Basal laminar deposit in the aging peripheral human retina

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Received: 6 October 1992 / Accepted: 18 February 1993

Abstract. A basal laminar deposit (BLD) in the human macula has been described as an early sign of age-related macular degeneration. In some eyes with a BLD in the macula, light microscopic sections of the peripheral retina revealed almost similar deposits between the retinal pigment epithelium and Bruch's membrane. Because the exact pathogenesis of age-related macular degeneration and the origin of the BLD are unknown, we studied the ultrastructure of these peripheral sub-RPE deposits. Parts of the equatorial and peripheral regions of the retina of ten human eyes, with BLD-like deposits between the retinal pigment epithelium and Bruch's membrane, were examined by electron microscopy. In eight of these ten eyes the ultrastructure of these deposits was amorphous and finely granular. Five of the eight deposits also contained small amounts of long-spacing collagen. Ultrastructurally, the deposits were similar to an early type BLD in the macula. In the remaining two eyes, the deposits appeared to consist of flat, elongated drusen. Our findings indicate that a BLD can develop not only in the macula but also in the peripheral region of the retina.

Introduction

Post mortem light microscopic examination of eyes from patients with clinical signs of age-related macular degeneration (ARMD) has shown an accumulation of extracellular material between the retinal pigment epithelial (RPE) plasma membrane and the inner side of Bruch's membrane in the macular area [12]. This material is called a basal laminar deposit (BLD) [12, 13, 16]. The presence of a BLD in the macula has been associated with RPE degeneration and decreased visual acuity [12, 13]. Light microscopically, a BLD appears as a discontinuous layer in the early stage of ARMD and as a

linear band or a continuous layer between the RPE and Bruch's membrane in a more advanced stage [16].

Ultrastructurally, a BLD is located between the RPE plasma membrane and its basement membrane [9, 14] in contrast to drusen, which lie between the basement membrane of the RPE and the inner collagenous zone of Bruch's membrane [4]. The early type of BLD is distributed in a patchy fashion between the RPE and Bruch's membrane and consists ultrastructurally of homogeneously stained, finely granular material interspersed with small amounts of banded material called "long-spacing collagen" (LSC) [3]. The late type of BLD, which generally occurs as a thick continuous layer between the RPE and Bruch's membrane, is composed mainly of LSC, which displays a characteristic fingerprint-like banded pattern with a periodicity of about 120 nm and is embedded in small amounts of homogeneously stained, finely granular material [14, 15]. Furthermore, some fibrillar material can be seen as well as a few vesicles. Material morphologically similar to that of a BLD has also been found in the inner and outer collagenous zones of Bruch's membrane in the macular region [1, 14].

A third type of BLD has been observed in eyes with long-standing macular degeneration at the edges of geographic atrophy. This is called a flocculent BLD, because of its multilaminar or cumuliform arrangement at the base of the RPE [13]. The ultrastructure has been described as a mixture of amorphous clumps, fibrillar material and small amounts of banded material [13].

In a previous light microscopical study [16], the equatorial retina was found to contain sub-RPE deposits, which had the same staining properties as a BLD in the macula but exhibited a slightly more compact structure. These deposits extended farther than hard drusen normally do and, in most eyes, they could be distinguished from drusen by both their shape and the difference in staining properties.

Most studies on ARMD focus on the degenerative changes in the macula [5, 6, 17, 18] since these changes have more important implications as far as function is

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concerned. Because the exact pathogenesis of ARMD as well as the origin of a BLD is still unknown, we examined the ultrastructure of BLD-like sub-RPE deposits and differences in ultrastructure of the RPE and Bruch's membrane and peripheral parts of the retina and choroid of eyes with a BLD in the macula.

