Molecular composition of drusen as related to substructural phenotype

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Age-related macular degeneration (AMD) is characterized in part by the deposition of extracellular deposits, including drusen, in the aging macula. A number of clinical studies have revealed a strong association between the number, size, and degree of confluence of drusen and AMD. Although a number of distinct morphological classes, or phenotypes, of drusen can be resolved at the ultrastructural level, very little is known about the compositional and etiological relationship between these phenotypes. A number of recent studies have begun to provide insight into the composition of drusen at the light microscopic level of resolution. Out of 33 extracellular matrix proteins evaluated, vitronectin was identified in hard and soft drusen [FASEB J 1999; 13:477-84]. Drusen have also been found to contain carbohydrate moieties which are labeled by wheat germ agglutinin (WGA), and Limax flavus agglutinin (LFA). We have recently extended these histochemical, immunohistochemical, and biochemical investigations to examine the relationship between substructural drusen phenotype and composition. The initial results of these observations, generated from a repository of human donor eyes processed within four hours of death, are reported herein. Five distinct substructural drusen phenotypes were identified in tissue sections from eyes of approximately 400 donors; all five phenotypes were observed in eyes from donors with and without clinically documented AMD. Interestingly, no strict relationship between size (one important discriminator between “hard” and “soft” drusen class) and morphology was noted for four out of the five drusen phenotypes. Sections from the same donors were incubated with antibodies directed against vitronectin and with the lectins WGA and LFA, three probes recently shown to label hard and soft drusen at the light microscopic level of resolution. As anticipated, all of these probes bound to all phenotypes of drusen examined. These data suggest that different phenotypes of drusen, although they may differ significantly with respect to their substructural morphology, may possess a similar complement of extracellular matrix-associated proteins and saccharides. Ongoing investigations are directed toward determining whether there exist specific drusen constituents, not yet identified, that impart phenotypic and/or ontogenic specificity to drusen. It is anticipated that a more complete understanding of drusen composition, as it relates to phenotype, will provide significant new insight into the biology and etiology of various clinically manifested forms of AMD.

Issues related to the pathobiology of the retinal pigmented epithelium-Bruch’s membrane-choroid interface were reviewed and discussed during the “Pathology of AMD” session held at the “Pathogenesis and Treatment of Age-Related Macular Degeneration” conference sponsored jointly by the Wilmer Ophthalmological Institute and the Foundation Fighting Blindness. Participants in this session were Drs. Christine Curcio, Paulus deJong, Richard Green, Gregory Hageman, John Marshall, and Shirley Sarks. Presentations included overviews related to classification systems for AMD, drusen, basal laminar and basal linear deposits, the choriocapillaris, and Bruch’s membrane. Information presented by some participants has been published recently [1-4], and/or is found in other papers in this volume (see accompanying papers by Drs. Green and deJong), and/or is incorporated into sections of this paper. The views and hypotheses presented herein, however, have not necessarily been derived by consensus of the participants.

A number of questions related to the role of drusen composition and biogenesis in age-related macular degeneration (AMD) were posed and discussed during the conference. These included:

- Is there evidence from pathologic studies that distinct drusen phenotypes exist?
- Can we develop and adopt a universally acceptable drusen classification scheme that can be used effectively by both ophthalmologists and vision scientists?
- What evidence is there that extracellular matrix abnormalities, including drusen, are associated specifically with the etiology of AMD? Are drusen derived as a consequence of a specific biological process? What genes and/or cells might be involved in drusen biogenesis?
- Can we develop a working hypothesis, based upon all existing data, to explain the role of drusen in the etiology of AMD?

In this paper, we have sought to address a number of the questions outlined above, to review current information pertaining to drusen classification, and to present new data related to drusen structure, composition, pathobiology, and association with AMD.

Natural History of Drusen: Drusen are extracellular deposits that accumulate between the basal lamina of the retinal
pigmented epithelium (RPE) and the inner collagenous layer of Bruch’s membrane in the human eye. They are typically associated with advancing age and are commonly observed in a variety of chorioretinal pathologies, including age-related macular degeneration (AMD), the leading cause of irreversible blindness in many countries. Although the presence of large, soft, and/or numerous macular drusen has been shown to be a significant risk factor for the development of AMD, precise information about their origin and composition is lacking.

