Apical-to-Basolateral Transcytosis of Photoreceptor Outer Segments Induced by Lipid Peroxidation Products in Human Retinal Pigment Epithelial Cells

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PURPOSE. Progressive accumulation of extracellular material at the basolateral side of the retinal pigment epithelium (RPE) is a key event in the pathogenesis of age-related macular degeneration (AMD). The authors previously demonstrated that modifications with lipid peroxidation products, such as 4-hydroxynonenal (HNE) and malondialdehyde (MDA), stabilize photoreceptor outer segment (POS) proteins against lysosomal degradation. Herein, they tested RPE cells for the basolateral release of undegraded modified POS proteins.

METHODS. Polarized cultures of the human RPE cell line ARPE-19 on permeable membranes were incubated with iodine-125–labeled POS on the apical side. After 24 hours, radioactivity was quantified in apical medium, cell lysates, and basolateral medium after separation of undegraded proteins by precipitation. Protein composition of basolaterally released POS material was analyzed by two-dimensional gel electrophoresis. C3a- and SC5b-9-specific enzyme-linked immunosorbent assays were used to assess complement activation by modified POS.

RESULTS. The amount of phagocytic uptake was similar for native and modified POS. Unmodified POS proteins were almost completely (98.1%) degraded, whereas degradation of HNE- and MDA-modified POS proteins was significantly reduced (47.2%; 56.5%). Undegraded POS proteins accumulated intracellularly (14.2%; 12.1%) and were trafficked through the cells to be released into the basolateral medium (38.5%; 31.5%). Protein composition of basolaterally released material matched the original POS preparations. Protein modifications did not confer increased complement-activating capacity to POS material.

CONCLUSIONS. Inhibition of lysosomal degradation by lipid peroxidation-related protein modifications induces apical-to-basolateral transcytosis of undegraded POS proteins by human RPE cells in vitro. This mechanism may contribute to sub-RPE deposits formation and drusen biogenesis in AMD. (Invest Ophthalmol Vis Sci. 2010;51:553–560) DOI:10.1167/iovs.09-3755

Progressive deposition of extracellular material between the basolateral side of the retinal pigment epithelium (RPE) and adjacent Bruch’s membrane is a hallmark of early-stage age-related macular degeneration (AMD).1,2 Among the various histologically and clinically distinguishable manifestations of sub-RPE deposits, basal linear deposits (BLinD) and soft drusen are recognized as specific for AMD.3,4 Both BLinD and soft drusen represent accumulations of material described as membranous debris5 between the RPE basement membrane and the inner collagenous layer of Bruch’s membrane and are, therefore, considered diffuse and focal manifestations, respectively, of the same lesion.1,3,6 The prognostic relevance of drusen size and number for the progression of AMD has been well established.7–9

When Heinrich Müller initially coined the term drusen in 1856, he already suggested that material deposition by the retinal pigment epithelium might contribute to their development.10 Today, more than one and a half centuries later, the mechanisms of sub-RPE deposit formation are still not completely resolved. However, proteomic studies revealed that drusen and aged Bruch’s membranes contain proteins that are covalently modified by products of lipid peroxidation, and these modifications were more abundant in AMD eyes than in age-matched control eyes.11 Lipid peroxidation is a mechanism of oxidative damage that predominantly affects tissues, such as the outer retina, that are exposed to high levels of oxidative or phototoxic stress and are highly enriched in polyunsaturated fatty acids (PUFAs). In lipid peroxidation, oxygen-derived free radicals interact with PUFAs double bounds, resulting in cleavage into a variety of highly reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE).12 Once formed, these lipid peroxidation products rapidly attach covalently to nearby proteins by forming adducts predominantly with cysteine, lysine, and histidine residues, a process that may interfere with protein functionality.12 Furthermore, modified proteins, also referred to as advanced lipid peroxidation end-products (ALE), are prone to aggregation by cross-linking of the adducts.12 By these mechanisms, lipid peroxidation products cause severe damage to cellular proteins and other macromolecules and thus impair cellular functions and survival.

