

Lutein and factor D: two intriguing players in the field of age-related macular degeneration

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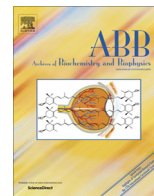
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Review

Lutein and Factor D: Two intriguing players in the field of age-related macular degeneration



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ABSTRACT

Age-related macular degeneration (AMD) is a progressive eye disease that impairs central vision among elderly populations in Western, industrialized countries. In this review we will focus on the role of factor D (FD) and lutein in AMD. FD is a rate-limiting enzyme of the alternative complement activation pathway that may play an important role in the development of AMD. Several independent studies have shown a significant increase in the level of a number of complement factors of the alternative pathway, including factor D in the blood of AMD patients as compared to healthy individuals, which suggests a systemic involvement in the pathogenesis of AMD.

FD, also called adipsin, is mainly produced by adipose tissue. Besides playing a role in the activation of the alternative pathway, FD is also known to regulate the immune system. Of interest is our preliminary finding that lutein supplementation of early AMD cases was shown to lower the level of systemic FD. If confirmed, these findings provide further support for the application of anti-factor D intervention as a new approach to control the development of this disease.

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Introduction

Age-related macular degeneration (AMD)¹ is a progressive eye condition that impairs central vision among elderly populations, particularly in those over 60 years of age in Western industrialized countries [1–3]. Increasing age is strongly associated with AMD in all-ethnic groups [4]. Additionally, both environmental and genetic factors contribute to the pathogenesis of AMD [5,6]. Of the environmental factors, light, smoking and nutrition (antioxidant intake, dietary fat, overall abdominal obesity) all play a role in the development of AMD [7–11]. Recently, evidence from both human and experimental animal studies is accumulating [12] that the pathogenesis of AMD is closely linked to the alternative pathway of the complement system [13,14]. The exact mechanisms explaining the role of complement in AMD are not yet clear. Recent studies have suggested that antioxidants might be involved in complement regulation [15,16], whereby Factor D (FD) was suggested to have a pivotal role [17–19]. Factor D is the rate-limiting enzyme of the

alternative pathway and is mainly produced by adipocytes [20], which remarkably is also the main storage site of carotenoids [21,22].

In this review, we will discuss the association of factor D with AMD, how it interacts with lutein, and we hypothesize how this knowledge may be applied to the prevention of AMD.

Does lutein affect age-related macular degeneration (AMD)?

The role of lutein in AMD can be addressed from a theoretical biological approach as well as from epidemiological and intervention studies. Lutein can act as a blue light filter absorbing between 390 and 540 nm, thereby protecting the underlying photoreceptors in the macula from photochemical damage [23]. As a powerful antioxidant, lutein may also protect the macula from oxidative stress [24–26]. Recent evidence shows that lutein may also have anti-inflammatory properties [27–29]. The three theoretical biological mechanisms described above might all be involved in the prevention or progression of AMD.

Epidemiological studies have shown that patients with AMD exhibit lower dietary intake of lutein compared to control subjects [30,31]. Large intervention studies have proven that intake of antioxidant supplements containing lutein can affect the progression of late AMD but that it does not affect worsening of early AMD [32–36]. Further, despite theoretical and epidemiological findings

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¹ Abbreviations used: AMD, age-related macular degeneration; AREDS2, age-related eye disease study 2; BMI, body mass index; CFH, complement factor H; CNV, choroidal neovascularization; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FB, factor B; FD, factor D; FI, factor I; GWAS, genome-wide association study.

concerning a preventive role for lutein in AMD, the intervention studies only support a role for lutein in the progression of AMD [36]. The explanation for these two discrepancies is unclear. It might be related to the genetical background of participants in the Age-Related Eye Disease Study 2 (AREDS2) study or the fact that protective levels of lutein were already present in the control groups. In the AREDS2 study the effects of oral supplementation of macular xanthophylls (lutein and zeaxanthin) and/or long-chain omega-3 fatty acids (docosahexaenoic acid) [DHA] and eicosapentaenoic acid [EPA] on AMD progression was evaluated. Patient groups in this study were not stratified concerning genetic background, which may have played a role in their response to the anti-oxidant supplements [37].

