ5 (P1F6). Figure 7 shows that 50 μg/ml anti-αvβ5 antibody (LM609) and normal mouse IgG did not affect ROS phagocytosis, whereas 50 μg/ml of anti-αvβ5 antibody inhibited the total ROS uptake by 65% (P < 0.001) and the ingestion of ROS by 45% (P < 0.01).

Similar results were obtained regardless of whether frozen or fresh ROS was used. For example, in one experiment, 1 mM GRGDSP peptide inhibited total uptake of fresh ROS by 52% ± 7% (mean ± SEM), and ingestion of ROS by 33% ± 4%, whereas when previously frozen ROS were used the inhibitions were 54% ± 8% and 25% ± 6%, respectively. The monoclonal antibody against integrin αvβ5 (P1F6) inhibited total uptake of fresh ROS by 51% ± 8% and the ingestion by 37% ± 12%. P1F6 inhibited uptake and ingestion of frozen ROS by 69% ± 8% and 48% ± 19%, respectively.
FIGURE 3. Immunofluorescence staining of cultured human fetal retinal pigment epithelium with monoclonal antibodies against human integrin αv subunit (A), αvβ3 (B), αvβ5 (C), and with normal mouse IgG (D). Confocal x, y sections (parallel to the monolayer) that transect the RPE microvilli are shown.

DISCUSSION

Expression of the Vitronectin Receptor

A previous study demonstrated the presence of one or more vitronectin receptors in cultured adult human RPE by immunofluorescence labeling and western blot analysis with a polyclonal antibody that recognized integrins αvβ3 and αvβ5. The same study detected integrin β3 mRNA but not β5 mRNA in rhesus monkey retina-RPE by northern blot analysis, suggesting that the RPE vitronectin receptor is the integrin αvβ5. This conclusion is supported by the immunochemistry and immunoprecipitation data presented here. Monoclonal antibodies against integrins αv and αvβ3 stained the apical membrane microvilli of native human fetal RPE explants (Figs. 2A, 2C) and cultured human fetal RPE (Figs. 3A, 3C). These monoclonal antibodies also immunoprecipitated two protein bands with molecular weights corresponding to integrin subunits αv and β3.

The integrin αvβ3 was not detected by staining native human fetal RPE or by immunoprecipitation of cultured human RPE membrane extracts. However, αvβ3 immunoreactivity was detected in a very small fraction of cultured human fetal RPE (<1%). One possibility is that the expression αvβ3 integrin is developmentally regulated and the cells expressing αvβ3 integrin were at a different developmental stage than the rest of the cells. However, by light microscopy no morphologic differences were apparent between the cells that expressed αvβ3 integrin and those that did not. Another possibility is that the RPE is not completely homogeneous and the αvβ3-positive cells may represent a small group of RPE cells that are functionally distinct. Nevertheless, it is clear that the αvβ5 vitronectin receptor is the predominant species found on the RPE apical microvilli.

Receptor-Mediated Rod Outer Segment Phagocytosis

Phagocytosis of shed disc membranes of ROS is crucial for maintaining the health and integrity of photoreceptors. However, ROS phagocytosis is a complex process that is not fully understood. Many cell surface proteins could be involved in this process. In the phagocytosis of apoptotic neutrophils by monocyte-derived macrophages, at least two different receptors on the macrophage cell surface are involved: the phosphatidylserine receptor (CD36) and the vitronectin receptor (integrin αvβ3). Multiple receptors are also used by macrophages in the phagocytosis of pathogens, including the immunoglobulin and complement factors, the integrin αMβ2 (CR3), and the mannose receptor.

Two RPE membrane receptors, CD36 and the mannose receptor, have been shown to mediate phagocytosis of ROS. The present study demonstrates that the RPE apical
Immunoprecipitation of integrins. (A) Membrane proteins of cultured human fetal retinal pigment epithelium were biotinylated and immunoprecipitated, and electrophoresis was performed on a 6% polyacrylamide gel under nonreducing conditions. Antibodies used were monoclonal antibodies against human integrin αv subunit (lane 1), αvβ3 (LM609, lane 2), and αvβ5 (P1F6, lane 3). Lane 4 is the negative control with normal mouse IgG. (B) Immunoprecipitation of biotinylated membrane proteins from MG-63 cells by monoclonal antibodies against human integrin αv subunit (lane 1), αvβ3 (LM609, lane 2), αvβ5 (P1F6, lane 3), and normal mouse IgG (lane 4). Molecular weight markers are indicated.

Membrane vitronectin receptor αvβ5 integrin also participates in the phagocytosis of ROS: The phagocytosis of ROS is inhibited by RGD-containing peptide and a function-blocking antibody against integrin αvβ3 (P1F6) (Figs. 6, 7). The phagocytosis was not affected by a function-blocking antibody against integrin αvβ3 (LM609).