We previously detected modifications with MDA and HNE on proteins isolated from RPE-derived lipofuscin, thus demonstrating the occurrence of these substances in the RPE lysosomal compartment and suggesting their contribution to lysosomal dysfunction and lipofuscinogenesis.13 Indeed, we observed that modifications of photoreceptor outer segment (POS) proteins with MDA and HNE significantly reduced their degradation by RPE cells in vitro.14 After normal phagocytic uptake, modified POS proteins exhibited increased stability.
against the proteolytic attack by lysosomal enzymes, resulting in their intracellular accumulation and long-term storage. In the present study, we further analyzed the processing of modified POS by human RPE cells in vitro and tested whether degradable POS components are disposed into the sub-RPE space, a process that may contribute to local immune processes and sub-RPE deposit formation in vivo.

**MATERIALS AND METHODS**

**RPE Cell Culture**

The human, spontaneously stable RPE cell line ARPE-19 (ATCC CRL-2302) was obtained from American Type Culture Collection (Rockville, MD) and was maintained in a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 medium (PAA Laboratories, Colbe, Germany) containing 2.5 mM L-glutamine, 0.5 mM sodium pyruvate, and 17.5 mM d-glucose and supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% heat-inactivated fetal bovine serum (PAA Laboratories) at 37°C in a humidified atmosphere of 5% CO₂ in air. Permeable polyester membrane cell culture inserts (Transwell-Clear, diameter 12 mm, pore size 0.4 μm; Corning, Lowell, MA) were coated with 2 μg/cm² laminin (Sigma-Aldrich, Munich, Germany) for 2 hours at 37°C according to the manufacturer's recommendation. Postconfluent stationary cells were trypsinized, seeded on laminin-coated membranes without cells were subtracted as background. RPE monolayers in our culture system reached TEER values of 63 ± 24 Ohm cm² (± SD) after 7 days of culture. Paracellular permeability was analyzed by means of marker dye leakage. We used FITC-dextran (molecular weight, 40 kDa; Sigma-Aldrich) as the marker substance because it was previously shown not to be transported transcellularly by RPE cells. FITC-dextran was added to the medium on the apical cell side of membrane-cultured RPE cell monolayers at a concentration of 100 μM. After 2 hours on a horizontal shaker (40 rpm) in a cell culture incubator, FITC-specific fluorescence in the basolateral medium was quantified using a microscope (Zeiss DSM 950; Carl Zeiss). For scanning electron microscopy (SEM) analysis, the specimens were fixed and dehydrated as described and then were treated with hexamethyldisilazane, air dried, sputtered with gold, and examined with a scanning electron microscope (Zeiss DSM 950; Carl Zeiss).

**Electron Microscopy Studies**

For transmission electron microscopy (TEM) analysis, cells were fixed with Karnovsky's solution in PBS (pH 7.3), postfixed in 1% OsO₄, dehydrated in ethanol, and embedded (Durcupan ACM Fluka; Sigma-Aldrich). Ultrathin sections were stained with uranyl acetate and lead citrate and were examined with a transmission electron microscope (Zeiss EM 109; Carl Zeiss, Oberkochen, Germany). For scanning electron microscopy (SEM) analysis, the specimens were fixed and dehydrated as described and then were treated with hexamethyldisilazane, air dried, sputtered with gold, and examined with a scanning electron microscope (Zeiss DSM 950; Carl Zeiss).

**POS Isolation**

Porcine eyes were obtained from a local slaughterhouse, and POS was isolated as previously described. Briefly, the eyes were dissected at the equator, and the retinas were removed. Isolated retinas were agitated in KCl buffer (0.3 M KCl, 10 mM HEPES, 0.5 mM CaCl₂, 1 mM MgCl₂) with 48% wt/vol sucrose at pH 7.0 and were centrifuged at 5000 g for 5 minutes. Supernatant containing the POS was filtered through sterile gauze, diluted 1:1 with KCl buffer without sucrose, and centrifuged at 40000 g for 7 minutes. The pelleted POS were repeatedly washed in PBS and stored at −80°C until use.

