ARTICLE

Structure and composition of drusen associated with glomerulonephritis: implications for the role of complement activation in drusen biogenesis

ROBERT F. MULLINS,
NATALIA APTSIAURI,
GREGORY S. HAGEMAN

Abstract

Purpose **The ocular fundi of many patients with membranoproliferative glomerulonephritis type II (MPGN-II) are characterised by the presence of deposits within Bruch's membrane that resemble drusen, hallmark lesions associated with age-related macular degeneration (AMD). Glomerulonephritis (GN)-associated drusen appear at a younger age, however, than do drusen in individuals with AMD. In light of recent evidence that immune-mediated events participate in drusen biogenesis and AMD, we examined the structure and composition of drusen in eyes obtained from human donors with two distinct glomerulopathies, both of which involve complement deposition within glomeruli. These features were compared with those of drusen from patients with clinically documented AMD.**

Methods **Eyes obtained from two human human donors diagnosed with membranous and post-streptococcal GN, respectively, were analysed histochemically, immunohistochemically and ultrastructurally.**

Results **Subretinal pigment epithelial (RPE) deposits in both types of GN are numerous and indistinguishable, both structurally and compositionally, from drusen in donors with AMD. GN-associated drusen exhibit sudanophilia, bind filipin, and react with antibodies directed against vitronectin, complement C5 and C5b-9 complexes, TIMP-3 and amyloid P component. Drusen from the membranous GN donor, but not the post-streptococcal GN donor, reacted with peanut agglutinin and antibodies directed against MHC class II antigens and IgG. The ultrastructural characteristics of these deposits were also identical with those of AMD-associated drusen.**

Conclusions **The composition and structure of ocular drusen associated with membranous and post-streptococcal/segmental GN are generally similar to those of drusen in individuals with AMD. In view of the recent data supporting the involvement of complement activation in drusen biogenesis and the pathobiology of AMD, further studies of the biological relationships between AMD and diseases associated with complement activation are warranted.**

Drusen, defined deposits that manifest within Bruch's membrane, are a hallmark feature of the early stages of age-related macular degeneration (AMD), often referred to as age-related maculopathy (ARM).[1] The number, size and degree of confluency of drusen in the macula represent major risk factors for the progression of AMD.[2–8] Drusen develop at a younger age in individuals with other retinopathies, including, for example, individuals with pattern macular dystrophy,[9,10] dominant drusen (Malattia Leventinese or Doyne's honeycomb retinal dystrophy)[11] and membranoproliferative (mesangiocapillary) glomerulonephritis type II (MPGN-II).[12–18] Drusen are often detectable in the second decade of life in patients with MPGN-II. MPGN-II is a member of a heterogeneous group of disorders, the glomerulonephropathies, in which abnormal glomerular deposits interfere with normal renal function, leading to proliferative, exudative and/or sclerotic changes within the kidney. It is noteworthy that a wide range of physiological insults (including microbial infections, systemic conditions such as diabetes and systemic lupus erythematosus, and neoplasms) can lead to glomerulonephritic changes. An autoimmune response has been implicated as a likely watershed event in these diseases.[19]

R.F. Mullins
N. Aptsiauri
G.S. Hageman
The University of Iowa
Center for Macular Degeneration
Department of Ophthalmology and Visual Sciences
The University of Iowa
Iowa City
Iowa, USA

Gregory S. Hageman, PhD ✉
Department of Ophthalmology and Visual Sciences
The University of Iowa,
11190E PFP
200 Hawkins Drive
Iowa City, IA 52240, USA
Tel: +1 (319) 384 9722
Fax: +1 (319) 353 7699
e-mail: gregory-hageman@uiowa.edu

