Analysis of Carbohydrate Structures in Basal Laminar Deposit in Aging Human Maculae

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Purpose. To analyze carbohydrate structures in basal laminar deposit (BLD), an extracellular material that accumulates between the retinal pigment epithelium (RPE) and Bruch’s membrane. BLD has been shown to correlate positively with visual loss in age-related macular degeneration.

Methods. Thirteen postmortem human maculae with BLD were histochemically examined by light microscopy using the monoclonal antibody HNK-1 and seven lectins: canavalia ensiformis (ConA), soybean agglutinin (SBA), wheat germ agglutinin (WGA), dolichos biflorus (DBA), ulex europaeus (UEA-I), ricinum communis agglutinin I (RCA-I), and peanut agglutinin (PNA). Three maculae were stained with polyclonal antibodies against laminin and collagen type IV.

Results. BLD was exclusively stained by DBA and SBA, whereas Con A, WGA, UEA-I, RCA-I, and HNK-1 stained various other structures in the human macula as well. The main part of the BLD adjacent to Bruch’s membrane stained with these lectins and the monoclonal antibody HNK-1, whereas only a small part of the BLD adjoining the RPE stained with antibodies against laminin and collagen type IV. Drusen stained neither with any lectin nor with any antibody.

Conclusions. DBA and SBA, which bind specifically to an α-D-GalNAc moiety, are specific markers for the light-microscopic detection of BLD in human macular tissue. Furthermore, the authors conclude that BLD contains several carbohydrate structures other than the carbohydrate moieties on laminin and collagen type IV. If drusen contain carbohydrate structures, these must be different from those in BLD. Invest Ophthalmol Vis Sci. 1994;35:2901–2905.

Age-related macular degeneration was first described by Haab in 1888 as an age-related abnormality of the macula lutea, affecting central vision.¹ The aging human macula shows varying degenerative histopathologic changes, for example, the presence of drusen,²⁻⁴ thickening of Bruch’s membrane,²⁻⁵ accumulation of lipofuscin in the retinal pigment epithelium (RPE),⁵ and basal laminar deposit (BLD).²⁻⁷ The amount of histologically detectable BLD, an extracellular material that accumulates between the RPE and Bruch’s membrane, is considered to be an indicator of the degree of photoreceptor degeneration and has been shown to correlate positively with visual loss.² Little is known about the origin or the composition of BLD.²⁻⁷ It has been assumed that BLD consists mainly of basement membrane material such as the glycoproteins laminin and collagen type IV, although no formal proof has been given.²⁻⁵⁻⁷⁻⁹ Besides the carbohydrate structures on laminin and collagen type IV, basement membranes contain many other glycoconjugates.¹⁰

The aim of this investigation was to analyze carbohydrate structures in BLD and to find a specific marker for BLD using histochemical and immunohistochemical techniques. Accordingly, we tested if BLD reacts with lectins with varying carbohydrate-affinities such as α-D-GalNAc (DBA and SBA), α-D-Mannose (CON A), β-D-GlcnAc (WGA), NeuNAc (WGA), β-D-Gal(1→3)-D-GalNAc (PNA), α-L-Fucose (UEA-I), and β-D-Gal > α-D-Gal (RCA-I). We also checked the monoclonal antibody HNK-1, which reacts with GlcUAβ1-3Galβ1-4GlcNAc moieties.¹¹⁻¹³

MATERIALS AND METHODS
Preparation of Macular Tissue
From a large series of human eye bank eyes used in previous studies on age-related macular degenera-
tion, we selected 13 maculae of 10 patients between 85 and 98 years of age (mean age, 91 years) on the basis of relatively large amounts of BLD class 2 and class 3. Class 2 BLD resembles a thin continuous layer, and class 3 BLD resembles a thick layer of at least half the height of the RPE. We did not use class 1 BLD (small solitary patches of BLD) because the amount of BLD here is too little to illustrate adequately the staining of BLD with the lectins and HNK-1. Although the specimens were also examined by electron microscopy in previous studies, we did not use these data to confirm the presence of BLD but made a new series of consecutive paraffin sections to be sure of the occurrence of BLD in all sections. BLD was histologically demonstrated by Mallory staining. Time between death and fixation ranged from 4.5 to 12 hours. After enucleation, the macula was dissected from the globe and hemisectioned in the direction of the optic nerve. Both halves were fixed in phosphate-buffered formaldehyde (4% vol/vol, pH 7.4, for 24 hours at room temperature) and embedded in paraaffin for histochemical and immunohistochemical studies. Methods for securing human tissue were humane, included proper consent and approval, and complied with the Declaration of Helsinki.