**HNE and MDA Modification**

HNE and MDA were prepared by acid hydrolysis from (E)-4-hydroxynonenal-dimethylacetal (Sigma-Aldrich) and 1,1,3,3-tetramethoxypropane (Sigma-Aldrich), respectively, and were used for POS protein modification as described. Briefly, POS were reacted with freshly prepared 5 mM HNE or 20 mM MDA at room temperature overnight on a shaker. Unbound HNE and MDA was removed by repeated washes in PBS. Total protein content was measured by Bradford assay (Bio-Rad Laboratories, Munich, Germany). Modified POS were stored at −80°C until use.

**Radioactive Labeling of POS Proteins**

POS (200 μg total protein) were incubated at room temperature for 10 minutes in PBS with 8.4 MBq carrier-free sodium iodide-125 (Na¹²⁵I; Amersham-Pharmacia Biotech, Freiburg, Germany) in the presence of one iodium reagent bead (Iodo-Bead; Pierce, Rockford, IL). Radio-labeling was stopped by removal of the bead. Labeled POS were separated from free iodine by repeated washes in PBS and were...
immediately used for experiments. Specific radioactivity was 10.4 MBq/mg protein.

Transcytosis Assay
Polarized RPE cell cultures on permeable membranes were incubated with radiolabeled POS (10 µg/cm² total POS protein; 11.7 kBq) in complete culture medium. In analogy to the in vitro situation, POS was added only to the upper (apical) compartment of the cell culture insert. Addition of 20 mM ammonium chloride (Sigma-Aldrich), acting as an inhibitor of lysosomal catabolic functions by raising lysosomal pH20, to both apical and basolateral medium was used in controls. After 24 hours, medium in the upper and lower (basolateral) compartment was collected separately. Cells were subjected to extensive washing to remove noninternalized POS and subsequently were lysed in 0.5 M NaOH. In medium samples and cell lysates, proteins were precipitated by addition of an equal volume of 10% trichloroacetic acid (TCA), and the resultant TCA-soluble (degraded) and TCA-insoluble (undegraded) protein fractions were separated by centrifugation (16,000g; 10 minutes). Degraded and undegraded protein fractions were transferred to liquid scintillation cocktail (Ultima Gold; PerkinElmer, Rodgau, Germany), and radioactivity was measured in a liquid scintillation counter (Tri-Carb 2500TR; PerkinElmer). Experiments were performed in triplicate, and results are presented as mean (±SD).

Two-Dimensional Gel Electrophoresis
Transcytosis assays were performed exactly as described except that serum content of the culture medium was reduced to 1% during the 24-hour POS incubation period to reduce interference of high-abundance serum proteins with the detection of POS proteins. For isoelectric focusing (IEF), basolateral medium samples resulting from these transcytosis experiments and samples of the original radiolabeled POS preparations were subjected to TCA protein precipitation. Proteins were extracted from the TCA-insoluble fraction in 7 M urea, 2 M thiourea, 4% Triton X-100, 65 mM dithiothreitol, and 0.8% carrier ampholyte (Pharmalyte; Sigma-Aldrich). Equal amounts of protein were separated in an immobilized pH gradient (IPG) strip (Immobiline DryStrip, pH 3–10, 11 cm; Amersham Pharmacia Biotech, Freiburg, Germany) using an IEF system (IPGphor; Amersham Pharmacia Biotech). For subsequent SDS-PAGE, the IPG strip was applied to a precast SDS gradient gel (8–18 ExcelGel; Amersham Pharmacia Biotech), and separation was performed on a horizontal electrophoresis unit (Multiphor II; Amersham Pharmacia Biotech). Phosphor screens were exposed to the dried gels and scanned on a phosphor imaging system (Cyclon Plus Phosphor Imager; PerkinElmer, Rodgau, Germany) using appropriate software (OptiQuant; AcroMetrix, San Francisco, CA).