Drusen were named after the German word for node or geode, and were first described by F.C. Donders in 1854 [5]. Donders, after studying the eyes of a 70 year old woman, described “reflective spheres” (reflektierenden Kugeln), surrounded by rings of hyperpigmentation. He observed that these deposits stained with iodine and were resistant to a number of chemical treatments (although these experiments appear to have been carried out after tissue fixation). He also noted that drusen often contain small spherules, presumably comprised largely of lipid. He concluded that these deposits were Coloidkugeln, or “spheres made of colloid”.

The nineteenth century histologist Heinrich Müller also made observations about drusen [6]. He referred to sub-RPE deposits as plaques or spherical, geode-like (drusigen) bodies, similar to the hard and soft drusen classes more thoroughly described later by Sarks and coworkers [4]. Müller confirmed Donders’ description of the frequent presence of hyperpigmentation around the rims of drusen, and suggested that they were likely due to the displacement of RPE cells away from the apices of drusen, now a commonly accepted paradigm. Alt [7] described excrescences in Bruch’s membrane as rare sites for ocular bone deposition, and noted that the RPE is intact only over the smallest of drusen.

More recent studies have revealed that drusen form as extracellular deposits between the RPE basal lamina and the inner collagenous layer of Bruch’s membrane. They cause lateral stretching of the RPE monolayer and physical displacement of the RPE from its immediate vascular supply, the choriocapillaris. This displacement likely creates a physical barrier that may impede normal metabolite and waste diffusion between the choriocapillaris and the neural retina. In this paradigm, wastes may be concentrated near the RPE and the diffusion of oxygen, glucose, and other nutritive or regulatory serum-associated molecules required to maintain the health of the outer retina and RPE are inhibited. It has also been suggested that drusen perturb photoreceptor cell function by placing pressure on rods and cones [8] and/or by distorting photoreceptor cell alignment [9].

Association of Drusen with AMD: A number of studies have concluded that the presence of macular drusen is a strong risk factor for the development of both atrophic and neovascular AMD [10-16]. In addition to macular drusen, extramacular drusen also appear to be a significant risk factor for the development of AMD [17]. In his monograph on macular diseases, Gass refers to drusen as “the earliest sign” of AMD [18]. Furthermore, Pauliekhoff and coworkers [19] demonstrated that the size, number, and extent of confluency of drusen are important determinants for the risk of developing AMD. The correlation between drusen and AMD is so firmly established as dogma that many investigators and clinicians refer to the presence of soft drusen in the macula, in the absence of vision loss, as “early AMD” [20,21], or “early age-related maculopathy” [22]. The presence of soft, large and/or confluent drusen is correlated to the occurrence of choroidal neovascularization, a clinically devastating manifestation of AMD. The risk of developing neovascular complications in patients with bilateral drusen has been estimated at 3-4% per year [23]. A recent report from the Macular Photocoagulation Study Group shows a relative risk of 2.1 for developing choroidal neovascularization in eyes possessing five or more drusen, and a risk of 1.5 in eyes with one or more large drusen [24].

Drusen appear to affect various aspects of vision prior to loss of visual acuity; these include changes in color contrast sensitivity, macular recovery function, central visual field sensitivity, and spatiotemporal contrast sensitivity [20,21,25-28]. A few recent studies have shown that visual acuity improves in some cases following laser photocoagulation and subsequent drusen regression [29,30]. These observations provide additional support for the concept of a correlation between drusen and vision loss in patients with AMD.

Classification of Drusen Phenotypes: There is no universally accepted classification system that incorporates aspects of drusen size, phenotype, and distribution. This stems largely from the facts that: (1) different modalities are employed for evaluating structural features of Bruch’s membrane deposits clinically than are used for assessment of donor eyes or on tissue sections; and (2) relatively scant information is available pertaining to the composition of drusen and other Bruch’s membrane deposits, making comparisons between different drusen phenotypes problematic. Consequently, the classification systems that have been proposed vary considerably due to different methods (i.e., light/scanning laser ophthalmoscopy, fluorescein/indocyanine green angiography, histology, histochemistry, and electron microscopy) employed for assessing drusen structure and appearance.