Pivotal role of complement activation in AMD

The complement system, originally recognized as a first line of defense against microbial intruders, is now known to play a central role during the immune response, bridging innate and adaptive immunity [38]. The system consists of over 40 proteins and regulators and is mainly found in the systemic circulation like blood or other body fluids. It plays a major role in clearing cellular debris and contributes to all phases of the inflammatory reaction, including the increase in vascular permeability, extravasation of leukocytes, and chemotaxis [39].

The complement system is activated through the classical, lectin, and alternative pathways. The alternative pathway shows the strongest association with development of AMD [40–42]. As shown in Fig. 1 [43], the alternative pathway directly activates C3 when it interacts with certain activating surfaces and involves C3, Factor B and Factor D [44–46]. The alternative pathway is able to become auto-activated because of a process termed “tickover” of C3 [47]. Auto-activation of C3 leads to the formation of C3b that covalently binds to a suitable surface. Surface bound C3b then interacts with factor B. This C3bB complex can be cleaved by factor D to form the alternative pathway C3 convertase (C3bBb) [48]. The complex is stabilized by binding of properdin and can cleave more C3 thus generating a feedback loop. This further binding of C3b leads to the formation of the C5 convertase (C3bBbC3b) and subsequent activation of the terminal complement pathway with generation of the effector molecules C5a and finally the membrane attack complex (C5b-9) (see Table 1).

The possible role of complement in AMD came from European immunohistochemical studies showing complement deposits in

Table 1
Complement gene polymorphisms associated with AMD [56,57].

SNP	Risk allele	Nearby genes	Combined	
			P	OR (95% CI)
rs10737680	A	CFH	$1 * 10^{-434}$	2.43 (2.39–2.47)
rs429608	G	C2-CFB	$4 * 10^{-89}$	1.74 (1.68–1.79)
rs2230199	C	C3	$2 * 10^{-41}$	1.42 (1.37–1.47)
rs4698775	G	CFI	$7 * 10^{-11}$	1.14 (1.10–1.17)
rs3826945	G	FD	$3.2 * 10^{-3}$	1.11 (1.01–1.23)

the retina's from AMD patients [49,50]. The Hageman group in the United States confirmed these observations later [51]. Photo-activation products in the retina have been shown to activate the complement system [52,53] and almost all complement system proteins as well as the regulators have been identified in drusen [40,51]. Drusen are considered the hallmark of early AMD and the fact that they contain complement proteins is one of the most important arguments in linking the complement system with AMD pathogenesis. Drusen are located on the RPE side of Bruch's membrane and complement is not only involved here but as shown recently also at the chorio-capillaris side [52]. Complement activation at the chorio-capillaris already starts early in life and is associated with choroidal thinning and development of AMD [54]. These authors showed that choroidal deposition of the membrane attack complex (sC5b-9) in humans was already evident at 5 years of age and that it increases markedly during life and with the presence of AMD. Quantitative analysis of sC5b-9 in retinal samples by ELISA showed that young donors had relatively low levels of sC5b-9. Retinal samples from AMD patients had variable but significantly higher sC5b-9 levels as compared to the age-matched control eyes [55]. Further support for a role of the complement system in AMD came from several genetic studies (see Table 2) that revealed that variants in complement system genes alter an individual's risk of developing AMD [56,57].

Highlight was the observation that a single nucleotide polymorphism in the gene encoding complement factor H was shown to be strongly associated with the development of AMD [58–60]. Further genetic studies revealed that other complement factors, such as factor B/C2, factor I and factor C3 are also associated with AMD [56,61,62]. A puzzling phenomenon is the fact that the complement loci identified so far contribute to both neovascular disease as well as geographic atrophy, two markedly different phenotypes of AMD. This suggests a role for complement in the common initial