C3a and SC5b-9 Enzyme-Linked Immunosorbent Assays
Human blood was drawn in anticoagulant-free tubes and was sedimented at room temperature for 30 minutes. Serum was separated by centrifugation (2000g, 5 minutes), and 100 µL serum was incubated with 20 µL POS (10 µg total protein) in PBS. In parallel, serum was incubated with 20 µL PBS without POS to serve as vehicle control. After 2 hours at 37°C, complement activation was assessed by detection of C3a and SC5b-9 in 1:104 and 1:10 diluted samples, respectively, using specific enzyme-linked immunosorbent assays (Quidel, San Diego, CA) according to the manufacturer’s recommendation. Experiments were performed in triplicate, and results are presented as mean (±SD).

RESULTS
POS Phagocytosis and Degradation
Polarized RPE cell cultures on permeable membranes were incubated with radiolabeled POS in the apical medium. At the beginning of the experiment, we confirmed that labeled POS proteins in the apical medium consisted almost exclusively of degraded proteins (99.6% of total POS protein; Fig. 2A). After 24 hours of incubation, approximately half the POS proteins were phagocytosed by the cells, as determined by reduction of the apical undegraded POS protein fraction (Fig. 2A). MDA and HNE modifications did not affect the amount of POS phagocytosis (51.8%; 50.9% of total POS protein) compared with unmodified POS (48.5%). When confining the analysis to phagocytosed POS proteins (by excluding the apical undegraded proteins), the fraction of apical, undegraded proteins was excluded in this alternative presentation of results.

FIGURE 2. Polarized RPE cells on permeable membranes were incubated on their apical side with unmodified POS (Control), MDA-modified POS (MDA), HNE-modified POS (HNE), or unmodified POS and ammonium chloride (NH4Cl). (A) After 0 and 24 hours of incubation, the radioactive POS label was quantified in extracts of total degraded (TCA-soluble) and degraded (TCA-insoluble) proteins from apical medium, cell lysates, and basolateral medium. At 0 hour, labeled proteins were almost exclusively detected as undegraded proteins in the apical medium, whereas 24 hours later, both degraded and undegraded POS proteins were found in all compartments of the cell culture system. The intracellular, degraded protein fraction was also detectable but too small to be visible in the diagram. (B) To illustrate the distribution of only the phagocytosed POS proteins, the fraction of apical, undegraded proteins was excluded in this alternative presentation of results.
graded protein fraction; Fig. 2B), unmodified POS proteins were almost completely degraded (98.1% of total phagocytosed POS protein), whereas protein degradation of MDA- and HNE-modified POS was significantly reduced (56.5%; 47.2%). Unspecific inhibition of lysosomal enzymes by ammonium chloride also reduced degradation (2.6%). In each group, total radioactivity of all protein fractions combined (6.9 × 10⁵ cpm) was in good agreement with radioactivity of initially added POS (7.0 × 10⁵ cpm), confirming that protein loss during the experimental procedure was minimal.

Basolateral Release of Undegraded POS

In cells incubated with MDA- and HNE-modified POS, some undegraded POS proteins accumulated intracellularly (12.1%; 14.2% of total phagocytosed POS protein; Fig. 2B). In both groups, however, most undegraded POS proteins were detected in the basolateral medium (31.5%; 38.6%). In ammonium chloride-treated cells, the amount of undegraded proteins was significantly higher, but the distribution between intracellular (42.3%) and basolateral (55.1%) compartments followed the same trend.