Various drusen classification systems have been described, reviewed and/or proposed recently by a number of investigators [2,15,31]. While it is not the intent of this paper to provide a full review of the literature related to these classification systems, a brief summary of their more salient features is provided below. The terminology most commonly used to distinguish drusen phenotypes is “hard” and “soft” (see, for example, [17,23,32-36]). Clinically, hard drusen are typically defined as small, distinct deposits behind the RPE. Histologically, hard drusen are round or hemispherical, possess distinct borders, and are comprised of homogeneous eosinophilic material. Soft drusen are larger and have indistinct, sloped borders. Unlike hard drusen, soft drusen are not usually homogenous. Histologically, they typically contain various inclusions and spherical profiles. The term diffuse drusen, or basal linear deposit, is used to describe the amorphous material that forms a layer between the inner collagenous layer of Bruch’s membrane and the RPE. This material can appear similar to soft drusen histologically, with the exception that it is not mounded. Klein and colleagues have described three
classes of drusen in the Wisconsin drusen grading system, based solely upon clinical funduscopic assessment [37]. In this scheme, “hard” drusen are defined as discrete drusen ranging from 1-63 µm in diameter and drusen are defined as being “soft” if they are larger than 125 µm in diameter, or if they are between 63 µm and 125 µm in diameter, with “visible thickness”. Soft drusen are further categorized as either “soft distinct” (large drusen with uniform density) or “soft indistinct” (large drusen with graded density and fuzzy edges).

In a histological study of eyes from AMD patients, Spraul and Grossniklaus [36] have proposed another system of histological nomenclature. Drusen are described as “hard” (small hyaline deposits), “large” (discrete drusen with diameters of greater than 63 µm), “soft” (drusen with pale staining characteristics and sloping borders), and “confluent” (coalescence of 3 or more individual drusen). It has also been suggested that drusen may fall into two defined classes, based not on size, but on histochemical (neutral lipid-rich or phospholipid-rich) and angiographic properties (discussed below) [38]. Similar classes of drusen, based upon their degree of hydrophobicity following indocyanine green angiography, were proposed recently [39]. A few attempts have been made to correlate clinical and histological classification schemes. Green and Enger, in a large histopathological study, were able to derive clinicopathological correlations in eleven patients and to map the pathologic characteristics of a few patients by serial section reconstruction [3]. Bressler et al. [15] have compared various histological and ultrastructural features of drusen with their clinical presentation in the same eyes. These data enabled direct comparisons between funduscopic appearance, fluorescein angiographic features, and morphology in two patients with AMD. In this scheme, hard drusen were defined as a function of size (less than 63 µm across) and soft drusen were divided into three distinct categories, based on ultrastructural characteristics.

In an attempt to bridge the gap between clinical and histological classification schemes, Curcio and coworkers have developed a system that correlates gross observations of human donor eyes with their histological and ultrastructural features. The classification system derived from these studies is referred to as the Alabama Age-Related Maculopathy Grading System [2]. In the Wilmer AMD conference, Curcio presented data derived from calculations of the sensitivity and specificity of membranous debris (basal linear deposit) and large drusen with diameters of greater than 63 µm. The investigators concluded that drusen contain, and may be derived from, RPE (although leukocytes were also noted in the vicinity of Bruch’s membrane). The presence of cellular constituents in drusen indicated a derivation from RPE. In a more recent cytochemical study [44], however, lysosomal enzyme activity within drusen was not verified. Burns and Feeney-Burns [45] examined drusen from nineteen donors fixed at various post-mortem times. They described the presence of “cytoplasmic debris” in small drusen, which they inferred was derived from the RPE (although leukocytes were also noted in the vicinity of Bruch’s membrane in some eyes). The investigators concluded that the presence of cellular constituents in drusen indicated a derivation from RPE cells. Feeney-Burns [46] later described a paucity of debris in Bruch’s membrane directly beneath drusen, and suggested that drusen may result from an inability of the choroid to clear debris from sites of drusen deposition. Ishibashi studied drusen formation in three eyes that were surgically enucleated for melanoma, and observed cellular extensions of the RPE that protruded through the RPE basal lamina and into Bruch’s membrane. Based on these data, he contended that drusen contain, and may be derived from, RPE reviewed at the Wilmer AMD conference. Definitions employed by Sarks and coworkers include “soft” drusen, which are discrete and rarely over 125 µm in diameter; drusen derived from the coalescence of hard drusen (termed “hard clusters,” “soft clusters,” and “confluent soft drusen derived from clusters”); and “soft membranous” drusen, which resemble confluent soft drusen but are composed of membranous coils. Based on their collective data, Sarks and colleagues propose that there are two pathways of drusen formation. They propose that soft drusen are derived from hard drusen, based on changes in fundus photographs and angiograms taken from the same patients over a number of years. Based on these observations, as well as ultrastructural images suggestive of drusen coalescence, they suggest that drusen soften and fuse over time [4,10,42].

