Is Basal Laminar Deposit Unique for Age-Related Macular Degeneration?

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- The ultrastructural nature and distribution of basal laminar deposit, considered to be a precursor of age-related macular degeneration, were studied in 42 human maculae. Basal laminar deposit was found from age 19 years on, not only between the retinal pigmented epithelial cells and their basement membrane but also more often on the choriocapillaris side of Bruch's membrane. No direct relationship was found with other aging changes, such as calcifications in Bruch's membrane, accumulation of lipofuscin granules, or drusen in the macular area. Material similar to basal laminar deposit can be found in the trabecular system, in the cornea, and also in many other organs and tissues. On a structural and morphometrical basis, we think that basal laminar deposit is similar to fibrous long-spacing collagen and thus does not seem to be a purely ocular abnormality.


Visual loss due to age-related macular degeneration (AMD) is an increasing problem in the Western world, owing to a rise in the average age of the population. In patients with clinical signs of AMD, postmortem light microscopic (LM) examination has shown progressive accumulation of extracellular material at the basal side of the retinal pigment epithelium (RPE) in the macular area. This has led to the postulation that these deposits might be a precursor of AMD. These basal deposits have been called basal laminar deposit(s), basal linear deposit(s), and linear basal deposit(s); all indicating identical materials. The basal deposits can be seen by LM as a linear band, actually representing a lamina between the RPE and Bruch's membrane. We prefer the singular expression, basal laminar deposit (BLD).

Ultrastructurally, BLD is located between the RPE cell membrane and its basement membrane and has a characteristic banded pattern, with a periodicity of about 120 nm. A second type of BLD has been observed in eyes with long-standing macular atrophy. It has been described as a mixture of amorphous clumps, fibrillar material, and a small amount of fibrous long-spacing collagen (FLSC). It was called "flocculent BLD," because of the multilaminar or flocculent arrangement at the base of the RPE. It is assumed that BLD might be a waste product of the RPE cells, and is secreted at the basal side of the cells.

The prevalence of BLD increases with age. A mild visual loss has been reported in patients with postmortem histopathological evidence of a moderate amount of BLD in the macular area. All patients with a large amount of BLD had serious visual loss during the last part of their life, and 14% had histopathological evidence of subretinal neovascularization. We studied the structure and distribution of BLD in postmortem human eyes by transmission electron microscopy (TEM), emphasizing advanced age groups.

MATERIALS AND METHODS

We obtained 145 randomly collected postmortem human eye bank and autopsy eyes. Nothing was known about the ocular history. The age of the patients ranged from 0 to 94 years, with an average of 70 years (SD, 15.8 years). Time between death and fixation ranged from 2 to 22 hours. Only one eye of each person was used for this study. After removing the cornea, the eyes were either fixed with a mixture of 1% glutaraldehyde (vol/vol) and 4% formaldehyde solution (vol/vol) or a 4% formaldehyde solution in a 0.1-mol/L phosphate buffer at pH 7.2.

After horizontal sectioning of the globe with a razor, the eyes were examined with a Zeiss binocular preparation microscope at x4 magnification. Only eyes without macroscopically gross pathological changes, apart from macular degeneration or drusen, were used, as BLD formation might be stimulated by various pathological conditions such as trauma or infection. The macular area was removed and cut into two equal parts. Half of the macula was embedded in paraffin following routine procedures for LM to select the eyes with BLD. The other half of the macula was divided into three equal parts for TEM and stored in fixative until use.

To compare the ultrastructure of maculae with and without BLD and of maculae of all age groups, we selected 42 maculae, 16 with LM evidence of the presence of BLD between the RPE and Bruch's membrane and 26 without LM evidence for the presence of BLD; these were distributed over all age decades. One part of these maculae was embedded, without osmium tetroxide postfixation, in epoxy resin (LX 112, Ladd Research Industries, Inc, Burlington, Vt) after dehydration with grading acetone. Semithin sections 1-μm thick were made for LM with a glass knife and stained with toluidine blue (1% wt/vol). Ultrathin sec-
tions of 70- to 80-nm thickness, made on an
Ultratome LKB IV (LKB, Stockholm, Swe-
den) with a diamond knife, were mounted
on unframed mesh 300 copper grids. After
staining for 30 minutes with uranyl acetate
and 2 minutes with lead citrate, the ul-
trathin sections were examined with a TEM
(Zeiss EM 902), with an acceleration volt-
age of 80 kV. Micrographs were made on
film (Kodak SO 163, Eastman Kodak, Roch-
ester, NY). An image-analyzing system
(IBAS 2000 Zeiss/Kontron, Oberkochen,
Federal Republic of Germany) was connect-
ed directly to the TEM for ultrastructural
measurements.

