Complementing apolipoprotein secretion by cultured retinal pigment epithelium

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Age-related macular degeneration (AMD) is a major cause of vision loss in the elderly, and it has two times the prevalence of Alzheimer disease in the United States. The underlying metabolic/vascular disease, with secondary neurodegeneration of photoreceptors, is still poorly understood, despite clinical success in treating neovascular complications. Most enigmatic have been drusen, the characteristic extracellular lesions that develop posterior to a support cell layer, the retinal pigment epithelium (RPE). Drusen are established ocular risk factors for progression to sight-threatening stages of disease. A major impediment to understanding drusen has been the scarcity of suitable experimental systems. In PNAS, the work by Johnson et al. (1) describes an RPE culture system exhibiting secretion of druse component apolipoprotein E, a cholesterol transporter, and activation of systemically derived complement, a pathway fingered in AMD by multiple genetic association studies.

For decades, the RPE's central role in AMD pathobiology has been suspected (2). This polarized monolayer has unusual properties dictated by its demanding dual responsibilities to both photoreceptors and choroidal vasculature. Its apical surface sits within the blood–retina barrier, and its basolateral surface is exposed to the systemic circulation across Bruch's membrane, a substratum that also serves as a vessel wall (Fig. 1). Polarized RPE functions include daily phagocytosis of photoreceptor outer segment (OS) tips, unusually oriented ion gradients, enzymes for plasma uptake and retina-side delivery of vision-critical retinoids, and secretion of growth factors, neuroprotectants, and chemokines (3–5).

Fifteen years of druse compositional studies have identified molecules and pathways well-known from other systems with functions in retina that are just being learned. Lipids, postulated as druse components in the mid-19th century (6), now include abundant esterified and unesterified cholesterol. Confirmed druse proteins include complement terminal complex C5b-9, fluid-phase regulator complement factor H (CFH), vitronectin, extracellular matrix regulator TIMP-3, apolipoproteins (E, B, and A–I), and scores of others. The synergy between complement localization in drusen and association of *CFH* sequence variants and other genes with AMD pointed strongly to a role for complement in the disease (7). Challenges to date include how constituents of local and systemic origin interact, both experimentally and clinically (8), because genes for many druse molecules are expressed by RPE, retina, or both (9). Another challenge is identifying the trigger(s) for complement deposition. A large cholesterol-rich lipoprotein containing apoB, secreted by the RPE and accumulating throughout adulthood within Bruch's membrane (where it can be toxically modified), is hypothesized to be a major trigger for age-dependent complement activation (10, 11).

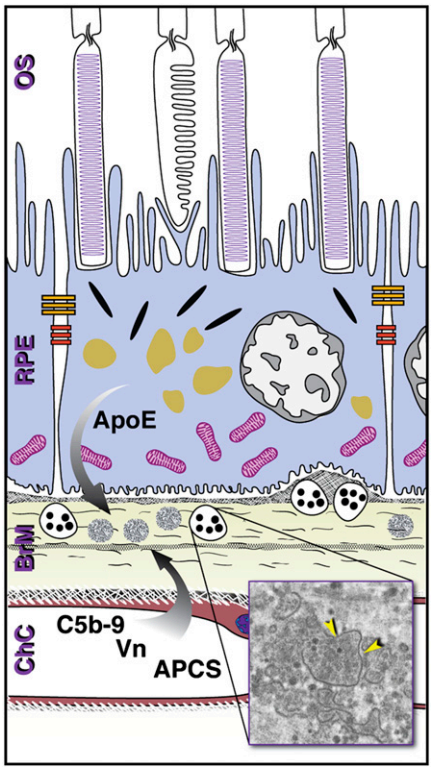

Fig. 1. Retinal pigment epithelium (RPE), photoreceptor (OS), Bruch's membrane (BrM), and choriocapillaris (Ch). Drusen are characteristic lipid- and protein-containing AMD lesions that form between RPE and BrM. Using differentiated human fetal RPE, the work by Johnson et al. (1) shows that druse-component apoE is secreted as part of multivesicular bodies (*Inset*; human BrM, 2 μm square), whereas other components C5b-9, vitronectin (VN), and serum amyloid P component (APCS) come from exogenously added serum.