Even though there is no general consensus regarding a universally accepted scheme for drusen classification, there is no dispute that an individual with many large/soft drusen is at a significantly higher risk of developing complications of AMD than an individual with no drusen or a few, small drusen. In the future, our ability to discriminate between drusen classes and, by extension, to elucidate the role(s) of different drusen phenotypes in AMD, should be enhanced significantly by new data in a number of areas. First, exhaustive characterization of drusen-associated constituents should help in the identification of similarities and differences between drusen classes at the molecular level. Second, a better understanding of drusen origin and development should facilitate our understanding of their role in the development of AMD. Third, genotype-phenotype correlations and rigorous clinicopathologic examination of drusen will help to determine whether distinct drusen phenotypes are associated with different clinical manifestations of AMD.

**Drusen Substructure and Origin:** Farkas and colleagues [43] examined drusen ultrastructure in five eyes enucleated for choroidal melanoma. They described drusen as consisting of numerous degenerating organelles, including what appeared to be lysosomes. Based on the observation that similar material was present on the RPE side of Bruch’s membrane prior to drusen formation, they suggested that drusen constituents were derived from the RPE. In a more recent cytochemical study [44], however, lysosomal enzyme activity within drusen was not verified. Burns and Feeney-Burns [45] examined drusen from nineteen donors fixed at various post-mortem times. They described the presence of “cytoplasmic debris” in small drusen, which they inferred was derived from the RPE (although leukocytes were also noted in the vicinity of Bruch’s membrane in some eyes). The investigators concluded that the presence of cellular constituents in drusen indicated a derivation from RPE cells. Feeney-Burns [46] later described a paucity of debris in Bruch’s membrane directly beneath drusen, and suggested that drusen may result from an inability of the choroid to clear debris from sites of drusen deposition. Ishibashi studied drusen formation in three eyes that were surgically enucleated for melanoma, and observed cellular extensions of the RPE that protruded through the RPE basal lamina and into Bruch’s membrane. Based on these data, he contended that drusen contain, and may be derived from, RPE...
cell constituents [47]. However, it should be noted that changes in RPE cytoskeletal organization and cell shape have been described in eyes with choroidal melanoma [48,49], potentially complicating the interpretation of these observations.

Whereas ultrastructural studies have shed some insight on our understanding of drusen biogenesis and composition, simultaneous compositional studies should facilitate interpretation of these ultrastructural data, and lead to a more thorough understanding of the mechanism(s) of drusen deposition within Bruch’s membrane.

**Drusen Composition:** Although the clinical manifestations of, and ophthalmoscopic changes associated with, AMD have been well-documented [18,50,51], it is surprising that relatively little is known about the molecular and biochemical alterations that accompany AMD. Because of the documented association of drusen with AMD, identification of their molecular components may provide crucial information toward understanding the etiology of AMD and, eventually, for identifying new treatments to arrest, delay, or prevent the development of this disease. This approach may be especially revealing if AMD represents multiple diseases with different clinical complications and responses to treatment. Only a few drusen constituents have been identified conclusively. Wolter and Falls [52] made the observation in two human donor eyes that drusen stain with oil red O, indicating the presence of neutral lipids in at least some drusen. Farkas et al. [53] performed a series of experiments in which drusen composition was analyzed by enzymatic digestion, organic extraction, and histochemical staining methods in order to provide information on drusen-associated molecules. They concluded that drusen are comprised of sialomucins (glycoproteins with O-glycosidically linked oligosaccharides), cerebrosides and/or gangliosides.