Immediately after sectioning, ultrathin
sections of two eyes with a large amount of
BLD were additionally stained for 6 hours
with phosphotungstic acid (10% wt/vol), a
selective collagen stain. In addition, we
embedded a piece of the anterior segment
of three eyes obtained at autopsy from
patients who were 59, 82, and 84 years of
age, including a small part of the cornea,
trabecular system, and ciliary body.

RESULTS

Basal laminar deposit was located
between the RPE cell membrane and
its basement membrane as a complex
of extracellular material (Fig 1). The
most prominent part of the BLD ap-
ppeared as irregularly oriented, small,
trapezoidal or spindle-shaped pieces of
material. Its most remarkable feature
was the fingerprintlike cross-banding
(Fig 1). This banding pattern consisted
of electron-dense bands with an aver-
age width of about 50 nm and electron-
lucent interbands with an average
width of about 80 nm (Fig 2). The
electron-dense bands consisted of two
parallel electron-dense bands, each
about 15 nm wide, and an electron-
luent band about 20 nm wide in be-
tween. This electron-lucent band was
not always clearly visible (Fig 2).
Within the interbands, a much finer
striation was observed perpendicular
to the electron-dense bands.

The bands ran in a strictly parallel
fashion, although there was a marked
variety in width of the banding pat-
tern, within and also between the dif-
ferent patches of BLD. Its periodicity
ranged from 115 to more than 140 nm.
Above 140 nm, the demarcation of the
electron-dense bands became so indis-
tinct that it was difficult to measure its
periodicity (Fig 2). Scattered between
the banded material were pieces of the
same size and shape, with a homoge-
neous, moderate electron density,
sometimes exhibiting the beginning of
a banded pattern at one of the edges
(Fig 2). This material seemed to con-
sist of bundles of fibers, sectioned per-
dicularly and thus not exhibiting the
banded pattern.

Most of the BLD was located close

Fig 1.—Electron micrograph of the retinal pig-
ment epithelium (top) and a large amount of basal
laminar deposit (BLD) between the cell membrane
(closed arrow) and its basement membrane
(open arrows). Note the fingerprintlike banding
and the irregular orientation of the material
(uranyl acetate–lead citrate, original magnification. × 3000).

Fig 2.—Higher magnification of basal laminar deposit with the epithelial basement mem-
brane (B), the banded material, homogeneous material (H), and electron-lucent spaces (E). Note the
electron-dense bands, with the electron-lucent center (large closed arrow) and the lighter
interbands with the longitudinally oriented fine striation (small arrows). Some bands seem wider,
probably owing to the angle of sectioning (open arrow). The homogeneous material seems to
consist of a bundle of fibers, sectioned perpendicularly, and merges gradually into a banded
pattern (phosphotungstic acid, uranyl acetate–lead citrate, original magnification. × 20 000).

to the RPE basal cell membrane and
between its basal infoldings. Basal
laminar deposit–like material was not
found within the RPE cytoplasm or
within the lipofuscin granules found in
the RPE. Between the banded materi-
al were areas filled with fibrils and
electron-lucent spaces, which seemed
"empty" with uranyl acetate–lead ci-
trate and phosphotungstic acid stain-
ing procedures (Fig 2). The fibrils
were sometimes seen to be connected
with the banded material (Fig 3). A
small number of vesicles and occasion-
ally a pigment granule were found
between the banded material.
Banded material, structurally similar to BLD and with the same periodicity of about 120 nm, was interspersed in the OCZ of Bruch’s membrane, especially on the choroidal side (Figs 5 and 6). In 33 (79%) of the 42 maculae examined, this BLD-like material was present in the OCZ (Fig 7), in some maculae in even larger amounts than between the RPE and its basement membrane (Fig 5). In 20 (48%) of the maculae, this BLD-like material was found in the OCZ, although no deposits could be found at the base of the RPE. The structure and periodicity of these deposits on the choroidal side of Bruch’s membrane were similar to those between the RPE and its basement membrane. The amount of BLD in the OCZ increased with age and BLD was seen already at age 19 years. The BLD between the RPE and its basement membrane was first seen at age 70 years.