Materials and methods

We selected ten eyes from a larger series of 50 post-mortem human eye-bank and autopsy eyes described previously [16]. These ten eyes were selected because light microscopy revealed the presence of a BLD class 2, a thin continuous layer, or class 3, a thick layer measuring at least half the height of the RPE between the RPE and Bruch's membrane in the peripheral retina [16]. The age of the subjects ranged from 72 to 90 years. The eyes were fixed with formaldehyde (4% volume/volume, pH 7.4). In five of these ten eyes tissue was taken from the stored but not embedded part adjacent to tissue in the paraffin block. Macroscopically, BLD could not be identified. In the remaining five eyes, the parts of the retina and choroid containing these deposits, as seen with light microscopy, were excised from the paraffin blocks. These blocks were subsequently deparaffinized with toluol and rehydrated in graded alcohols. After additional post-fixation with osmium tetroxide (1% wt/vol in buffer, pH 7.4, room temperature, overnight) and subsequent dehydration with graded acetone, all tissue samples were embedded in LX 112 (Ladd Research Industries, Burlington, Vermont) as previously described [14]. Semithin sections (1 µm thick) were cut with a glass knife and stained with toluidine blue for light microscopy. Ultrathin sections (30–40 nm thick) were cut with a diamond knife on an ultratome (LKB, Stockholm, Sweden) and mounted on unfilmed 200-mesh copper grids. After staining with uranyl acetate and lead citrate, the ultrathin sections were examined with a transmission electron microscope (Zeiss TEM 902, Oberkochen, Germany), with an acceleration voltage of 80 kV. Micrographs were made on sheet film (Kodak SO 163, Eastman Kodak, Rochester, N.Y.). The ultrastructure of the sub-RPE deposits in peripheral retinas was compared with some micrographs of the ultrastructure of BLD in maculae from a previous study [14].

Results

The ultrastructure of the RPE and Bruch's membrane in the peripheral retina differed in some aspects from the ultrastructure of similar structures in the macula.

The RPE in the peripheral part of the eye (Fig. 1a) was more attenuated and contained fewer melanin, melanolipofuscin and lipofuscin granules than that in the macula (Fig. 1b). These granules are mainly located in the apical part of the RPE cells. The mitochondria were more evenly distributed throughout the cytoplasm of the RPE cells and not merely in the basal part of the cells (Figs. 1a, 2), as in the macula (Fig. 1b). The apical villi were long and numerous and often appeared to have branches and connections (Fig. 2). Basal infoldings of the RPE cells were numerous (Fig. 1a), as can be seen in the macula (Fig. 1b). In both the macular and the peripheral retina the basement membrane of the RPE did not follow the basal infoldings of the cell membrane, but continued in a straight line (Fig. 1a, b).

The most striking difference between Bruch's membrane in the macula and outside the macula was the presence, in the periphery, of a thick elastic lamina,

Fig. 1. **a** Electron micrograph of the retinal pigment epithelium (RPE) (*top*) and Bruch's membrane (*bottom*) in the *equatorial* retina of an 84-year-old subject. Most apical villi (*V*) are seen tangentially or in cross-section. The melanin (*M*) (oval) and lipofuscin (*L*) (round) granules are located in the apex of the cells. Mitochondria (*Mi*) can be seen throughout the cytoplasm. At the base of the cells are numerous basal infoldings of the cell membrane. Note that the basement membrane (*arrows*) does not follow the basal infoldings. The inner collagenous zone (*ICZ*) of Bruch's membrane is slightly thickened. The thick elastic layer (*EL*) of Bruch's membrane is frequently interrupted. *OCZ*, Outer collagenous zone. **b** Ultrastructure of the RPE (*top*), Bruch's membrane and choriocapillaris (*bottom*) in the *macula* of an 81-year-old subject. The elastic layer of Bruch's membrane (*arrows*) is very thin and indistinct. The *ICZ* and *OCZ* are filled with vesicles, membranous material and long-spacing collagen (*LSC*). *LSC* is mainly located close to the thickened basement membrane (*B*) of the choriocapillaris. The lipofuscin granules (*L*) in the RPE are less electron-dense, because this part of the macula was not post-fixed with osmium tetroxide. There are many basal infoldings (*arrowheads*), some of which are filled with basement membrane-like material (*asterisks*). *Mi*, Mitochondrion

Fig. 2. Electron micrograph of the RPE and Bruch's membrane in the *equatorial* retina of a 72-year-old subject. The apical villi (*V*) of the RPE seem to branch out and connect with each other. An amorphous, finely granules deposit can be seen between the RPE cell membrane and the ICZ of Bruch's membrane. The basal infoldings have become wider than in RPE cells without the deposit. Close to the basal cell membrane of the RPE, the deposit appears fibrillar (*asterisks* and *inset*)

Fig. 3. Amorphous, finely granular deposit between the RPE (*top*) and Bruch's membrane *halfway between the macula and the equator* of a 90-year-old subject. Long-spacing collagen (*LSC*) is interspersed within this material. A second thin basement membrane can be seen along the basement membrane of the RPE (*arrows*).