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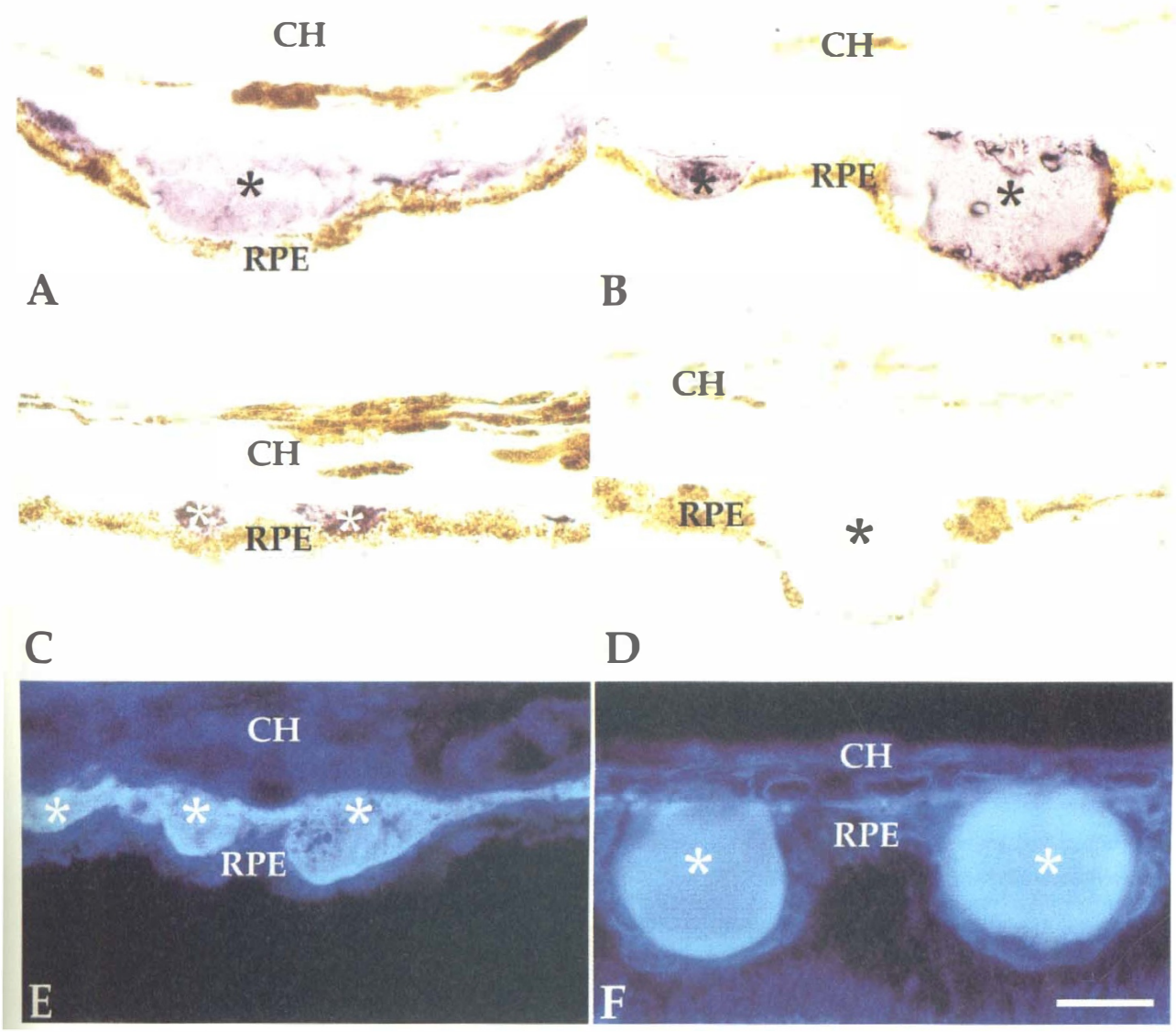

