Immunobiology 221 (2016) 650–656

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Increased serum IgA concentration and plasmablast frequency in patients with age-related macular degeneration

Honghua Yu[a,b,1], Ling Yuan[c,1], Yahan Yang[a,1], Suihong Ma[d,*], Lianghong Peng[b], Yong Wang[b], Chu Zhang[b], Tao Li[a,*]

[a] State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510275, China
[b] Department of Ophthalmology, General Hospital of Guangzhou Military Command of PLA, Guangzhou 510010, China
[c] Department of Ophthalmology, The First Affiliated Hospital of Kunming Medical College, Kunming 650031, China
[d] Guangzhou No. 1 People's Hospital, Guangzhou 510180, China

ARTICLE INFO

Article history:
Received 9 October 2015
Received in revised form
30 December 2015
Accepted 12 January 2016
Available online 16 January 2016

Keywords:
IgA
Plasmablast
Age-related macular degeneration

ABSTRACT

Age-related macular degeneration (AMD) is the leading cause of blindness among senior citizens of developed countries, with currently unknown etiology. Despite the close associations between AMD development and inhibitory complement factor H mutations, the first step of complement activation, which is the antibody response in AMD patients, has not been studied. Here, we obtained blood and tear samples from AMD patients and Non-AMD controls. We found that compared to Non-AMD controls, AMD subjects had increased IgA titers in serum and tear, and had elevated levels of circulating antibody-secreting plasmablasts. The increase in antibody titer was limited to the IgA isotype, since no significant differences were observed in IgM and IgG isotypes between AMD patients and Non-AMD controls. Interestingly, this increased antibody response in AMD patients was correlated with disease severity, as late AMD patients had increased IgA titers in serum and tear, as well as elevated plasmablast frequency after staphylococcal enterotoxin B stimulation, compared to early AMD patients. Together, our results implicated a role of overreactive IgA responses in AMD pathogenesis.

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of vision loss in seniors of developed countries (Lim et al., 2012; de Jong, 2006). The early, "dry" form of AMD occurs when immunologically active molecules, such as apoliproteins and members of the complement system, and other lipid and proteins build up to form yellow or white drusen between the retina and the choroid, causing atrophy and scarring of the retina (Rickman et al., 2013). This early dry form can progress into a more advanced stage of dry AMD. About 1 in 10 patients will also have a more severe, "wet" form of AMD, which occurs when abnormal blood vessels begin to grow underneath the retina, which may leak fluid or blood, causing blurring or distortion of the central vision (Kulkarni and Kuppermann, 2005). No cure is yet available for this condition.

The exact cause of AMD is still unknown, but several lines of evidence suggest that the immune system play a key role. Aside from the immunologically active components in drusen (Wang et al., 2010; Anderson et al., 2004), AMD risk factors, including hypertension, obesity, oxidative stress, and smoking, are known to induce a proinflammatory immune status (Haas et al., 2011; Klein et al., 2010; Evans, 2001). More directly, polymorphisms in complement factor H, an inhibitory molecule in the alternative complement system that accelerates C3 convertase degradation, greatly increase the risk of AMD development (Maller et al., 2007; Klein et al., 2005; Edwards et al., 2005). The complement system consists of a host of plasma proteins that are activated sequentially in response to an antibody–pathogen complex, leads to a series of signal amplifications and results in the activation of the cell-killing membrane attack complex (Ricklin et al., 2010). Deposition of antibodies, including IgM, IgG and IgA subtypes, are a key first step in complement activation cascade. This, and the discovery that retinal tissue-specific autoantibodies were present in AMD patients (Morohoshi et al., 2012; Cherepanoff et al., 2006; Tamm et al., 2001), imply that antibodies and antibody-induced complement activation may play a role in AMD pathogenesis.

Since antibody–antigen complexes could directly activate the complement system through binding, in this study, we sought to determine whether the antibody responses in AMD patients was

* Corresponding authors.
E-mail addresses: suihongma@sina.com (S. Ma), taoligz@126.com (T. Li).
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.imbio.2016.01.004
0171-2985/

altered, which could lead to higher complement activation. The three most studied antibody subtypes are IgM, IgG and IgA, which are most recognized for high avidity antibody–antigen interactions, high specificity and affinity bindings, and gastrointestinal microorganism neutralizations, respectively. All of them could activate the complement system independently (Hiemstra et al., 1987; Monteiro and Van De Winkel, 2003; Taylor et al., 2000). In our study, we found that AMD patient serum IgA level was elevated, together with tear IgA concentration. In addition, the frequencies of plasmablasts, a rare population of antibody-secreting B cells in blood, were significantly upregulated in the peripheral blood of AMD patients. Moreover, both serum IgA level and plasmablast B cell frequency were increased in patients with more advanced stages of AMD. Together, our data demonstrated a possible link between AMD pathogenesis and B cell activation.

Table 1
Subject information.

	AMD ($n=20$)	Non-AMD ($n=15$)	P
Age, years	57 (50–63)	58 (50–64)	>0.05
Gender, n			>0.05
Male	9	7	
Female	11	8	
BMI, kg/m^2	27.1 (24.5–28.8)	26.8 (23.2–28.3)	>0.05
AMD stage		–	
Early	10		
Late	10		
CRP, mg/L	2.4 (0.7–5.8)	1.5 (0.6–4.2)	>0.05
ESR, mm/h	13 (2–24)	11 (3–19)	>0.05
Total cholesterol, mg/dL	189 (156–234)	174 (146–231)	>0.05
LDL cholesterol, mg/dL	88 (69–145)	90 (71–139)	>0.05
Triglycerides, mg/dL	133 (95–285)	147 (102–230)	>0.05

Age, BMI, CRP and ESR were expressed as median (range).