Adjacent to this basement membrane the deposit appears fibrillar (*asterisks*). The original basement membrane is still parallel to the ICZ of Bruch's membrane (*arrowheads*). (Ultrathin section recovered from paraffin-embedded material)

Fig. 4. An early type BLD in the *macula* of an 88-year-old subject. The major component of this type of BLD is the homogeneous material (*H*). Small amounts of long-spacing collagen (*LSC*) are present. Basement membrane (*arrowheads*)

Fig. 5. Large BLD in the *macula* of an 88-year-old subject. The major component is long-spacing collagen. Only small amounts of homogeneous material can be found (*H*). Basement membrane (*arrowheads*). Many lipofuscin (*L*) and melanolipofuscin (*MI*) granules can be seen in the RPE cytoplasm

which was often discontinuous (Fig. 1a). In the macula this elastic lamina was composed of a very thin meshwork of elastic fibers (Fig. 1b). The structure of both the inner collagenous zone (ICZ) and the outer collagenous zone (OCZ) of Bruch's membrane seemed to be more open in the peripheral retina than in the macula (Fig. 1a, b). In the ICZ several types of small vesicles, dense granules and membrane fragments could be seen

(Fig. 1a), although these were not as abundant as in the macula (Fig. 1b). The OCZ was often thinner in the peripheral retina than the ICZ and contained fewer vesicles and membrane fragments than the ICZ. In this series the OCZ hardly seemed to have thickened with advanced age. In contrast the macular OCZ contained abundant cellular debris accumulated during the life (Fig. 1b). This resulted sometimes in a three-fold in-

Fig. 6. **a** Deposit between the RPE cell membrane and Bruch's membrane in the *peripheral retina* of an 82-year-old subject, consisting of lightly stained amorphous material with electron-dense, irregularly shaped clumps (*arrows*). **b** Higher magnification of the deposit. The electron-dense parts are not surrounded by a membrane

crease in total thickness, especially between the capillaries of the choriocapillaris.

In eight of the ten eyes, amorphous material was found at the location of the BLD-like deposits revealed by light microscopy in the paraffin sections. Ultrastructurally, it was localized outside the RPE cells between the basal infoldings of the cell membrane, which appeared wider when associated with these deposits (compare Fig. 1a with Fig. 2). This material was continuous with the RPE basement membrane and had the same electron density. When close to the RPE cell membrane, the deposits often had a fibrillar structure (Fig. 2, inset).

In five of these eight eyes banded material or long-spacing collagen was observed in the deposits (Fig. 3). The proportion of the amount of banded material to

Fig. 7. Two large vacuoles (*asterisks*) and several small vacuoles (*arrows*) in the RPE in the *equatorial region* of a 90-year-old subject. The right vacuole is filled with amorphous, finely granular material as are several small vacuoles (*arrows*). The large vacuole on the left is partly filled with membranous debris. Small sub-RPE deposit (*open arrow*) consisting of lightly stained material and electron-dense material. Small pieces of long-spacing collagen (*LSC*) can be seen in the outer collagenous zone of Bruch's membrane

the amount of finely granular material was low, as was found for the early-type BLD in the macula (Fig. 4). The late-type BLD in the macula consisted almost entirely of LSC (Fig. 5).

The extracellular deposits in five of the eight eyes contained randomly scattered and irregularly shaped clumps of amorphous, more electron-dense material (Fig. 6a). These clumps consisted of a similar amorphous material, but were more compact. This material was not surrounded by a membrane (Fig. 6b).

Large vacuoles were found in the cytoplasm of several RPE cells in the peripheral retina (Fig. 7). Some vacuoles were filled with a finely granular amorphous material, similar to that observed between the RPE cells and Bruch's membrane. Smaller vacuoles, filled with morphologically the same type of material, could be seen in the basal parts of the RPE cells. Frequently, these vacuoles were continuous with the extracellular RPE basement membrane (Figs. 1b, 7–9). Still other vacuoles were electron-lucent and seemed to be empty or were partially filled with even smaller vacuoles containing membranous cellular debris (Fig. 7). Sometimes the deposits appeared to be lined with a basement membrane on the RPE side as well as on the side of Bruch's membrane (Fig. 8).

In only two of the eight eyes with sub-RPE deposits was material similar to the LSC component of BLD found in the OCZ of Bruch's membrane in the peripheral retina (Fig. 7). Only a few fragments of LSC were sometimes seen in the ICZ.