Fig. 1. *Sections of the retinal pigment epithelium (RPE)–choroid (CH) interface from donors with membranous (A, C, E) and segmental/post-streptococcal (B, D, F) glomerulonephritis (GN). Drusen (asterisks) associated with both diseases are similar in composition to drusen associated with age-related macular degeneration. These drusen react with antibodies directed against vitronectin (A, B), as well as a variety of other drusen-associated proteins including TIMP-3, C5, C5b-9, amyloid P and fibrinogen (not shown). Interestingly, drusen associated with membranous GN (C), but not post-streptococcal GN (D), react with antibodies directed against HLA-DR. A similar pattern is observed with IgG and PNA (not shown). Both types of drusen are also bound by filipin[27] (E, F), a cholesterol-binding probe, and Sudan black B (not shown). Scale bar represents 30 μm.*

In view of the fundus similarities between individuals with various forms of glomerulonephritis (GN) and AMD, as well as the description of neovascular changes in MPGN-II,[15,20] it is conceivable that these disorders share a common, or related, aetiology. In this report we compare the ultrastructural and compositional features of the drusen in eyes with AMD and those associated with membranous and segmental GN.

Materials and methods

Human donor eyes

Eyes from two human donors, diagnosed with membranous GN (donor 125-97, 49 years of age) and segmental/post-streptococcal GN (donor 82-00, 45 years of age), were obtained and processed within 5 and 7 h of death, respectively. An inferior sagittal wedge from the macula to the ora serrata was processed from each donor for light microscopy.[21] An additional inferior sagittal wedge, and a macular punch (4 mm, centred at 3 mm from the fovea), were processed from each donor for transmission electron microscopy.[22] Fixatives and buffers were employed as described previously.[22] Use of human tissue conformed to the Declaration of Helsinki and the policies of The University of Iowa Institutional Review Board.

Histochemistry/immunohistochemistry

Histochemical and immunohistochemical studies were performed as described previously.[21–26] Antibodies directed against vitronectin were obtained from Gibco-BRL (Rockville, MD) and antibodies directed against C5, C5b-9 complex, amyloid P component, fibrinogen and HLA-DR/DP/DQ (clone CR3/43) were obtained from Dako (Carpinteria, CA). Peroxidase-conjugated antibodies directed against human IgG and IgM were obtained from Sigma Chemical (St Louis, MO).

Table 1. *Comparison of drusen phenotypes*

Ultrastructure	Age-related drusen[21,24–27,29–33] Variable; five principal phenotypes (I–V)	Membranous GN Type III phenotype; heterogeneous, numerous inclusions of varying diameter	Post-streptococcal/segmental GN Type I and II phenotypes; largely homogeneous
TIMP-3 IR	+	+	+
VN IR	+	+	+
C5 IR	+	+	+
C5b-9 IR	+	+	+
Amyloid P IR	+	+	+
HLA-DR IR	Most drusen +; some labelling restricted to 'cores'	+; some labelling restricted to 'cores'	−
Fibrinogen IR	− to ±	− to ±	− to ±
IgG IR	− to +	+	−
IgM IR	−	−	−
PNA (post-neuraminidase)	+ in cores (27%)	+ in cores (30%)	−
Filipin	+	+	+
Sudan black B	+	+	+

IR, immunoreactivity; GN, glomerulonephritis; VN, vitronectin; C5, complement component C5; C5b-9, terminal complement complex C5b-9.

Antibodies directed against TIMP-3 were obtained from Chemicon International (Temecula, CA). The Vectastain Elite Universal ABC kit with the Vector VIP substrate was used to visualise antibody labelling (Vector, Burlingame, CA). Other sections were pretreated with neuraminidase and labelled with fluorescein-conjugated peanut agglutinin (PNA), as described previously.[24]

Transmission electron microscopy

Studies were conducted to evaluate the ultrastructural characteristics of drusen and the Bruch's membrane–retinal pigment epithelium–choroid interface. Tissue biopsies were collected from the maculas and inferior equatorial regions located 10–14 mm from the macula. Thin sections were obtained and imaged as described previously.[22] Tissues from the two donors with GN were compared with the phenotypes of drusen observed in aging and AMD.[25]

Results

Histochemistry/immunohistochemistry

Drusen associated with both types of GN examined in this study tended to be dome-shaped to spherical, with average height-to-width ratios of approximately 0.3 in the membranous GN donor and 0.7 in the post-streptococcal GN donor. Drusen greater than 100 μm in diameter were observed frequently in both donors. Drusen in both the GN donors were remarkable for both size and number, when compared with age-matched controls.