In none of the eyes examined was BLD present on the capillary side of the endothelial basement membrane of the choriocapillaris. In seven maculae, small amounts of similar banded material were found in the inner collage nous zone (ICZ) of Bruch’s membrane.

In seven maculae, an early stage of BLD was found between the basal infoldings of the RPE cell membrane and the RPE basement membrane (Fig 5). This consisted of globular deposits with a homogeneous, moderate electron density. In most cases these deposits were confluent. Interspersed between these deposits were found a few small pieces of 120-nm banded material, small amounts of fibrillar material, and nonhomogeneous granular material in varying amounts. The floculent type of BLD was not seen in our study, probably because none of the eyes examined exhibited an advanced stage of macular degeneration.

No connection was observed between the presence of BLD and the location of other aging changes of the macular area, such as the amount of accumulated lipofuscin granules in the overlying RPE cells, loss of retinal pigment granules, or calcifications within Bruch’s membrane. No relation was found between the fixation delay and the prevalence of BLD.

In the trabecular system (Fig 8), a varying amount of BLD-like, banded deposits was found in the trabeculae of all three eyes, located close to the basement membrane of the trabecular endothelial cells. The periodicity of the banding pattern was also about 110 to 120 nm and was structurally similar to the banded material of the BLD in the macular area, especially when sec-
Sarks showed that the amount of BLD is positively correlated with visual loss and is a good indicator of the degree of RPE degeneration. However, BLD cannot be detected directly by ophthalmoscopy or fluorescein angiography. The first clinically visible signs of AMD are pigment changes in the macular area, due to alterations in the RPE cells. In this stage, there is already a slight visual loss.

It is generally assumed that BLD is produced by the RPE cells, probably owing to the fact that BLD was initially found between the RPE and its basement membrane. However, in this study, BLD was also found by TEM within the ICZ and OCZ and between the OCZ and the basement membrane of the choriocapillaris endothelium, as has been mentioned by others. This might be explained by diffusion of precursor material from the RPE through the layers of Bruch’s membrane, before polymerization into BLD. However, in 20 maculae we found BLD located only between the OCZ and the endothelial basement membrane of the choriocapillaris, without evidence of BLD in relation to the RPE. This could be an argument against BLD production by the RPE and suggests a multifocal origin of BLD or a complete diffusion through Bruch’s membrane of the precursors of the banded material. Also, the idea that BLD might originate exclusively from the choriocapillaris seems unlikely, because in the trabecular system, which is avascular, banded deposits have also been described. In our study, the trabecular deposits were located adjacent to the trabecular endothelial basement membrane and were structurally similar to BLD in the macular area. The periodicity of the banded pattern in both was about 110 to 120 nm. The trabecular banded material was located in an area of a homogeneous and slightly electron-lucent substance, from which it seemed to originate (Fig 8). The longitudinally directed striations were, however, more pronounced than in BLD, and the electron-lucent center of the electron-dense bands was absent. As in the BLD, deposits with a higher periodicity were also seen here, coupled with broader and more indistinct striations. This is probably due to a different angle of sectioning. We can assume that when the periodicity is smallest and the bands are most distinct, the angle of sectioning is approximately 90°.

In the trabecular system, both the RPE and blood vessels are absent.

Fig 5.—Electron micrograph of the retinal pigment epithelium (top) and Bruch’s membrane with the inner collagenous zone (ICZ), elastic layer (EL), and outer collagenous zone (OCZ). Between the coarse basal infoldings of the retinal pigment epithelium and the basement membrane is a large amount of an early stage of basal laminar deposit (asterisks), with a small amount of banded material. Within the OCZ on the choroidal side, there is a large amount of banded material (arrows), which has the same structure and banding pattern as basal laminar deposit. Note calcifications (C) in Bruch’s membrane. CC indicates choriocapillaris (uranyl acetate–lead citrate, original magnification, × 4400).