2. Materials and methods

2.1. Participants and samples

All study procedures adhered to the tenets of the Declaration of Helsinki for biomedical research and was approved by the Institutional Review Boards of The First Affiliated Hospital and General Hospital. Participants aged 50 years and older were recruited. For each participant, two 45° digital retinal centered on the fovea were obtained at both eyes, using the Non-Mydratic Retinal Camera CR6-45NM (Canon). This step was performed in both AMD and Non-AMD participants to confirm the presence or absence of symptoms. Early and late AMD grading was performed by four experienced ophthalmologist using the University of Wisconsin Age-Related Maculopathy Grading System (Klein et al., 1991). Subjects with active immune disorders, including autoimmune diseases, type 2 diabetes, hepatitis B, traumatic injury and tumor, were excluded from the study.

Peripheral blood was drawn from all participants by venipuncture and was collected in Vacutainer tubes supplemented with citrates (BD). Blood were then centrifuged at 300 × g for 10 min

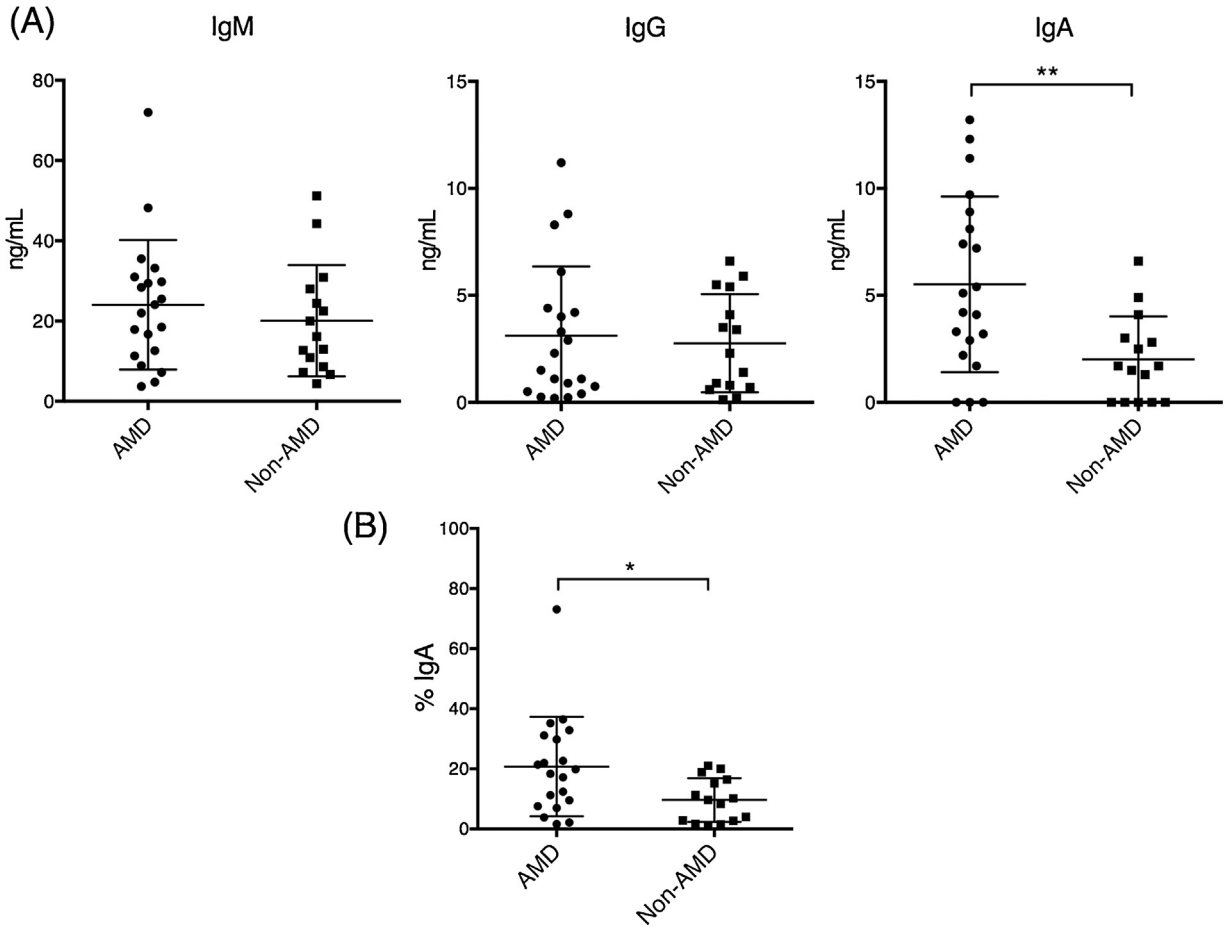

Fig. 1. Serum antibody composition in 20 AMD subjects and 15 Non-AMD control subjects.
Fresh subject sera were diluted at 1:10 dilution. The concentrations of IgM, IgG and IgA in sera were measured by ELISA. (A) The serum IgM, IgG and IgA titers in all study subjects. (B) The relative concentration of IgA in all study subjects, as calculated by the serum titer of IgA divided by the titers of IgA, IgG and IgM combined. Data were represented as mean ± standard deviation. Mann–Whitney test. $^*P<0.05$. $^{**}P<0.01$.