**Light Microscopy**

Paraffin sections (5 μm) were mounted on 3-aminopropyl-tri-oxysilane (AAS, Sigma, St. Louis, MO) coated glass slides. After deparaffinization and rehydration, they were rinsed with water and phosphate-buffered saline (PBS). The slides were placed in a Sequenza Immunostaining Workstation (Shandon Scientific, Cheshire, UK) and were incubated for 1 hour with either the monoclonal antibody HNK-1 (American Tissue Type Culture Collection, 1:10 dilution) or one of the seven biotinylated lectins mentioned below. These lectins were canavalia ensiformis (ConA, concanavalin A, lot 80718), glycine max (SBA, soybean agglutinin, lot 70326), triticum vulgaris (WGA, wheat germ agglutinin, lot A0201), dolichos bifloris (DBA, lot 80705), ulex europaeus (UEA-1, agglutinin I, lot 80713), ricinus communis agglutinin I (RCA-1, lot 80721) and arachis hypogaea (PNA, peanut agglutinin, lot 80629). All were commercially obtained (Vector Laboratories, Burlingame, CA) and were used at a concentration of 20 μg/ml PBS. After washing with PBS and incubation for 30 minutes with alkaline phosphatase (AP)-conjugated streptavidin (Biogenex, San Ramon, CA; dilution 1:50), the slides were rinsed with PBS and finally with 0.2 M Tris-HCl, pH 8.0, before staining for 30 minutes with 0.3% new fuchsin/Tris-HCl (Sigma). The sections were counterstained with Mayer’s hematoxylin for 15 seconds, rinsed for 10 minutes with water, and air dried. In case of incubation with the HNK-1 monoclonal antibody, the slides were incubated for 30 minutes with biotinylated secondary antibodies (Multilink, Biogenex; 1:100 dilution) before incubation with AP-conjugated streptavidin. Other steps were identical as described for lectin staining. In negative controls, the lectins were replaced by PBS or incubated with a specific blocking sugar (0.2 M α-D-GalNAC for DBA and SBA) for 15 minutes before application to the tissue sections. The monoclonal antibody HNK-1 was replaced by normal mouse serum (DAKO, Glostrup, Denmark; dilution 1:10). We also used macular parts with no BLD as an internal negative control. RPE served as an internal positive control for ConA and WGA, blood vessels of the choroid were positive controls for UEA-1 and RCA-1, and ganglion cells of the human retina for HNK-1. Human kidney tissue, which is known to contain epitopes for all these lectins, was used as an external positive control for SBA, DBA, and PNA.

Consecutive slides from three maculae with BLD class 3 were also stained with antibodies against laminin and collagen type IV according to a previous study.

**RESULTS**

The binding of a series of lectins and the monoclonal antibody HNK-1 with BLD in 13 human maculae is

| Table 1. Staining Characteristics of Basal Laminar Deposit in 13 Human Maculae |
|---------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Macula number                | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| BLD class                    | 2 | 3 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 3 | 3 |
| Specificity                   | α-α-Man (CON A)       | + | + | + | + | - | - | + | + | + | + | - | + |
| α-D-GalNAc (SBA)             | ± | + | + | + | - | - | - | + | + | - | + | ± | ± |
| β-D-GlCNAC and NeuNAc (WGA) | + | + | + | + | + | + | + | + | + | + | + | + | + |
| α-D-GalNAc (DBA)             | ± | + | + | + | + | + | + | + | + | + | + | + | + |
| α-1-Fucose (UEA-1)          | - | + | - | - | - | - | - | - | - | - | - | - | - |
| β-D-Gal > α-D-Gal (RCA-1)    | - | ± | + | - | - | - | - | - | - | - | - | - | - |
| β-D-Galβ(1-3)- α-D-GalNAc (PNA) | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Gl(UA)β[1-3-Galβ1-4GlCNAc (HNK-1) | + | - | - | - | - | - | - | - | - | - | - | - | - |

Staining: + = positive; ± = faintly positive; − = negative. BLD = basal laminar deposit; CON A = canavalia ensiformis; SBA = soybean agglutinin; WGA = wheat germ agglutinin; DBA = dolichos bifloris; NEA-1 = ulex europaeus; RCA-1 = ricinus communis agglutinin; PNA = arachis hypogaea.
shown in Table 1. BLD stained positive with WGA and DBA in all specimens. In 12 out of the 13 maculae, BLD stained with HNK-1. The main part of the BLD strongly stained near Bruch's membrane, and the coloring fainted toward the RPE (Fig. 1); this was the case with all lectins used (except for PNA) and also with the monoclonal antibody HNK-1. We did not find any difference between the binding of BLD class 2 or 3. PNA neither stained BLD nor any other structure in the macula.

Of the structures in the posterior part of the human eye, BLD stained exclusively positive with DBA and SBA (Table 2, Figs. 1 to 3). However, in three maculae, the media of the blood vessels (for example, in the retina and the choroid) also stained with DBA and SBA. These blood vessels in the same eyes also stained with HNK-1. Besides BLD, Con A, WGA, UEA-I, RCA-I, and HNK-1 stained varying structures of the retina (for example, basal membranes, photoreceptors), choriocapillaris, optic nerve, and/or RPE (Table 2, Fig. 4). Hard and soft drusen found in 5 of 13 specimens did not stain with any of the lectins nor with the monoclonal antibody HNK-1. All positive controls were affirmative. Negative controls revealed no specific staining. No change in staining properties was seen in parts of the maculae that lacked BLD.