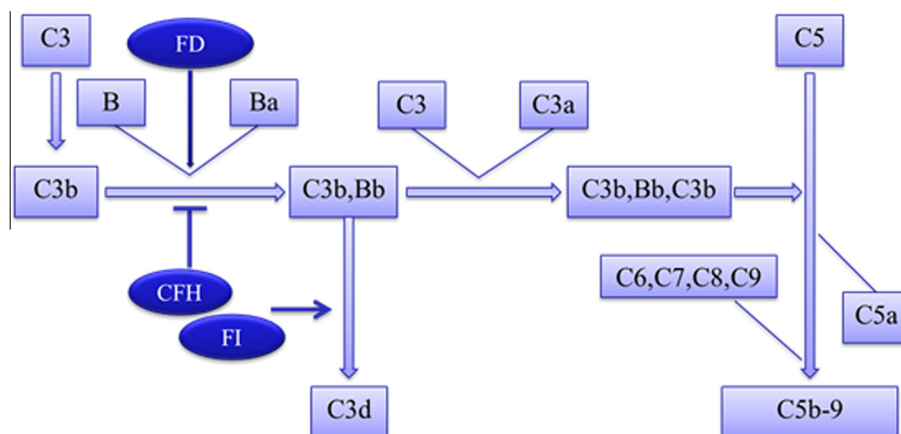


Fig. 1. Schematic representation of the cascade of the alternative pathway. During this cascade, the alternative complement pathway has a positive and a negative regulation: inhibition by complement factor H (CFH) (in combination with factor I, FI) and enhancement by factor D (FD) [43].

Table 2

Level of plasma complement factors increased in AMD patients compared with age matched healthy controls. The median concentration of the complement components were increased in the AMD patients compared with the controls in three studies. Factor H levels were not altered [41,43,63].

Complement protein	Units	Scholl et al., 2008			Hecker et al., 2010			Smailhodzic et al., 2012		
		Controls	AMD	<i>P</i>	Controls	AMD	<i>P</i>	Controls	AMD	<i>P</i>
Factor B	ug/ml	642	803	0.02	985	1103	0.13	15.9	16.9	<0.001
Factor D	ug/ml	0.95	1.26	<0.001	1.16	1.5	0.004	–	–	–
C3a	ng/ml	14.3	15.5	0.03	–	–	–	–	–	–
C5a	ng/ml	1.67	1.85	0.04	4.06	4.28	0.3	0.15	0.22	<0.001
Ba	ug/ml	1.09	1.33	<0.001	0.78	1.07	0.0006	–	–	–
C3d	ug/ml	46.9	55.2	<0.001	35.8	40.7	0.009	11.2	15.6	<0.001
C5b-9	units	159	188	0.01	–	–	–	–	–	–
Factor H	ug/ml	515	546	0.21	668	681	0.19	24.5	24.9	0.654

pathway in AMD pathogenesis but also suggests a further role for other environmental or genetic factors that may predispose an individual to react with a neovascular response or not.

The genome-wide association study (GWAS) data mentioned above did not show an involvement of FD gene polymorphisms in AMD. An earlier two stage study investigating the role of factor D in AMD in a combined cohort of 4765 cases and 2693 controls, showed an odds ratio of 1.11 ($P = 0.032$), whereby the association was mainly confined to female patients [57]. Both the odds ratios as well as the P values were not very strong (not reaching GWAS thresholds) and more studies are needed to prove the exact role of FD gene polymorphisms in AMD.

Further support for the pivotal role of complement in AMD pathogenesis came from a series of studies showing that plasma levels of certain complement proteins (FD, FB) and activation products (C3a, C3d, C5a, Ba, sC5b-9) were increased in AMD patients compared to age matched healthy controls [41,43,63] (see Table 2).

Additional evidence showing the important role of complement in AMD pathogenesis came from the experimental animal model of CNV. Various mouse studies whereby the complement system was inactivated in vivo with cobra venom factor showed diminished laser induced choroidal neovascularization (CNV) [64]. The targeted deletion of complement genes in experimental animals also affected the outcome of laser induced CNV [65]. Application of complement inhibitors was able to ameliorate the outcome of laser induced CNV [66,67]. Taken together, the animal models confirm human data implicating the complement system in AMD pathogenesis.

Successful targeting of Factor D in AMD?

In view of the important role of the complement system in AMD, many clinical trials have been started to target complement factors for the treatment of AMD [68–71]. Most of these studies have however not been successful [72], except a recent study whereby monoclonal antibodies against FD were injected into the vitreous of AMD patients [73]. In the following we will focus on FD and its role in AMD.

