Anti-amyloid therapy protects against retinal pigmented epithelium damage and vision loss in a model of age-related macular degeneration

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AUTHOR SUMMARY

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness among the elderly in Western industrialized countries. AMD affects more than 1.75 million people in the United States alone. The strongest known risk factors for AMD are advanced age and cigarette smoking, with additional risk conferred by body mass index and diets high in fat (1). Currently, there are no effective treatments for early-stage AMD, and treatments for late-stage disease are limited. AMD is characterized by the accumulation of extracellular lipid- and protein-containing deposits between the retinal pigmented epithelium (RPE) and a layer of tissue beneath the retina known as Bruch's membrane. These sub-RPE deposits contain activated components of the complement system, which boost host defense against invading pathogens (e.g., C3b and C5b-9), in addition to molecules involved in the acute-phase inflammatory response (e.g., amyloid P component) and proteins that modulate the immune response [e.g., complement factor H, vitronectin, clusterin/apolipoprotein J, apolipoprotein E (apoE), and amyloid-β (Aβ)] (2). The deposits may be focal (drusen) or diffuse and likely contribute to disease pathogenesis and progression, which has been documented for extracellular deposits that typify other pathologies such as Alzheimer's disease. Because the presence of Aβ in sub-RPE deposits has been implicated in AMD pathogenesis (3) and because immunotherapies targeting Aβ have been successfully applied to mouse models of Alzheimer's disease, we hypothesized that immunotherapy targeting Aβ would protect against ocular disease in a mouse model of AMD. Our murine model combines multiple factors known to increase AMD risk, and it exhibits morphologic and functional hallmarks of both early and late AMD. The early stage of AMD is characterized by RPE changes and deposits containing lipids and proteins known as soft drusen, whereas the late-stage disease is distinguished by either an atrophy of the RPE and retina (i.e., geographic atrophy) or choroidal neovascularization. We compared the efficacies of unique anti-Aβ antibodies, which target the two most common forms of Aβ (i.e., Aβ40 and Aβ42).

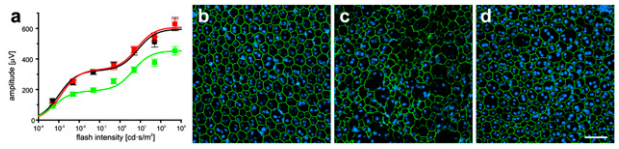

Fig. P1. Visual function and RPE histology are protected in anti-Aβ40/42–treated *APOE4*-HFC mice. (*A*) Scotopic electroretinogram flash responses show the stimulus response curves of b-wave amplitudes. Baseline electroretinograms were obtained from *APOE4*-ND controls (black) and affected *APOE4*-HFC vehicle-treated controls (green). b-Wave amplitudes were fully preserved in *APOE4*-HFC mice that received weekly 3 mg/kg i.p. anti-Aβ40/42 antibody injections (red), with no significant difference from *APOE4*-ND controls. (*B–D*) Confocal fluorescence images showing flat mounts of the central RPE from (*B*) a normal 80-wk *APOE4*-ND mouse, (*C*) an age-matched affected *APOE4*-HFC mouse, and (*D*) an anti-Aβ40/42–treated *APOE4*-HFC mouse stained with Hoechst 33342 (cyan) to counterstain RPE nuclei and labeled with antizona occludens 1 (ZO-1, green) imaged RPE apical side up with the neural retina removed. (*B*) Anti ZO-1 labeling of tight junctions (green) reveals typical hexagonal shape of RPE cells, which are also largely binucleate (cyan) in normal *APOE4*-ND mice. In *APOE4*-HFC mice (*C*), there are many more enlarged and multinucleate cells, whereas normal RPE morphology is largely maintained in the anti-Aβ40/42–treated APOE4-HFC mice (*D*). (Scale bar: 50 μm.)

Consistent with a pathogenic role for Aβ in AMD, we show that systemic administration of an anti-Aβ40/42 bispecific antibody targeting the C termini of Aβ40 and Aβ42 preserves retinal function and protects RPE morphology in this model. Our results indicate that treatment with anti-Aβ antibodies has potential therapeutic value in the treatment of both early and advanced stages of AMD.