In two of the ten eyes the sub-RPE deposits, identified by light microscopy as being similar to BLD, appeared

Fig. 8. Small deposit between the RPE (*top*) and Bruch's membrane (*bottom*), just *outside the macula* of a 90-year-old subject. Note that the original basement membrane is still parallel to the ICZ of Bruch's membrane (*closed arrows*). A second, thinner basement membrane is adjacent to the RPE cell membrane (*open arrows*). The ICZ and OCZ of Bruch's membrane are markedly thickened. EL, elastic layer. (Ultrathin section of tissue recovered from paraffin-embedded material)

Fig. 9. Elongated, drusen-like deposit (*asterisk*) between the RPE basement membrane (*closed arrows*) and the ICZ in the *peripheral* part of the retina of an 84-year-old subject. In the cytoplasm of a RPE cell is a vacuole filled with phagocytized discs of a photoreceptor outer-segment (*curved arrow*). Note the basal infoldings overlying the drusenlike deposit; some are filled with material, which is confluent with the basement membrane (*open arrow*)

ultrastructurally to be located between the RPE basement membrane and the ICZ of Bruch's membrane (Fig. 9). These deposits consisted of the same mixture of cellular debris, as can be seen in hard drusen and in the thickened OCZ of Bruch's membrane in the macula (Fig. 1b), but LSC was not seen. Although the ultrastructure of these deposits was similar to that of hard drusen, light microscopy revealed that this material was deposited in an elongated and flat way, thus imitating a more extensive BLD. The ultrastructure of the RPE cells overlying the flat deposits was normal (Fig. 9).

Discussion

As previously described in a light microscopy study [16], sub-RPE deposits similar to a BLD in the macula can be seen in the peripheral retina. Although their staining properties are similar, the deposits in the peripheral retina have a more compact structure than a BLD in the macula. A positive correlation between the presence of these deposits in the macula and the peripheral retina of the same eyes was found [16]. Ultrastructurally, both differences and similarities were found. The main component of the deposits in the peripheral retina was a homogeneously stained material, only rarely interspersed with LSC. In contrast, a BLD in the macula consisted of small amounts of homogeneously stained material embedded in large amounts of LSC [14] (Fig. 5). However, an early type BLD in the macula closely resembled the deposits in the peripheral retina [14] (Figs. 3, 4).

It is not clear why there is a difference in the ratio of the two components of these deposits, depending on the site in the same eye. Several hypotheses have been proposed. The most striking difference between the macula and the peripheral retina is the distribution of rods and cones, with the cones dominating in the macular region. Because there are no indications that a BLD is comprised of degradation products of the photoreceptor outer segments [2, 15], it is unlikely that this is the determining factor. Another difference between the macula and the peripheral retina is the difference in the structure of RPE cells [7]. Although several authors think that the sub-RPE deposits are produced by the RPE [6, 9, 12], it is not clear why differences in cell morphology would result in a difference in the composition of the deposits. One might postulate that differences in the composition of Bruch's membrane between the macula and the peripheral retina could result in a more or less well-developed chemical and mechanical barrier between the RPE and the choriocapillaris in the peripheral part of the region [8]. This might lead to differences in the composition of the extracellular fluid and, subsequently, in the composition of the BLD. The OCZ in the peripheral retina contained only a few age-related deposits, such as curly membranes, vesicles of various sizes, dense granules and LSC, in contrast to the OCZ in the macula. This does not support a possible role of the choriocapillaris endothelial cells in the secretion of LSC in the peripheral retina, as was suggested for the macula [14].

The deposits in the macula as well as in the peripheral parts of the eye ultrastructurally seemed to consist of

basement membrane material, which was often continuous with the basement membrane of the RPE. In the macula these deposits were also found adjacent to and sometimes connected with the endothelial basement membrane of the choriocapillaris [14]. Recently, the presence of laminin in BLD in the macula has been described [10], supporting the hypothesis that it consists of basement membrane material.

The intracellular vacuoles in the RPE cells seemed to be filled with homogeneous material that was morphologically the same as that found directly outside the cytoplasm of the RPE cells (Fig. 7). This can be interpreted as the production and possible secretion of this material by the RPE cells. It can also be a misleading image of very large basal infoldings, that are sectioned obliquely. A few vacuoles were filled with membranous material, which can also be found in aged Bruch's membrane and in soft drusen [13].

The more electron-dense, irregularly shaped material (Fig. 6) has not been described in the macula. Perhaps this material condenses or polymerizes into the banded material, as is suggested in Fig. 3.

The sub-RPE deposits sometimes seemed to be lined by two basement membranes, one on either side, suggesting first the deposition of material between the RPE cell membrane and the basement membrane and afterwards the production of a new basement membrane on the RPE side (Fig. 8). This production of a new basement membrane has been explained as a defense mechanism of the RPE against macrophages and an attempt to repair breaks in Bruch's membrane [11].