Factor D is a serine protease that cleaves factor B in the C3bB complex leading to the formation of the alternative pathway C3 convertase (C3bBb) [74]. FD is present in human plasma in very low concentrations (1–2 ug/ml) and is a rate-limiting factor in the activation of the alternative pathway [75,76]. Small changes in the circulating FD level will thus have profound effects on the activation of the alternative pathway and may represent a safeguard to prevent over activation of this pathway. How FD levels are controlled is unknown. Of interest is the fact that most complement proteins are synthesized in the liver whereas FD is mainly produced by adipocytes [20,77].

FD is a small protein (23.5 kd) that is synthesized at a high rate but on the other hand is also rapidly excreted by the kidney [78]. Changes in the amount of adipose tissue may affect FD levels as

evidenced by the fact that FD levels are associated with body mass index (BMI) [79,80].

Adipose tissue is no longer considered to be only important for energy storage, but with its release of pro-inflammatory and anti-inflammatory cytokines, it is now seen as a metabolically active immune organ [77,81]. FD is also produced by macrophages and low mRNA expression has been shown in the choroid [40], which may be due to the dense concentration of macrophages in this tissue [82,83]. The local presence of FD in the eye and in view of its important role in alternative pathway regulation opened new venues to control its level in AMD via intravitreal injection of anti FD antibodies. The earliest clues that controlling FD might affect retinal disease came from a mouse model where it was shown that photoreceptors were protected from light induced damage in FD knockout animals [84]. Based on these promising findings, Genentech, Inc. (San Francisco, CA) has developed a humanized IgG Fab murine anti-factor D antibody (FCFD4514S) [85]. In vitro studies showed that this monoclonal antibody blocked the FD mediated proteolytic activation of its C3bB substrate [85]. The antibody was given the name Lampalizumab and a phase 1 dose escalation study in patients with geographic atrophy showed that a single-dose intravitreal injection was safe and well tolerated and did not show adverse events [73]. These data support a multi dose safety and tolerability assessment of FCFD4514S in geographic atrophy. This was followed by the so-called MAHALO study which is a phase 2 study whereby intravitreally injected Lampalizumab was shown to slow down atrophy progression in eyes of patients with geographic atrophy. A 20% reduction rate in the atrophic area was observed at 18 months in advanced dry AMD patients [86]. The studies described above showed positive results concerning local inhibition of the alternative pathway by blocking FD. In view of the fact that systemic complement activation may also play a role in AMD [43], a systemic control of FD might be an approach to control the alternative pathway [40]. A recent study from our group provided evidence for the fact that lutein supplementation was associated with a decrease in circulating FD levels and the downstream activation products C3d, C5a and sC5b-9 (Fig. 2) [15,16].

In this study seventy-two subjects with signs of early AMD were randomly assigned to a placebo or a 10 mg lutein supplement during twelve months. A significant 0.11 ug/ml monthly decrease in plasma FD was observed in the lutein group, resulting in a 51% decrease from 2.3 ug/ml at baseline to 1.0 ug/ml at the one-year time point. In the placebo group we found a significant 14% decrease in FD levels from 1.9 ug/ml at baseline to 1.4 ug/ml at the one-year time point. The reason for this decrease was not clear. Before entering the trial, the subjects were informed about the nature and background of the study, which may have led to a change in their dietary habits.

Although C3d levels observed in this study also decreased with time during lutein supplementation, a small not statistically significant rise was seen between the 8 and 12 month time points (14.3 ± 9.4 versus 14.4 ± 10.6 ug/ml). The placebo group showed a