Antibodies directed against vitronectin, complement C5, complement C5b-9 (membrane attack complex), TIMP-3 and amyloid P component reacted intensely with drusen in both GN donors, similar to the reactivity observed in AMD-associated drusen (Fig. 1A, B; Table 1). The lipid-binding probes Sudan black B and filipin (Fig. 1E, F) also reacted with drusen in both GN donors, as well as drusen associated with AMD.[27] Weak and sporadic labelling of some drusen with the fibrinogen antibody was observed in both GN donors. Antibodies directed against IgM did not label drusen associated with AMD or either type of GN. Several drusen in the membranous GN donor possessed PNA-binding 'core' domains after neuraminidase digestion, as described previously for age- and AMD-related drusen.[24] Drusen in this donor were also bound by HLA-DR (Fig. 1C) and IgG antibodies. In contrast, the drusen in the post-streptococcal GN donor did not possess PNA-binding core domains, nor did they exhibit HLA-DR (Fig. 1D) or IgG immunoreactivity.

Transmission electron microscopy

The ultrastructural features of drusen in the maculas and inferior quadrants of both GN donors were evaluated. Large deposits were noted between the RPE basal lamina and the inner collagenous layer of Bruch's membrane, in the same extracellular location occupied by AMD-related drusen.[28]

Drusen in the donor with membranous GN were dome-shaped and comprised largely of amorphous material containing a number of large electron-dense and electron-lucent inclusions (Fig. 2A, B). Thus, they resemble drusen phenotype III, as described previously.[25] Occasional banded polymeric structures (which resemble fibrin) were observed in some of these deposits, similar to structures observed occasionally in drusen of AMD donors.

In the eye from the donor with segmental/poststreptococcal GN, drusen were large (often > 100 μm) and hemispherical to spherical (Fig. 2C). They resembled drusen phenotypes I and II described previously,[25] comprised largely of homogeneously distributed particles and no significant debris or heterogeneous profiles.

