The American Journal of Pathology, Vol. 184, No. 11, November 2014

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The American Journal of PATHOLOGY

ajp.amjpathol.org

VASCULAR BIOLOGY, ATHEROSCLEROSIS, AND ENDOTHELIUM BIOLOGY

The Membrane Attack Complex in Aging Human Choriocapillaris

Relationship to Macular Degeneration and Choroidal Thinning

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Accepted for publication July 10, 2014.

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Age-related macular degeneration (AMD) is a common disease that can result in severe visual impairment. Abnormal regulation of the complement system has been implicated in its pathogenesis, and *CFH* polymorphisms contribute substantially to risk. How these polymorphisms exert their effects is poorly understood. We performed enzyme-linked immunosorbent assay (ELISA) analysis on young, aged, and AMD choroids to determine the abundance of the membrane attack complex (MAC) and performed immunofluorescence studies on eyes from 117 donors to evaluate the MAC in aging, early AMD, and advanced AMD. Morphometric studies were performed on eyes with high- or low-risk *CFH* genotypes. ELISA confirmed that MAC increases significantly with aging and with AMD. MAC was localized to Bruch's membrane and the choriocapillaris and was detectable at low levels as early as 5 years of age. Hard drusen were labeled with anti-MAC antibody, but large or confluent drusen and basal deposits were generally unlabeled. Labeling of retinal pigment epithelium was observed in some cases of advanced AMD, but not in early disease. Eyes homozygous for the high-risk *CFH* genotype had thinner choroids than low-risk homozygotes ($P < 0.05$). These findings suggest that increased complement activation in AMD and in high-risk genotypes can lead to loss of endothelial cells in early AMD. Treatments to protect the choriocapillaris in early AMD are needed. *(Am J Pathol 2014, 184: 3142—3153; http://dx.doi.org/10.1016/j.ajpath.2014.07.017)*

Age-related macular degeneration (AMD) is a complex disease that frequently results in loss of visual acuity. AMD, the most common cause of irreversible blindness in the elderly, is projected to affect 3 million Americans by 2020.[1,2] Clinically, the early stages of AMD are characterized by structural abnormalities in the posterior pole that include an abnormal appearance of the retinal pigment epithelium (RPE) and the presence of drusen, which are extracellular deposits that form between the RPE and its blood supply, the choriocapillaris.[3] Although for most patients early AMD does not progress to severe end-stage disease, a subset of patients will develop extensive degeneration of photoreceptor cells and RPE cells in the macula, caused by either pathological invasion of new blood vessels from the choroid into the sub-RPE or subretinal spaces [ie, choroidal neovascularization (CNV)] or by idiopathic loss of the macular RPE and photoreceptor cells (ie, geographic atrophy).

Although our understanding of the pathophysiology of AMD is incomplete, recent genome-wide association studies

Supported in part by NIH grants R01EY017451 (R.F.M.), R01EY016822 (E.M.S.), and DP2-OD007483-01 (B.A.T.); the Howard Hughes Medical Institute (E.M.S.); The Elmer and Sylvia Sramek Charitable Foundation (R.F.M. and B.A.T.); the Doris Duke Clinical Research Fellowship Program (D.S.); an unrestricted grant to the Department of Ophthalmology and Visual Sciences from Research to Prevent Blindness; the Hansjoerg E. J. W. Kolder, M.D., Ph.D., Professorship in Best Disease Research (R.F.M.); and the Stephen A. Wynn Foundation.

Disclosures: None declared.