More recently, Pauleikhoff and colleagues [38] used lipid-based histochemical approaches to show that drusen contain either phospholipids or neutral lipids. These investigators proposed that drusen are either hydrophobic or hydrophilic, and that different drusen classes may be indicative of significantly different pathologies. The “hydrophilic” drusen phenotype contains phospholipids, bound fluorescein, and reacted with an anti-fibronectin antibody. Collectively, these data imply the existence of different compositional classes of drusen, not solely based on morphology (i.e., hard and soft).

In an immunohistochemical study employing a small battery of antibodies, Newsome and collaborators [34] described...
labeling of soft drusen with antibodies directed against fibronectin, and both hard and soft drusen with antibodies directed against IgG and IgM. Other investigators have been unable to reproduce the observation of an association of fibronectin and immunoglobulin with drusen [54]. In more recent studies, a weak reaction of drusen with antibodies directed against beta amyloid [55] and complement factors (C1q, C3c, C3d, and C4) [54] was observed. More intense labeling with antibodies directed against ubiquitin [56], TIMP-3 [57], and advanced glycation end products [58] has been reported. No obvious single explanation can account for the discrepancies between the results of the various immunohistochemical studies. It is important to point out, however, that: (1) there is no universally agreed upon nomenclature or classification system for drusen; this makes comparisons of data from different laboratories difficult; (2) some studies employed dehydrated, paraffin-embedded tissues (potentially resulting in the extraction of some drusen constituents), whereas others used frozen sections; (3) different antibodies directed against different epitopes of the same protein can produce disparate binding patterns; and (4) post-mortem time can create artifacts in drusen composition, structure, and antigenicity.

We have recently asked questions pertaining to drusen composition employing a large battery of lectins and antibodies. These studies have resulted in the identification of a number of drusen-associated carbohydrate moieties, specifically binding sites for Concanavalin A, LFA, WGA, and RCA-I [59]. In a study directed toward a thorough characterization of extracellular matrix molecules associated with drusen, vitronectin was found to be a major constituent of histologically hard and soft drusen [1]. We have also identified other molecules, including amyloid P component and apolipoprotein E, as drusen constituents [60].

METHODS

In order to compare the compositions of a number of different ultrastructural “phenotypes” of drusen, human donor eyes, with and without clinically-documented AMD and with distinct drusen morphologies, were employed for simultaneous transmission electron microscopical and immunohistochemical observation. Eyes used in this study were selected from a repository of approximately 2,000 pairs of human donor eyes (between 0 and 102 years of age) obtained from MidAmerica Transplant Services (St. Louis, MO), the Iowa Lions Eye Bank.
(Iowa City, IA), the Heartland Eye Bank (Columbia, MO) and the Virginia Eye Bank (Norfolk, VA) and were processed within four hours of death. The gross pathologic features of all eyes, as well as the corresponding fundus photographs and angiograms, when available, were read by a retina surgeon. Approximately 18% of the donors had some form of clinically diagnosed AMD; these included eyes with macular pigment changes, macular drusen, geographic atrophy, choroidal neovascularization, and/or disciform scars. Eyes with and without clinically documented AMD, were employed in this study.

Eyes from approximately 400 of these donors possessing drusen were processed for transmission electron microscopic examination as described previously [61]. Tissues were fixed in one-half strength Karnovsky’s fixative within four hours of death for a minimum of 24 hours, and transferred to 100 mM sodium cacodylate buffer, pH 7.4, prior to dehydration, embedding, sectioning, and photomicrography.

Tissues from the same eyes processed for electron microscopy were processed for light histological, lectin histochemical, and immunohistochemical studies as described previously [1,59,62]. Anti-vitronectin antibody was obtained from Telios (San Diego, CA); wheat germ agglutinin and peanut agglutinin (PNA) were obtained from Vector (Burlingame, CA); LFA was obtained from EY Laboratories (San Mateo, CA).