In the *APOE4* high-fat, cholesterol-enriched (HFC) diet murine model of AMD used in these studies (*APOE4*-targeted replacement mice expressing the E4 human apoE isoform, aged to over 65 wk then fed a HFC diet for 8 wk; T.D. 88051; Harlan Teklad), multiple factors that are known to modify AMD risk combine to manifest an AMD phenotype with striking concordance with human pathology, including histopathologic changes in the RPE and formation of apoE-, complement-, and Aβ-containing deposits basal to the RPE (4). Importantly, there is also a robust, reproducible loss of visual function associated with the development of the AMD-like phenotype that mimics electrophysiological abnormalities observed in AMD patients. We likewise detected the Aβ peptide deposition observed in human AMD (3) in sub-RPE basal deposits and neovascular lesions (4) in these mice. Moreover, in response to the HFC diet, plasma Aβ levels were significantly elevated in *APOE4*-HFC

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mice compared with age-matched controls fed a normal diet (*APOE4*-ND). We hypothesized that, in this *APOE4*-HFC mouse model of AMD, Aβ accumulation initiates ocular damage at the level of the RPE/choroid and immunotherapy targeting Aβ could, thus, protect the *APOE4*-HFC mouse from developing ocular disease. We assessed the efficacy of monoclonal anti-Aβ antibodies constructed to target different forms of Aβ without triggering complement activation or antibody-dependent cell-mediated cytotoxicity. These anti-Aβ antibodies were designed to target the C termini of Aβ peptides, because these antigenic determinants are normally buried in the lipid bilayer in uncleaved amyloid precursor proteins and are predominantly exposed in the pathological state. These antibodies were also designed with a modified constant region (Fc) that does not bind to C1q complement receptors or Fcγ receptors, hence losing the ability to trigger complement activation or antibody-dependent cell-mediated cytotoxicity. The lack of antibody-induced complement activation by these Fc-modified anti-Aβ antibodies may be of high clinical relevance in the context of AMD treatment, because complement pathway dysfunction is strongly implicated in AMD pathogenesis (5). We show that systemic administration of an anti-Aβ40/42 bispecific antibody that simultaneously targets both Aβ40 and Aβ42 reduces Aβ load in sub-RPE deposits, maintains normal RPE morphology, and preserves retinal function (Fig. P1*A*). We quantified RPE damage in *APOE4*-HFC mice using RPE flat mounts, which allowed us to view the apical side of the RPE tissue surface. RPE cells were enlarged and were frequently observed to be multinucleate rather than mono- or binucleate in vehicle-treated *APOE4*-HFC mice compared with control *APOE4*-ND and anti-Aβ40/42 antibody-treated *APOE4*-HFC animals (Fig. P1 *B–D*). Examination of RPE flat mounts from human donor eyes with and without documented AMD revealed similar RPE abnormalities to those abnormalities found in the affected *APOE4*-HFC mouse eyes; in the perimacular region of AMD eyes, multinucleate RPE cells were more common, and RPE cell area was consistently larger than in the donor eyes without history of ocular disease. Our findings support the hypothesis that reducing Aβ levels in the eye is sufficient to protect the RPE and suggest that amyloid-induced RPE damage contributes to RPE compromise, photoreceptor dysfunction, and vision loss in AMD. This tenet is also supported by our observations in the *APOE4*-HFC mice that early histopathologic changes are specific to the RPE. In addition, the rod photoreceptor-driven, but not the cone photoreceptor-driven, component of the electroretinograms is attenuated, correlating with rod-driven electrophysiological abnormalities observed in early-stage human AMD patients.

Using the *APOE4*-HFC model, the current study strongly implicates Aβ in the pathogenesis and progression of AMD, providing evidence that this protein contributes to RPE damage, sub-RPE deposit formation, and complement activation. The results suggest that Aβ may trigger the complement cascade, leading to inflammatory processes and RPE damage. Consistent with such a role is the remarkable protection from histopathologic changes and visual dysfunction afforded by anti-Aβ immunotherapy.

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