To analyze whether the difference in basolateral occurrence of undegraded POS proteins between the unmodified and modified POS groups resulted from paracellular leakage in the latter, we compared paracellular permeability of cell monolayers for the marker substance FITC-dextran (Fig. 3). We detected no significant differences in apparent permeability coefficients (P_app) after 24 hours of incubation with MDA- and HNE-modified POS (4.4 × 10⁻⁶ cm/s; 3.8 × 10⁻⁶ cm/s) compared with unmodified POS (4.3 × 10⁻⁶ cm/s). Similarly, TEER values after POS incubation were similar in all groups (data not shown). In summary, these results suggest that the observed apical-to-basolateral translocation of undegraded modified POS was not a result of paracellular leakage but rather of transcellular transport processes such as transcytosis.

Characterization of Modified POS Material

To assess whether transcytosis and basolateral release of modified POS material was selective for specific POS proteins, we compared protein compositions of the original, purified POS preparations with those of basolaterally released, undegraded POS material using two-dimensional gel electrophoresis (Fig. 4). Given that the previous experiment had already established that virtually no undegraded POS proteins are released after incubation with unmodified POS, the two-dimensional gel analysis was confined to the groups of HNE- and MDA-modified POS. The use of radiodetection for imaging of the gels ensured that the visualized proteins were derived exclusively from the radiolabeled POS.

The analysis demonstrated a virtually identical protein composition of POS material in the basolateral medium and the original POS preparations, indicating that POS proteins are basolaterally released in a nonselective fashion. Because protein extraction for this analysis was performed by TCA precipitation, low-molecular weight protein degradation fragments were eliminated in the process and, thus, do not appear on the gels.
FIGURE 5. Complement activation was assessed by quantification of complement factor 3 activation fragment C3a and terminal complement complex SC5b-9 in human serum after incubation with vehicle alone (Control), unmodified POS (Unmodified), HNE-modified POS (HNE), and MDA-modified POS (MDA).

gels. Larger protein degradation fragments and cross-linked protein aggregates were not detected because of their random molecular weights and isoelectric points.

Finally, we evaluated the capability of modified POS material to activate the complement system. We measured generation of two key complement components, C3 activation fragment C3a and terminal complement complex SC5b-9, after incubation of POS with human serum (Fig. 5). Unmodified, HNE-modified, and MDA-modified POS significantly amplified the generation of C3a (4.5-, 4.7-, and 4.2-fold) and SC5b-9 (2.2-, 1.8-, and 2.0-fold) compared with vehicle control. However, complement activation was not increased for modified POS compared with native POS.

DISCUSSION

Transcytosis is the vesicular transport of molecules and particles across cellular barriers. After uptake by endocytosis on one side of the cell, the cargo is moved through the cell and released on the opposite side by fusion of the transport vesicle with the plasma membrane. Transcytosis is a common feature of various epithelial cell types, such as intestinal epithelium, microvascular endothelium, and respiratory epithelium. Several examples of transcytosis of phagocytosed particles across epithelial barriers exist and regulated exocytosis of phagosomal contents by macrophages has also been reported. In RPE cells, transcytosis of undegraded residual material derived from phagocytosed POS has previously been described morphologically in different animal models. In the present study, we provide evidence that polarized human RPE cells in vitro make use of transcytosis of undegested POS material when lysosomal capacity has been exceeded and that these processes can be induced by lipid peroxidation-related modifications of phagocytosed POS proteins.