RESULTS

Ultrastructural Drusen Phenotypes: Ultrastructural examination of donor eyes fixed within relatively short postmortem times reveals five distinct morphological “classes” of drusen in eyes with and without clinically documented AMD. For the purposes of this study, no attempts have been made to correlate these drusen classes to those phenotypes described in the literature. The purpose of this investigation was simply to examine the composition of all ultrastructural classes of drusen observed in eyes obtained from a large number of donors, representing a significant age range and a variety of AMD phenotypes.

One class is manifest by drusen exhibiting a uniformly homogenous substructure. Drusen of this phenotype range in

![Figure 5](http://www.molvis.org/molvis/v5/p28)

**Figure 5.** Electron micrograph of a third, highly heterogeneous drusen phenotype. A third class of drusen is highly heterogeneous and contains electron dense and electron lucent inclusions, spherical profiles, fibrin-like profiles, curvilinear profiles, and, perhaps, cellular debris (A and B). They are also characterized by the presence of a calcium-containing inclusions (B and Figure 6A).

![Figure 6](http://www.molvis.org/molvis/v5/p28)

**Figure 6.** High magnification transmission electron micrograph of the third and fifth drusen phenotypes. The third drusen phenotype exhibits granular material with large amounts of electron dense and electron lucent material, as well as calcium-containing inclusions (A). A fifth phenotype of drusen exhibits numerous membrane-bounded vesicles (B and Figure 7B) and possesses material with a periodicity similar to that of basal laminar deposit/long-spacing collagen, which is interspersed within these vesicles (B). No granular material or other characteristics typical of the other four drusen phenotypes is observed in this class.
size between 1 µm to >200 µm (Figure 1) and are comprised largely of approximately 20 nm diameter “granules” (Figure 2A). They typically exhibit a rounded to hemispherical morphology similar to what has been described previously as “hard” drusen. However, these results suggest that drusen of this substructural phenotype can be significantly larger than hard drusen described in most clinical grading schemes (e.g., [37]). In addition, drusen with this morphology can be confluent over large areas.

A second class is comprised of drusen that are uniformly homogenous at the ultrastructural level of resolution (Figure 3A), but in addition to the “granular” material described above, contain small (80 nm diameter), electron lucent, spherical elements (Figure 2B). The density of these spherical elements can vary significantly; they are often more dense in the periphery of the drusen. Drusen of this class have been observed that are >200 µm in diameter. A distinct subclass of drusen in this category possesses, in addition to the features described above, numerous curvilinear profiles (polymerized collagen) and larger, spherical elements (Figure 3B). Some of the latter are membrane-bounded and all are electron dense (Figure 4).

A third class consists of drusen that are highly heterogeneous based on ultrastructural examination (Figure 5). Electron dense and electron lucent inclusions, spherical profiles of various diameters, fibrin-like profiles, curvilinear profiles, and, perhaps, cellular debris are present within these drusen (Figure 6A). These drusen are further distinguished by large calcium-containing inclusions (Figure 5 and Figure 6A). Intact lipofuscin and/or melanin granules are not typically identified in this or any other class of drusen, however, material that could be interpreted as fragments of these RPE-associated organelles have been observed and are common to this ultrastructural class of drusen. These drusen always possess sloped borders are typically wider than they are high, and can exist in confluent layers.

A fourth class of drusen is comprised primarily of electron lucent, 50-400 nm diameter membrane-bounded vesicles (Figure 7A). These drusen typically have extremely sloped borders and often exist as a confluent syncitium. They are most similar in appearance to basal linear deposits that have been described previously.

A fifth class of drusen is observed, but only rarely. Drusen of this phenotype contain numerous membrane-bounded vesicles that vary between 80 and 300 nm in diameter (Figure 7B). Material with periodicity similar to that of basal laminar deposit/long-spacing collagen, and small calcified inclusions,
is interspersed within these vesicles (Figure 6B). No granular material or other characteristics of the other four drusen phenotypes is observed. These drusen are typically small; they have not been observed to exceed 30 µm in diameter.