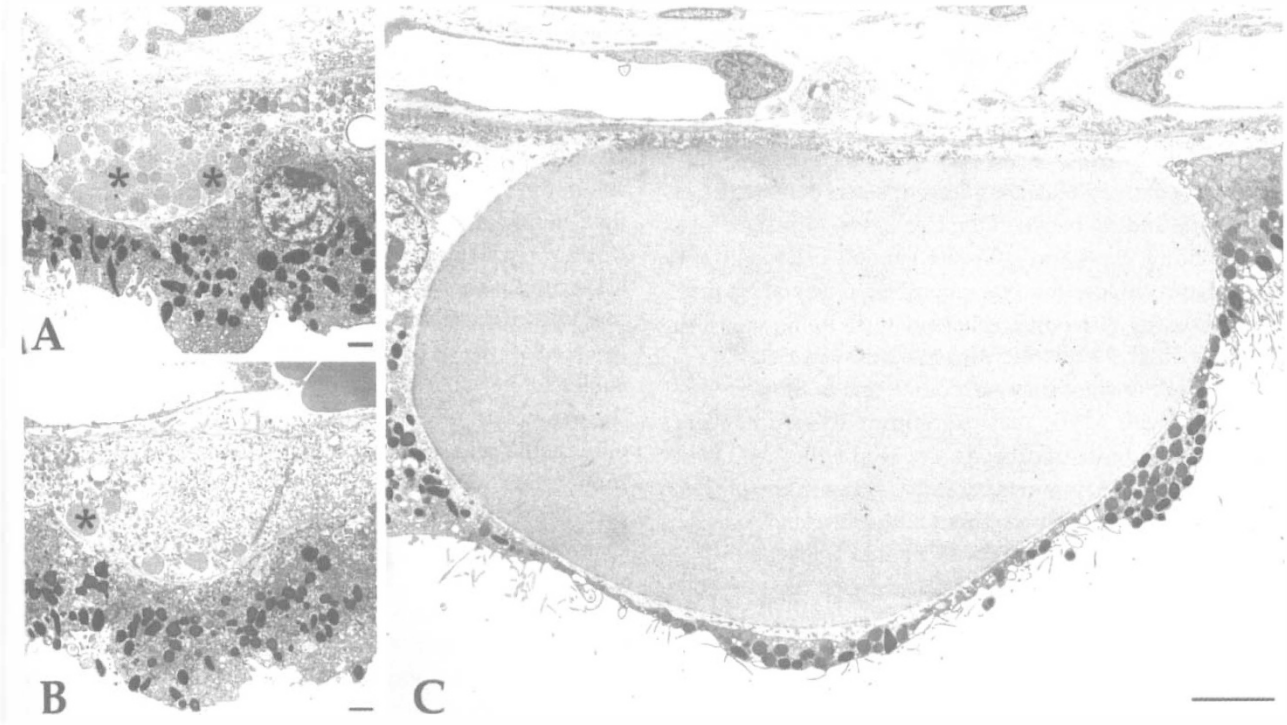

Fig. 2. *Transmission electron micrographs depicting drusen associated with membranous (A, B) and post-streptococcal/segmental (C) glomerulonephritis (GN). Drusen associated with membranous GN were dome-shaped and comprised largely of amorphous material containing a number of large electron-dense and electron-lucent inclusions (asterisks). Two small drusen are depicted (A, B). Drusen associated with segmental GN tended to be large (> 100 μm) and hemispherical to spherical (C). These drusen were comprised largely of homogeneously distributed particles without significant debris or heterogeneous profiles. Scale bars represent 2 μm (A, B) and 5 μm (C).*

Discussion

A number of the compositional characteristics of all phenotypes of drusen associated with ageing and AMD have been identified.[21–23,26,27,29–33] Clinical data accumulated from a number of investigations have revealed that patients with MPGN-II frequently also develop drusen-like deposits beneath the RPE.[12–18] To determine whether GN-associated Bruch's membrane deposits are of similar composition to those associated with AMD – with a goal of determining whether the pathogenesis of these deposits may involve similar pathways – eyes from donors with GN were evaluated for their immunoreactivity to several distinct markers of AMD-associated drusen. In this study, we have found that two distinct cases of GN, both characterised by the accumulation of complement-rich deposits in renal glomeruli, possessed significant accumulations of extracellular, drusen-like accretions in Bruch's membrane by the fifth decade of life. Compositional and ultrastructural analyses have revealed that these deposits are remarkably similar to AMD-associated drusen in a number of respects. Although GN-associated drusen develop at an earlier age, they contain cholesterol, amyloid P component, vitronectin and complement complexes, just as do those associated with AMD. Thus a more thorough understanding of the origin of these deposits may provide additional insights into the aetiology of AMD.

The observation that drusen associated with GN are similar to drusen associated with advanced age and AMD suggests a common pathogenesis of drusen in these disorders. Data presented here on the composition of early-onset drusen in GN add to those of Duvall-Young *et al.*,[12] who examined the morphology of these Bruch's membrane deposits in a 19-year-old patient diagnosed with MPGN-II. In contrast to the current study, Duvall-Young and colleagues did not detect complement deposits in the GN-associated drusen, although the authors noted that, as each eye represents only a single timepoint in disease progression, it was impossible to rule out completely a role for complement in these deposits. In a large study on drusen composition, we have recently identified complement C5 and C5b-9,[26] as well as inhibitors of complement such as vitronectin,[21] clusterin and Factor H (unpublished results), as drusen constituents. These late-stage complement molecules (including the membrane attack complex, C5b-9) were also detected in the drusen deposits associated with GN, and may suggest that aberrant complement activation or regulation represents a mechanism sufficient to result in the development of drusen in Bruch's membrane in both AMD and GN.