Several lines of evidence indicate an involvement of lipid peroxidation processes in the development of the outer retinal changes observed in AMD. Protein modifications with the lipid peroxidation product carboxyethylpyrrrole (CEP) were detected in drusen isolated from AMD eyes. HNE-derived protein modifications in retinal tissue have been demonstrated as a result of light damage and aging, and peroxidized lipids have been shown to accumulate in Bruch’s membrane with age. HNE and MDA represent two of the most abundant products of lipid peroxidation and are predominantly generated from docosahexaenoic acid (MDA only), arachidonic acid, and linoleic acid, the three most prevalent PUFAs in POS membranes. Our previous observation that RPE-derived lipofuscin contains proteins modified with HNE and MDA documents that these substances occur within the RPE lysosomal compartment and suggests their contribution to lysosomal dysfunction and lipofuscinogenesis. Indeed, we demonstrated that POS proteins modified with HNE and MDA become stabilized against the proteolytic attack in the RPE lysosomal compartment and reduce activities of lysosomal proteases by a competitive mechanism. Our finding that phagocytosis of modified POS was unaffected while internalized POS were no longer degraded raised the question of how the RPE cells deal with the increasing intracellular load of modified POS material. In the present study, we addressed this question in an in vitro model using radiolabeled POS.

Polarized differentiation and sufficient barrier properties of the cellular monolayer are prerequisites for meaningful analysis of transcytosis processes in vitro. Our experiments were performed using the cell line ARPE-19, a nontransformed human RPE cell line that forms polarized monolayers and intercellular tight junctions under the culture conditions used in this study and that is capable of phagocytosis and degradation of POS in vitro. Although prolonged culture periods and the use of specialized culture media were reported to further increase barrier properties of this cell line, we found our culture conditions to be sufficient to induce morphologic features of a polarized RPE monolayer and barrier properties that resulted in almost complete blockage of paracellular passage of undegraded POS proteins from the apical to the basolateral compartment (99.5%; Fig. 2, control group). The degree of POS protein modifications yielded by the methods used in this study has been reported previously and was chosen to correspond to the range of carbonyl modifications detected in human cells in vivo, such as aged human erythrocytes, because quantitative data for human POS modifications in vivo is not yet available.

In accordance with our previous results, we found phagocytosis to be unaffected by POS modifications, as evident from similar amounts of POS uptake after incubation with unmodified, MDA-, and HNE-modified POS. After phagocytosis, unmodified POS proteins were almost completely degraded. In contrast, degradation of modified POS proteins was markedly reduced; consequently, a significant amount of undegraded POS proteins accumulated within the RPE cells. The predominant fraction of undegraded POS proteins, however, was detected in the medium on the basolateral side of the cells, indicating translocation of undegraded POS material across the RPE cell monolayer. Similar results were obtained when cells were incubated with unmodified POS in the presence of ammonium chloride, an inhibitor of intralysosomal proteolysis. Paracellular permeability assays did not provide evidence for increased paracellular leakage and thus imply transcellular transport as the underlying mechanism. Protein composition of basolaterally released material from RPE cells incubated with modified POS matched that of the original HNE- and MDA-modified POS preparations, suggesting that the apical-to-basolateral POS transport was not specific for selected proteins but...
may involve basolateral exocytosis of complete POS material. In summary, our findings demonstrate that RPE cells in vitro use transcellular apical-to-basolateral transport (transcytosis) of undegraded POS proteins with subsequent release into the sub-RPE space.

Because of the remaining nonphagocytosed POS in the apical medium at the end of the experiments, apical release of undegraded POS proteins could not be tested for and, thus, cannot be excluded. However, our data indirectly indicate that apical release did not contribute significantly, if it did at all, to the overall release of undegraded POS material. In cells incubated with unmodified POS, degradation of phagocytosed POS proteins was almost complete; hence, all remaining undegraded POS proteins in the apical medium (51.5%) most likely resulted from nonuptake because of exceeded phagocytic capacity. The amount of undegraded apical POS proteins was similar for MDA- and HNE-modified POS (48.2%; 49.1%), suggesting that this assumption applies as well to these groups given their previously established similar rate of phagocytic POS uptake.14 These results imply that in our experimental setting, release of undegraded POS occurred predominantly on the basolateral cell side.