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http://dx.doi.org/10.1016/j.ajpath.2014.07.017

Table 1 Characteristics of Donor Eyes Used in Enzyme-Linked Immunosorbent Assay Study

Donor	Donor age (years)	MAC concentration (ng/mL)	Cause of death	D–P time (hours)	Allele	
					Y402H	V62I
Young (<50 years of age)						
1	23	2.75	Methane asphyxia	6.75	HY	VV
2	48	18.68	Cancer	6.5	HY	VV
3	45	15.67	Respiratory failure	6.5	HY	VV
4	21	30.71	Hodgkin lymphoma	6	HY	VV
5	34	26.78	Intercranial hemorrhage	6.5	HY	VV
6	46	4.46	Cardiopulmonary arrest	<8	YY	IV
7	46	45.34	Pneumonia	2.75	YY	II
8	43	34.19	Lung cancer, renal failure	5.25	HY	IV
9	46	19.97	Renal disease	6.5	HY	VV
10	45	1.64	Pneumonia	6.75	YY	IV
Aged control, without AMD						
11	77	38.77	Pulmonary embolism	5.5	YY	VV
12	71	28.72	Intracerebral hemorrhage	7	HY	IV
13	82	83.68	Heart failure	5.5	YY	II
14	83	68.07	Respiratory failure	5.75	YY	IV
15	96	30.16	Respiratory failure	7	HY	IV
16	85	47.80	Metastatic bladder cancer	4.75	HY	VV
17	79	52.29	Congestive heart failure	5.25	HY	IV
18	95	43.95	Stroke	7	YY	VV
19	82	49.05	NA	6	YY	II
20	78	43.72	Lung cancer	6	YY	IV
Aged AMD						
21	89	185.44	NA	4.5	HH	VV
22	88	221.13	Myocardial infarction	6	HH	VV
23	99	26.96	Stroke	5.75	YY	IV
24	78	113.91	Respiratory failure	5	HH	VV
25*	77	89.88	NA	6	HH	VV
26*	85	114.65	Acute coronary syndrome	7	HY	VV
27	82	40.67	Stroke	5.5	HY	IV
28	93	165.16	Urosepsis	5.5	HY	VV
29	89	70.34	Congestive heart failure	7.25	HY	VV
30	91	77.24	Perforated bowel	5.5	HY	IV

*Earlier punches from these donor eyes were analyzed in a previous study.[10]

AMD, age-related macular degeneration; D–P time, maximum time between death and preservation of eye in liquid nitrogen; NA, not available; sC5B-9, soluble terminal complement complex.

and candidate gene approaches have led to the identification of various polymorphisms that affect the risk of AMD at all stages. These include single-nucleotide polymorphisms in a number of genes encoding members and regulators of the complement pathway, including *C3*, *CFI*, *C2* and/or *CFB*, and *CFH* (recently reviewed by Khandhadia et al[4]). One polymorphism in the *CFH* gene (rs1061170) increases risk of AMD by approximately twofold to sevenfold, depending on the population studied.[5–8] This variant results in the substitution of histidine for tyrosine at amino acid residue 402. The effect of this polymorphism in the human eye is not well understood, although adults harboring the Y402H polymorphism show increased choroidal C-reactive protein[9] and increased membrane attack complex (MAC).[10]

Formation of the MAC is the final event in the terminal portion of the complement cascade and results from the binding of C5b to plasma complement proteins C6, C7, C8, and multiple molecules of C9. MAC forms transmembrane channels that lead to cell lysis and death. The MAC has been found in drusen of older eyes with AMD.[11] However, the relative abundance and distribution of MAC in aging, early AMD, and advanced AMD have not been comprehensively studied. Inhibition of MAC components such as C6 can inhibit CNV,[12] and other complement pathway inhibitors are in active clinical trials for the treatment of AMD.[13] Because it is the ultimate downstream effector of the complement pathway, understanding the role of the MAC in the pathophysiology of AMD is important for the development of new therapies.

We evaluated the MAC in a large series of donor eyes. MAC was present in Bruch's membrane and choriocapillaris in very young eyes, but the concentration increased with age; we observed the highest levels in eyes with AMD. We further evaluated the MAC in a series of eyes from young and old donors, and from donors with early and advanced AMD. Although in early AMD the MAC is associated exclusively

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