Significantly, no strict relationship between size (one important discriminator between “hard” and “soft” drusen class) and substructural morphology is noted for four out of the five drusen phenotypes described above. The diameters of drusen belonging to the first four drusen classes described above range between 63 µm (the often-employed “clinical cutoff” between hard and soft drusen) and >200 µm. Drusen that characterize the fifth substructural class are all <63 µm in diameter.

Drusen Composition as Related to Ultrastructural Phenotype: Previous light level histochemical and immunohistochemical investigations using a battery of lectins and antibodies led to the identification of a distinct array of proteins and carbohydrates commonly associated with hard and soft drusen phenotypes as classified at the light microscopic level of resolution [1,59,60]. The studies described below were conducted to determine whether all drusen phenotypes possess this same complement of constituents bound by three representative molecules (anti-vitronectin antibody, WGA, and LFA) known to react with constituents associated with hard and soft drusen phenotypes. These probes were employed herein to examine the relationship between ultrastructural drusen phenotype and composition. Examples of drusen labeling are depicted in Figure 8. As anticipated, all three probes bound to all five drusen phenotypes.

DISCUSSION

A number of clinical, histological, and clinicopathological drusen classification schemes have been proposed. As has been noted [2,15], the difficulties inherent in developing a unified scheme for classification are primarily due to different means of data collection and the resolution of the various techniques employed. In fact, it may be impossible to develop a universal technique that bridges the barrier between fundamentally different means of data collection and analysis. Furthermore, without reliable information concerning drusen composition, origin, and substructure, classification schemes are inherently limited to gross morphological descriptors.

Examination of eyes from over 400 donors (fixed within a shorter interval after death than that typically employed in other published studies) that possessed drusen reveals five distinct, definable drusen phenotypes at the ultrastructural level of resolution. Because these substructural phenotypes are observed in drusen of various sizes, shapes, and degree of confluency, we propose that clinical drusen classification schemes that rely on size alone are not a reliable indicator of drusen morphology. Furthermore, the observations described herein suggest that drusen of all ultrastructural phenotypes may possess a common set of constituent proteins and saccharides, as represented in this study by vitronectin, WGA, and LFA. Additional studies, employing a larger battery of probes to drusen-associated constituents, should help to clarify this suggestion.

Why, then, if all drusen are compositionally similar, do hard and soft drusen confer different risks for choroidal neovascularization? Or, in fact, do they? One explanation is that drusen may be comprised of other proteins, not yet identified, that play a role in determining the pathologic characteristics of these deposits. An alternative explanation is that, even though a number of protein and carbohydrate constituents are common to all classes of drusen, their lipid composition may vary considerably and thus determine their roles in the etiology of AMD. Indeed, Pauleikhoff and colleagues have suggested that lipid composition may determine the risk posed by sub-RPE deposits [38]. Furthermore, the lipid composition of drusen could determine their size, shape, tendency to coalesce, and/or their permeability. In a somewhat related argument, one cannot exclude the possibility that it is not “hard” or “soft” drusen (i.e. drusen size), as defined by most clinical classification schemes, that confer the risk for choroidal neovascularization. Rather, it is the composition of drusen that confer this risk. If this proves to be the case, more sophisticated techniques will be required in order to evaluate drusen clinically if one hopes to diagnose various AMD clinical phenotypes.

One approach for determining the full complement of drusen-associated molecules will be to employ more sensitive biochemical assays for detecting the presence of proteins.
and lipids. One goal of this laboratory has been to develop a reliable method for collecting drusen-enriched preparations from human donor eyes. Recent preliminary experiments suggest that enriched drusen preparations are bound by the same probes that bind to drusen in situ, suggesting that these preparations can be obtained without a loss of drusen-associated constituents. An example of one such enriched preparation is depicted in Figure 9.

The observations made in this, and related, studies suggests that, even though a number of ultrastructural classes of drusen can be distinguished, no significant differences in composition between these phenotypes have yet been detected. Although considerable attention has been paid to clinical and morphological appearance of drusen phenotypes, future studies toward determining the molecular composition of drusen should provide fresh insight into drusen biogenesis and function. As always, a number of significant questions pertaining to the role of drusen in the development of AMD remain. However, it is likely that significant progress will continue to be made in this field over the next few years.

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