Previous studies have demonstrated that lipid peroxidation products interfere with the cellular vesicle transport machinery by blocking vesicular fusion events or inducing trafficking defects.55–58 Interestingly, in an experimental model similar to ours, simultaneous challenge of RPE cells with POS and lipid peroxidation product/protein complexes (oxidized LDL) also resulted in the inhibition of POS degradation and the release of phagosomal contents such as pro-cathepsin D into the extracellular space.55,57 Furthermore, intracellular accumulation of oxidized LDL inhibited phagosome maturation and phagosome/lysosome fusion,55,57 raising the possibility that similar effects of HNE and MDA-modified POS proteins may contribute to POS transcytosis in our model. However, our observation that lysosomal inhibition by ammonium chloride alone is sufficient to induce POS transcytosis, even in the absence of lipid peroxidation-related protein modifications, argues for lysosomal dysfunction as the predominant underlying factor for the induction of transcytosis in our system.

Whether and to what extent RPE cell transcytosis contributes to AMD pathogenesis in vivo remains to be elucidated. The lack of detectable POS-specific proteins in drusen, at least in one study,11 and differences in the cholesterol composition between drusen and POS argue against the substantial direct contribution of unprocessed POS material to sub-RPE deposits. However, damage by lipid peroxidation and related mechanisms as well as intracellular processing during transcytosis could result in extensive alterations to POS components that might have precluded their detection in drusen in the past. Furthermore, modified POS components may not necessarily contribute quantitatively to drusen material but rather trigger local immune processes, such as complement activation, that result in chronic low-grade inflammation with progressive deposition of additional, inflammation-related material in the sub-RPE space and proangiogenic stimuli that eventually induce choroidal neovascularization.40–42

Complement activation has been implicated in the pathogenesis of AMD based on the detection of activated complement components such as C3b and C5b-9 in drusen and the association of AMD with genetic variants in the complement factors CFH, CFB, C2, and C3.44,45 Damaged cellular components, such as modified lipids and proteins, sequestered from compromised RPE cells into the sub-RPE space have been suggested as local triggers of complement activation in AMD.41,42 Indeed, in atherosclerosis models, complement activation by MDA-modified, low-density lipoprotein (LDL) with resultant generation of C3a and SC5b-9 have been reported.46 Other studies, however, detected no, or only minor, complement activation by oxidized LDL.47–49 We analyzed the effects of modified POS on key components of the complement system and found no evidence that modifications with HNE and MDA confer increased complement-activating capacity to POS material.

Although these results suggest that lipid peroxidation-related modifications may not directly act as complement activators, they could, however, generate new antigens that induce the formation of autoantibodies and subsequent immune complex-mediated complement activation. Autoantibody formation against lipid peroxidation-derived modifications on LDL has been demonstrated, and the resultant immune complexes have been implicated in chronic local inflammation in atherosclerosis.50 A role for immune complex-mediated complement activation in AMD has previously been suggested based on the colocalization of immunoglobulins and C5b-9 in drusen.51 Furthermore, serum from patients with AMD has been reported to contain increased levels of autoantibodies against the lipid peroxidation product CEP.52 and mice immunized with CEP-modified albumin develop sub-RPE complement depositions and RPE cell damage.53 Clearly, more research is needed to identify the mechanisms that trigger complement activation in patients with AMD.

In the present study, we provide evidence that human RPE cells in vitro use apical-to-basolateral transcytosis as a means of disposing undegraded POS material in situations when lysosomal capacity has been exceeded. Furthermore, we demonstrate that lipid peroxidation-related modifications of POS proteins are capable of inducing this process, most likely by their previously demonstrated capacity to induce lysosomal dysfunction in RPE cells. Future studies will have to examine whether these mechanisms can be generalized from the in vitro to the in vivo situation and to determine their role in sub-RPE deposit formation in AMD. The further elucidation of the mechanisms of drusen biogenesis and associated complement activation is crucial for the understanding of AMD pathogenesis and the identification of novel therapeutic targets for prophylaxis and treatment of this blinding disease.

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References


