AMD as an Inflammatory Disease

An understanding of the role of the immune system in AMD may lead to new therapeutic approaches.

BY VICTOR L. PEREZ, MD

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly in industrialized countries.[1,2] A risk factor for AMD and an early sign of its development are drusen, focal areas of extracellular material that accumulate beneath the retinal pigment epithelium (RPE).

It has recently been recognized that complement factor proteins are present in drusen in eyes with AMD,[3-7] and that genetic variations in several genes related to the complement system are associated with AMD.[7-12] These findings suggest that inflammation plays an important part in the pathophysiology of AMD. However, how the immune system becomes involved in the disease process in AMD is not completely understood.

We have investigated the possibility that carboxyethylpyrrole (CEP), a unique oxidation fragment of the docosahexaenoic acid (DHA), serves as an initiating signal for inflammation in AMD. This article summarizes some of our findings and their potential implications. These findings were presented recently at the Macula 2012 meeting in New York.

BACKGROUND

The phospholipid DHA is abundant in the outer retina, where high oxygen tension and a high degree of light exposure provide a rich environment for oxidation. CEP forms when fragments of DHA, the most oxidizable of the long-chain fatty acids, interacts with certain amino acids in tissue proteins.

Previous investigations have shown that CEP-modified proteins are more abundant in the outer retina of AMD donor eyes than in age-matched controls.[3] Also, there are more CEP-adducted proteins and CEP antibodies in plasma from AMD patients than in control samples.[13] These findings regarding this oxygenation-generated hapten are notable because of the long-recognized association of AMD with oxidative damage.[14]

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MOUSE STUDY

We know that DHA is concentrated in the RPE and photoreceptor cells, and therefore it is probable that these tissues are a source of CEP adducts in the course of aging. Serum albumin is one of the proteins most commonly modified by CEP adduction in AMD patients.

We therefore immunized mice with CEP-modified mouse serum albumin (CEP-MSA) in order to boost the animals' sensitivity to endogenous CEP. We hypothesized that immunization with these CEP-modified self-proteins would sensitize autoreactive T-cells and generate a stronger immune response to CEP adducts. This would make the outer retina more vulnerable to immune-related damage and induce AMD-like pathology in the mice.[15]

In our experimental design, mice were immunized with CEP-MSA or MSA without CEP (controls) with complete Freund's adjuvant. At 10 days, the mice were challenged with incomplete Freund's adjuvant. Short-term (2 to 3 months) experiments showed that CEP-antibody titers were 6 to 8 times higher in the CEP-MSA–immunized mice compared with controls or naïve mice. Similar results were seen in longer-term (12 to 14 months) experiments. CEP-MSA–immunized Rag-deficient mice, which lack mature T cells and B cells, did not demonstrate the changes observed in normal mice.

These findings demonstrated that CEP-MSA can generate an antibody-mediated immune response. In addition, a close relationship was seen between CEP-specific

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antibody titers and the level of outer retinal pathology. Histology showed multiple AMD-like RPE lesions in the CEP-MSA eyes, including vesiculation and swelling of cells, cell lysis, pyknosis, and the presence of monocytes in the interphotoreceptor matrix. In some eyes, focal areas of RPE were missing and overlying photoreceptors were swollen. Histology also showed the presence of macrophages near some lesions.

We also evaluated levels of C3d, a degradation product of C3b, which is a key complement protein in the classical, lectin, and alternate pathways. C3d was seen in Bruch membrane in mice that had received CEP-MSA immunization. Immunofluorescence in Bruch membrane in these mice was 10 times higher than in control mice.

DISCUSSION

In summary, these investigations showed that mice mount an antibody-mediated response to CEP-MSA. In addition, the immune system responds by depositing complement below the RPE, as the location of C3d in Bruch membrane demonstrated. The response requires an intact immune system, as suggested by the absence of these changes in Rag-deficient mice.

The fact that macrophages were found near some RPE lesions suggests that macrophages may play a role in the pathogenesis of AMD, although whether this is a protective or a damaging role, or both, is not known.

It remains to be seen how these findings regarding inflammation will mesh with other current investigations of the role of the complement system in the pathogenesis of AMD. Most recently, Weismann and colleagues[16] recently showed that complement factor H (CFH) binds malondialdehyde (MDA), a common lipid peroxidation product that accumulates in many pathophysiologic processes, including AMD. In mice, CFH can block the uptake of MDA-modified proteins by macrophages and MDA-induced proinflammatory effects in vivo. Further, they found that a CFH polymorphism strongly associated with AMD (H402) reduces the ability of CFH to bind MDA, indicating a causal link to the etiology of the disease. This nominal observation links how abnormalities in the complement system can have an effect in the role of innate / adaptive "inflammatory" responses. We can speculate that similar to MDA, CEP also have proteins that bind and neutralize its ability to mount an antibody response.[17]

As more is learned about the roles of inflammation and the immune system's response to oxidative stress in the pathogenesis of AMD, we will gain important insights into novel potential immuno-therapeutic approaches to this important sight-threatening disease. ■

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