Fig 6.—Electron micrograph of Bruch’s membrane and the choriocapillaris (CC) of an 86-year-old person. The difference between the layers of Bruch’s membrane is not clearly visible in the macular area. The inner and outer collagenous zones (ICZ and OCZ) are filled with small vesicles between the collagen fibers. Within the OCZ, especially on the choroidal side, one may see banded material similar to basal laminar deposit (B), trilaminated curvy membranes (open arrows), and an electron-dense granule (closed arrow). EL indicates elastic layer (uranyl acetate–lead citrate, original magnification, × 7000).
Fig. 7.—Age distribution of the 42 patients and the ultrastructural distribution of basal laminar deposit (BLD) in the macula. Darker shaded bars indicate all eyes; lighter shaded bars, the BLD located in the outer collagenous zone (OCZ) of Bruch’s membrane; and open bars, the BLD between the basement membrane of the retinal pigment epithelium (RPE) and its cell membrane. Note the presence of BLD in the OCZ in young persons, in contrast to BLD between the RPE and its basement membrane.

Fig. 8.—Tangential section through a trabecular fiber near the limbus of an 82-year-old patient. Two layers of trabecular endothelial cells (TEC) with a basement membrane (arrows) are covering the connective tissue core. Adjacent to this basement membrane, banded material (B) with a periodicity of 110 to 120 nm is seen (uranyl acetate-lead citrate, osmium tetroxide, original magnification, x 12,000).

This suggests another origin of the banded material or the uptake of precursor molecules from the chamber fluid, followed by polymerization into banded deposits. McMenamin et al found large amounts of banded material in the trabecular system of eyes of aged patients, but this material has also been described in small amounts in patients from 6 years old on.

Ultrastructurally, BLD resembles FLSC. Gradually mentioned four types of FLSC, all of which can be made in vitro, but only FLSC III resembles in vivo FLSC. Influences such as pH, PO₂, and the concentration and type of glycosaminoglycans may be the reason that only type III is formed in vivo. This might also be influenced by age and pathological or traumatic changes in the tissue.

Fibrous long-spacing collagen type III has been found thus far in a variety of normal and pathological tissues and organs, listed in the Table. The slightly different ratio of collagen to glycosaminoglycans and other external factors may determine the small differences of length and width of the FLSC in the various tissues.

Authors have used confusing names for FLSC in TEM images, such as banded structures, curly collagen, wide banded collagen, long-spacing collagen, broad banded striated bodies, lattice collagen, sheath collagen, kollagenoid, gitterkollagen, and Lise bodies. This might be due to its diverse TEM appearances as a result of diverse fixation techniques, staining techniques, and measuring methods of the periodicity of the banding pattern, or it might be due to the kind of tissue examined.

Our findings suggest that the most characteristic substance of BLD, the
banded material found between the RPE and its basement membrane, within Bruch's membrane, and within the trabecular system, is the same as FLSC III. Structurally, BLD is similar to FLSC III, with a banding periodicity of 100 to 120 nm. Both have an extracellular location, close to an epithelium with an adjacent basement membrane. Both BLD and FLSC III are found in tissue with aging or degenerative changes (Table). To the best of our knowledge, it is still impossible to identify BLD and FLSC with immunological techniques.

Electron microscopically, BLD is surrounded by electron-lucent material, which is referred to by Loeffler and Lee as “empty space, possibly in vivo filled with fluid” (Fig 5). Another explanation might be that this is electron-lucent material that does not stain with routine TEM staining techniques or with phosphotungstic acid.

**CONCLUSION**

We think that the formation of BLD is neither a unique process nor a purely ocular disease. In the eye, it is most often found in the vicinity of Bruch's membrane in the macular area but it can also be found elsewhere, as in the trabecular system. The location of BLD in the macula, not only between the RPE cell membrane and its basement membrane but also within Bruch's membrane on the choroidal side, suggests a multifocal origin or a polymerization of smaller particles, eg, t ropocollagen or basement membrane material. This may diffuse through the tissue and polymerize to collagen or to BLD, depending on the microenvironment. The production of an excessive amount of glycosaminoglycans might be a determining factor. Therefore, BLD might be a symptom of general degenerative changes. Further research is needed to investigate the role of the RPE and the exact composition of BLD.

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