Corresponding to the results of Van der Schaft et al., only a small part of the BLD near the RPE in one macula (out of three) stained positively with antibodies against laminin (Fig. 5A) and collagen type IV (not shown, but almost identical with Fig. 5A).

**DISCUSSION**

Our results indicate that BLD contains different carbohydrate moieties with varying expression. The most consistent accessible carbohydrate residues in BLD were α-D-GalNAc, β-D-GlcNAc, NeuNAc, and the HNK-1 epitope. Variability in lectin binding is probably due to other carbohydrate epitopes on the same or adjacent glycoconjugates, which can enhance or inhibit the binding of lectins. It is also possible that carbohydrate structures in BLD are slightly different from one patient to another because of enzymatic digestion or modification.

BLD was exclusively stained by DBA and SBA. The positive staining of the blood vessels in three speci-

**TABLE 2. Lectin and HNK-1 Binding in the Human Eye (n = 13)**

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Bruch's Membrane</th>
<th>Choroid*</th>
<th>RPE*</th>
<th>Retina*</th>
<th>Optic Nerve*</th>
<th>BLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON A</td>
<td>-</td>
<td>-</td>
<td>+ (13)</td>
<td>+ (13)</td>
<td>+ (13)</td>
<td>+ (10)</td>
</tr>
<tr>
<td>SBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ (9)</td>
</tr>
<tr>
<td>WGA</td>
<td>-</td>
<td>+ (13)</td>
<td>+ (13)</td>
<td>+ (13)</td>
<td>+ (13)</td>
<td>+ (13)</td>
</tr>
<tr>
<td>DBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ (13)</td>
</tr>
<tr>
<td>UEA-I</td>
<td>-</td>
<td>+ (13)</td>
<td>+ (13)</td>
<td>+ (13)</td>
<td>-</td>
<td>+ (3)</td>
</tr>
<tr>
<td>RCA-I</td>
<td>-</td>
<td>+ (9)</td>
<td>+ (7)</td>
<td>+ (4)</td>
<td>-</td>
<td>+ (3)</td>
</tr>
<tr>
<td>PNA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HNK-1</td>
<td>-</td>
<td>-</td>
<td>+ (13)</td>
<td>+ (13)</td>
<td>+ (13)</td>
<td>+ (12)</td>
</tr>
</tbody>
</table>

Staining: + = positive; − = negative. RPE = retinal pigment epithelium; BLD = basal laminar deposit. Values in parentheses are the number of positive specimens. See Table 1 for abbreviations.

* Varying parts in this structure stained.
mens can be related to blood group A as reported by Kiveli and Tarkkanen. In our study, these clinical data were not available.

None of the hard and soft drusen seen in our specimens stained with either the lectins or the monoclonal antibody HNK-1. Other studies on BLD using antibodies against laminin, collagen type IV, and heparan sulphate proteoglycan also reported no staining of drusen with these antibodies.

Ultrastructurally, the main components of BLD show similarity with fibrous long-spacing collagen (FLSC) and a homogeneous material with the same electron density as basement membranes. FLSC is a polymer of collagen, with a banded ultrastructure and a periodicity between 100 and 120 nm that in vitro precipitates in the presence of chondroitin sulphate. It has been demonstrated that BLD reacts with antibodies against basement membrane elements, such as collagen type IV, heparan sulphate proteoglycans, and laminin. The basal extracellular matrix of the normal RPE in vivo and in vitro is also positive for antibodies against basal membrane elements. However, the positive staining with antibodies against laminin and collagen type IV occurred only in the homogeneous part of the BLD in a small rim adjacent to the RPE cell surface (Fig. 5A). The FLSC did not react with any of the abovementioned antibodies against basement membranes. In contrast, the lectins and the monoclonal antibody HNK-1 in our study were bound to the main part of the BLD, and this was most prominent near Bruch’s membrane (Fig. 5B). Unfortunately, it is impossible to distinguish between the FLSC and the homogeneous part of BLD with light microscopy. However, the relatively thick layer of positive staining with the lectins and the monoclonal antibody HNK-1 seems to be suggestive of the FLSC component of BLD. Further electron microscopic investigation is required to support this concept.

It has been demonstrated that lipids accumulate in Bruch’s membrane in the aging human macula. However, it is unlikely that BLD consists of glycolipids because processing to wax dissolves carbohydrates linked to fat. Therefore, we assume that BLD contains carbohydrate structures that are most likely part of glycoproteins or part of complex carbohydrate molecules. We also conclude that the main part of BLD contains several carbohydrate structures different from carbohydrate structures on laminin and collagen type IV.
Carbohydrate Structures in Basal Laminar Deposit

In conclusion, we found that BLD contains α-D-GalNAc, which in our study was not detectable in other structures of the macular tissue. Therefore, DBA or SBA can be used as a specific marker for the detection of BLD. Further analysis of the composition of BLD-associated carbohydrate structures may provide information about the structure and origin of BLD in age-related macular degeneration.

**Key Words**
age-related macular degeneration, maculopathy, basal laminar deposit, drusen, lectins

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**References**