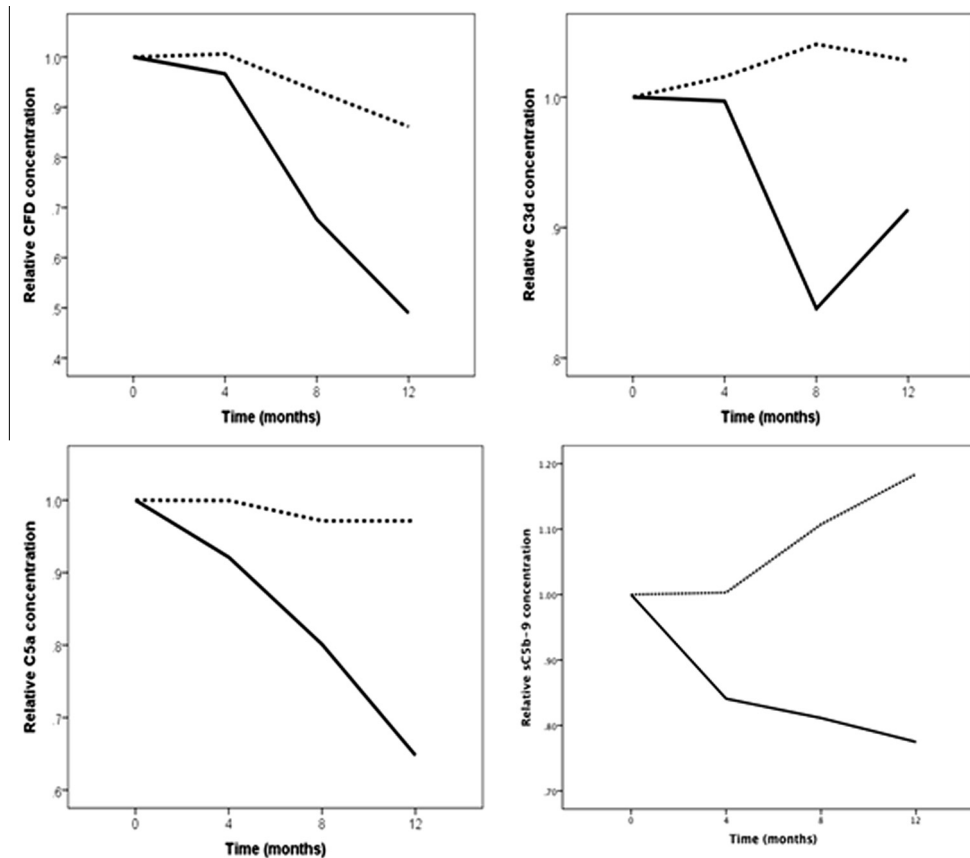


Fig. 2. Change in the relative concentration of factor D (FD), C3d, C5a and C5b-9, whereby the baseline level at time 0 is taken as 1.0. Dotted lines show results for the placebo group and solid lines for the lutein group. These graphs are modifications from graphs shown in [15,16].

small but insignificant increase in the C3d and sC5b-9 levels. Our observations concerning the effect of lutein supplementation on the level of various complement factors have to our knowledge not yet been repeated and as such they should be regarded as preliminary findings. Measurement of complement levels in blood may be affected by the sampling method and in our study care was taken to prepare plasma as soon as possible after venipuncture and to store samples at -80°C until the analysis of the individual complement components.

The mechanism whereby lutein potentially affects FD levels in the circulation is not yet known. Of interest is the fact that adipose tissue, which is the main source of FD, is also an important storage site for carotenoids such as lutein, zeaxanthin and meso-zeaxanthin [87]. Whether lutein affects FD expression or release from adipocytes is not known. It is possible that carotenoids such as lutein, by blocking the translocation of nuclear factor κB to the nucleus, inhibits the synthesis of many cytokines and that it may regulate FD synthesis in a similar manner [88]. Given the anti-oxidant properties of zeaxanthin and meso-zeaxanthin it seems likely that these xanthophylls might also affect adipocyte FD release although this has formally not yet been investigated.

Recent studies concerning the FD levels in AMD have not controlled whether the included patients were taking lutein supplements and this might have affected the data presented [63,89]. The differences between controls and patients may thus be even larger than reported. It may also explain that some studies did not observe a higher FD level in blood samples from AMD patients [63]. Whether this may also explain the observation that FD levels were only raised in female AMD patients is not clear and may also be related to differences in adipose tissue in older females as compared to males [57].

Due to the key role of FD in the activation of the alternative pathway of the complement system the early findings presented above show that both local intraocular as well as systemic control of FD might be promising tools in the treatment of AMD.

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