The sub-RPE deposits in two of the ten eyes were located between the RPE basement membrane and the ICZ of Bruch's membrane. Ultrastructurally, they consisted of hard drusen, but they were flatter than normal and extended farther. Presumably, under the light microscope, these very flat drusen were confused with a BLD. Another possibility is that the deposits seen by light microscopy were not present in the adjacent tissue, embedded for electron microscopy.

From our observations we can conclude that the ultrastructure of the sub-RPE deposits in the peripheral retina is morphologically similar to that of basement membrane material. Relatively large amounts of this homogeneously stained basement membrane material are interspersed with some LSC, as in the early-type BLD in the macula. This hypothesis is further supported by the fact that a statistically significant correlation between the presence of a BLD in the macula and the occurrence of these sub-RPE deposits in the peripheral retina has been established [16]. Therefore, we postulate that the deposits found in the peripheral part of the retina can be classified as early-type BLD. Because of the almost exclusive localization of these deposits in the

peripheral retina, between the RPE plasma membrane and the basement membrane, they seem to arise from the RPE cells. The differences in composition between the deposits in the macula and those in the peripheral retina cannot be explained. Light microscopically, a BLD in the equatorial region can easily be confused with elongated, flat drusen. However, ultrastructurally differentiation of these drusen is easy.

References

1. Feeney-Burns L, Ellersieck MR (1985) Age-related changes in the ultrastructure of Bruch's membrane. *Am J Ophthalmol* 100:686-697
2. Feeney-Burns L, Gao Chung-Ian, Tidwel M (1987) Lysosomal enzyme cytochemistry of human RPE, Bruch's membrane and drusen. *Invest Ophthalmol Vis Sci* 28:1138-1147
3. Ghadially FN (1988) Ultrastructural pathology of the cell and matrix, 3rd edn. Butterworth, Stoneham, Mass, pp 1234-1241
4. Green WR (1985) In: Spencer WH. *Ophthalmic pathology*, vol II, 3rd edn. Saunders, Philadelphia, pp 841-843, 936-961
5. Green WR, Key SN (1977) Senile macular degeneration: a histopathologic study. *Trans Am Ophthalmol Soc* 75:180-254
6. Green WR, McDonnell PJ, Yeo JH (1985) Pathologic features of senile macular degeneration. *Ophthalmology* 92:615-627
7. Hogan MJ, Alvarado JA, Weddel JE (1971) *Histology of the human eye*. Saunders, Philadelphia, pp 154-182; 344-372
8. Killingsworth MC (1987) Age-related components of Bruch's membrane in the human eye. *Graefes Arch Clin Exp Ophthalmol* 225:406-412
9. Loeffler KU, Lee WR (1986) Basal linear deposit in the human macula. *Graefes Arch Clin Exp Ophthalmol* 224:493-501
10. Marshall GE, Konstas AGP, Reid GG, Edwards JG, Lee WR (1992) Type IV collagen and laminin in Bruch's membrane and basal linear deposit in the human macula. *Br J Ophthalmol* 76:607-614
11. Pollack A, Korte GE, Heriot WJ, Henkind P (1986) Ultrastructure of Bruch's membrane after krypton laser photocoagulation. II. Repair of Bruch's membrane and the role of macrophages. *Arch Ophthalmol* 104:1377-1382
12. Sarks SH (1976) Ageing and degeneration in the macular region: a clinico-pathological study. *Br J Ophthalmol* 60:324-341
13. Sarks JP, Sarks SH, Killingsworth MC (1988) Evolution of geographic atrophy of the retinal pigment epithelium. *Eye* 2:552-577
14. Van der Schaft TL, Bruijn WC de, Mooy CM, Ketelaars GAM, Jong PTVM de (1991) Is basal laminar deposit unique for age-related macular degeneration? *Arch Ophthalmol* 109:420-425
15. Van der Schaft TL, Bruijn WC de, Mooy CM, Ketelaars GAM, Jong de PTVM de (1992) Element analysis of the early stages of age-related macular degeneration. *Arch Ophthalmol* 110:389-394
16. Van der Schaft TL, Mooy CM, Bruijn WC de, Oron FG, Mulder PGH, Jong PTVM de (1992) Histologic features of the early stages of age-related macular degeneration: a statistical analysis. *Ophthalmology* 99:278-286
17. Yanoff M, Fine BS (1989) *Ocular pathology*, 3rd edn. Lippincott, Philadelphia, pp 301-305
18. Young RW (1987) Pathophysiology of age-related macular degeneration. *Surv Ophthalmol* 31:291-306