Although the pathogenesis of the various glomerulopathies is poorly understood, a common active pathway appears to be the aberrant activation or regulation of the immune/complement systems, either due to genetic factors (e.g. Goodpasture's syndrome[34]) or following infection (post-streptococcal or postviral GN[19]). Autoantibodies are likely to participate in some forms of GN, in which both complement and IgG are detected in renal lesions. In contrast, the glomerular deposits in other forms of GN do contain complement complexes but do not contain immunoglobulins,

suggesting that complement in these lesions may be activated by the alternative pathway. Interestingly, in the two cases we have evaluated in this study, terminal complement complexes were a consistent finding in all drusen, but Ig was detected only in the membranous GN donor. Hence, whereas complement activation was a consistent finding between the two cases, HLA-DR, PNA-binding cores and IgG were variable between these phenotypes, suggesting that complement activation may be induced by different mechanisms.

Interestingly, while the drusen associated with GN were generally similar to each other and to those associated with AMD, a few significant differences were noted. The majority of drusen associated with AMD tend to have HLA-DR immunoreactivity, either in distinct core-like domains or throughout entire drusen.[26] Although this was the case with drusen from the membranous GN donor, who also exhibited considerable IgG immunoreactivity and PNA-binding 'cores', drusen in the segmental/post-streptococcal GN donor did not possess PNA-binding cores or exhibit immunoreactivity for IgG or HLA-DR. This may suggest the existence of two (or more) distinct processes in the biogenesis of drusen, one of which involves a cell-mediated process[35] and all of which culminate in the activation and deposition of complement complexes. In this case, it is possible that AMD in eyes without HLA-DR- or PNA-positive drusen represent a specific disease phenotype. It is alternatively possible that the differences in PNA-binding sites, IgG and HLA-DR represent temporal differences in drusen development. Clearly, compositional studies on the drusen of additional donors with these and other glomerulopathies will be beneficial in understanding these relationships.

In view of (1) the compositional and ultrastructural similarities between GN-associated and age-related drusen, (2) the compositional similarity between dense deposits and drusen,[26] and (3) the similarity of GN (MPGN-II)-associated macular disease with end-stage AMD,[15,16,20] it appears likely that a similar mechanism of pathogenesis may exist between early AMD and some forms of renal disease. In this context, one might expect a high rate of coincidence of renal disease and AMD, which has not been described in a number of large epidemiological studies. However, it is possible that drusen in ageing and AMD, rather than resulting from a systemic condition, result from local complement activation. Along these lines, it is significant that a number of complement components and inhibitors, including C3, C5, C9,[26] vitronectin[21,36] and clusterin (unpublished data), are synthesised by ocular cells adjacent to Bruch's membrane, and may imply a role for local activation and deposition in AMD (as opposed to systemic activation and deposition as in other, related conditions). It is also interesting to speculate that, in the event that an active process of immune complex formation occurs,[37] a specific autoantigen may be shared between the kidney and the eye.

These data further implicate a role for the complement system in the development of deposits associated with AMD. We have previously noted that drusen possess terminal complement complexes (C5b-9),[26] and that these complexes may lead to injury of the RPE in a similar fashion to glomerular injury in GN. It is also possible that, in response to complement-mediated injury, the RPE up-regulates the expression of protective, drusen-associated molecules such as vitronectin, leading to increased drusen deposition and/or begins to promote the breakdown of Bruch's membrane. Interestingly, sublethal complement injury of synoviocytes promotes the expression of collagenase,[38] and sublytically injured endothelial cells secrete factors chemotactic for monocytes;[39] a similar set of responses by the RPE could conceivably lead to increased degeneration of Bruch's membrane and, subsequently, choroidal neovascularisation.

From the current study it appears that distinct forms of glomerulonephritis with distinct aetiologies may all result in the development of large drusen at a relatively early age. This may suggest that the commonality between MPGN-II and other forms of glomerulonephritis is sufficient to lead to the development of Bruch's membrane deposits indistinguishable from drusen. We propose that further studies into the possible role of complement activation in drusen biogenesis and AMD are warranted.

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