The elegant and straightforward experiments by Johnson et al. (1) are notable for the simplicity and biological relevance of the model system itself. Monolayers of human fetal RPE (hfRPE) are becoming an industry standard (12–14). Techniques for culturing hfRPE have been rationalized, and the experimental advantages of well-differentiated cells are more widely recognized. Here, laminin-coated culture well inserts acted as a surrogate Bruch's membrane to entrap cellular secretions. This material was then directly visualized, along with binding to human serum components introduced into the medium, through immunofluorescence and confocal microscopy.

The findings brought together divergent threads of druse biology–apoE-mediated lipid transport and complement. In this system, apoE, an abundant component secreted by RPE (15, 16) but uncharacterized morphologically, was localized to a particulate material retained in a spatially distinct pattern within the faux Bruch's membrane. ApoE travels on small, high-density lipoproteins that export cellular cholesterol from brain and macrophages as well as larger apoB-containing lipoproteins that circulate in plasma (17, 18). Here, hfRPE-secreted apoE took two distinct ultrastructural forms—a multivesicular body and an electron-dense aggregate. The multivesicular body resembled the coated membrane-bounded bodies, bags containing 60- to 80-nm lipoprotein particles, found with their apparent fragments in aging human Bruch's membrane (19–21) (Fig. 1). Furthermore, phagocytosis was not required for hfRPE apoE secretion (10). It is time to rethink the widely held concept that druse contents reflect mechanisms to offload byproducts of photoreceptor OS phagocytosis to the choroid (22). Rather, the RPE may have separate apical and basolateral recycling circuits for lipids and other components of both OS and dietary origin (11).

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The findings added new twists to the evolving role of the complement system in producing drusen. Here, cultured hfRPE was exposed to human serum with selective deletion of complement proteins. In this setting, only complete serum given exogenously yielded binding of druse components C5b-9 terminal complexes, vitronectin, and amyloid to the apoE-immunoreactive secretions (Fig. 1). RPE cell surfaces, medium, and support matrix were unlabeled. Equally of interest, given recent focus on the alternative complement pathway, was evidence that the classical pathway is also involved. Colocalization of C5b-9 and apoE in the RPE secretions occurred only when C1q was restored to C1q-depleted serum. The classical pathway is activated primarily when C1q recognizes and binds antigen-bound immunoglobulins, but it can also binds to C-reactive protein, lipoprotein-derived cholesterol, and amyloid, all found in drusen. A gene encoding a C1 inhibitor, *SERPING1*, has been investigated for AMD associations (23), with the encoded glycoprotein, C1INH, localizing mainly to photoreceptors (24).

New questions arise from these results. What is the full range of proteins and lipids secreted by RPE and their morphologies? In particular, are the multivesicular structures secreted in vitro the release route for Bruch's membrane lipoproteins in vivo? Is there a unique epitope or molecular configuration in some individuals that predisposes to C1q binding and complement activation? Could extended cultures create entire drusen or basal linear deposits, a flat form of drusen in the same compartment? Might coculture experiments disclose a requirement for cells other than RPE in lesion formation (25)?

Experiments by Johnson et al. are notable for the simplicity and biological relevance of the model system itself.

Recent authors advocated for improved cell systems for AMD-relevant RPE physiology (11, 13, 14). Close attention to differentiation status and polarity in such systems is essential, because the RPE secretes growth factors and cytokines to both photoreceptors and choriocapillaris (5, 26), and drusenoid deposits form on the apical as well as the basolateral RPE aspect in AMD eyes (27). The complexity of local vs. remote factors is exemplified by vitronectin, a fluid phase acute responder expressed in outer retina (28). However, vitronectin binding to hfRPE-secreted apoE was revealed only as part of human serum, supporting systemic involvement without definitively excluding the local factors.

Fortunately, many hypotheses about lesion biogenesis can now be addressed in the hfRPE system implemented in the work by Johnson et al. (1). Eventually, it will be possible to exploit this system to efficiently evaluate agents to modulate either the secretion of or the response to cholesterol-rich material by RPE. These two strategies are newly available to reduce the burden of drusen and their sequelae in the clinic (11), a prospect indeed welcome for AMD patients and the physicians and scientists poised to help them.

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