In a previous study, in which a different monoclonal antibody against integrin αv was used, Ryeom et al. did not...
Figure 6. Effects of peptides GRGDSP and GRGESP on the total rod outer segment (ROS) uptake (externally bound and ingested) and ingestion of ROS by the cultured human fetal retinal pigment epithelium. Data from four experiments are expressed as a percent of the total ROS uptake under the control condition. GRGDSP at 0.1 mM and 1 mM reduced the total ROS uptake and the ingested ROS, but 1 mM GRGESP had no significant effect.

observed any inhibition of ROS phagocytosis by cultured human RPE. This discrepancy could be attributed to differences in antibody specificities or the experimental procedures. In that study, the monoclonal antibody 69-6-5 was preincubated with RPE for 30 minutes at 37°C before ROS was added. In our study, preincubation of antibody P1F6 with the RPE culture for longer duration (approximately 30 minutes) at 37°C greatly reduced its inhibitory effect, possibly because of degradation or internalization of the antibody. Thus, we limited the preincubation to 10 minutes at room temperature (see Materials and Methods section). Consistent with our results, Miceli et al.26 reported inhibition of phagocytosis by adult human RPE using the same P1F6 antibody used here.

The phagocytosis of ROS by RPE involves several different steps. The initial step is attachment of ROS to the RPE apical membrane, which requires the recognition and binding of ROS by the RPE. The bound ROS are then internalized and digested by the RPE. Under the conditions of our assay, about 20% of the bound ROS are internalized. The RGD-containing peptide and anti-αvβ5 antibody had more significant inhibitory effects on the ROS binding at the RPE surface than on their internalization by the RPE. RGD peptide (1 mM) and P1F6 reduced ROS internalization by 37% and 43%, respectively, and the externally bound ROS (the total uptake minus ingested ROS) by 62% and 58%, respectively. This differential inhibition suggests that αvβ5 is involved in the initial binding event. The internalization step that follows binding might involve the recruitment of additional receptors11-13 and the assembly of accessory intracellular factors involved in endocytosis and the formation of the phagosome.

Anti-αvβ5 antibody and RGD peptide only partially inhibited ROS phagocytosis, supporting the idea that there are other receptors involved besides integrin αvβ5. αvβ5 may cooperate with CD36 in a fashion similar to αvβ3 in other cells. In the phagocytosis of apoptotic neutrophils by monocyte-derived macrophages, antibodies against integrin αvβ3 and CD36 applied together caused synergistic inhibition of the phagocytosis.17 Although some functional differences have been noted,34,36 integrins αvβ3 and αvβ5 bind similar ligands and may play similar roles in phagocytosis.

A study by Heth and Marescalchi37 showed that ROS stimulate the inositol triphosphate (IP3) pathways in Long Evans rat RPE, but not in the RCS rat RPE (which lacks phagocytosis capability), even though the IP3 pathway is intact in the RCS rat RPE. Stimulation of the IP3 pathway causes the elevation of intracellular calcium.38 It has been shown that binding of ligands or antibody to αv vitronectin receptors leads to intracellular calcium mobilization.39,40 Thus, the ROS-induced
Role of Integrin αvβ5 in ROS Phagocytosis

Figure 7. Effects of anti-αvβ3 (LM609) and anti-αvβ5 (P1F6) antibody on the total rod outer segment (ROS) uptake and ingestion by cultured human fetal retinal pigment epithelium. Concentrations of all antibodies were 50 μg/ml, and data from three experiments are expressed as a percent of the total ROS uptake under the control condition. The total ROS uptake and ingested ROS were reduced by P1F6 but not by LM609 or normal mouse IgG.

Ligands for Integrin αvβ5

The ligand for integrin αvβ5 in ROS phagocytosis has not been identified. Previous studies had shown that in vitro ROS phagocytosis is dependent on the presence of serum, which indicates that some serum components are crucial for phagocytosis. Vitronectin, a ligand for vitronectin receptor, is abundant in serum (approximately 300 μg/ml), and a recent study reported that vitronectin is responsible for serum-stimulated ROS uptake by adult human RPE cells. We examined whether vitronectin helps mediate phagocytosis of ROS by the RPE. In contrast to the findings of Miceli et al., we found that supplements of 50 to 250 μg/ml vitronectin did not increase phagocytosis in the absence of serum. In the absence of serum, total ROS uptake was only 6% ± 1% of the control, and ROS ingestion was only 17% ± 4% of control. Addition of purified human vitronectin at 250 μg/ml (nearly 10 times the amount of vitronectin in the control medium [10% fetal calf serum]) did not result in any significant restoration of phagocytosis (5% ± 1% total uptake, 17% ± 6% ingestion) compared with control in the presence of serum. Differences in results may be due to the age of the cultures or the sources and purity of the vitronectin used. The interphotoreceptor matrix may contain other as yet unidentified serum component that may act as a ligand for αvβ5.

More work will be necessary to define how αvβ5 and other receptors participate in the complex process of RPE phagocytosis. It is not yet clear how many receptors play a role in the process, and in vivo experiments to test their functions have not yet been carried out for any of the candidate receptors. It is possible that RPE αvβ5 and other integrins may play important roles in RPE-photorceptor interactions other than phagocytosis, such as retinal attachment. Current work is in progress to further elucidate the functions of RPE αvβ5 integrin and to identify its ligand or ligands.

Note

While this study was under revision, Finnemann et al. reported results supporting a role for αvβ5 in ROS phagocytosis by rat and human RPE cell lines.

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in the Long Evans rat RPE could be caused by the binding of ROS to integrin αvβ5. Any mutation that affects integrin αvβ5 signal transduction domain or the downstream signal transduction pathway that activates phospholipase C might explain the absence of IP3 mobilization and the lack of phagocytosis by the RCS rat RPE.

While this study was under revision, Finnemann et al. reported results supporting a role for αvβ5 in ROS phagocytosis by rat and human RPE cell lines.

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