Alterations in the Distribution of Fibronectin and Laminin in the Diabetic Human Eye

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The distribution of fibronectin and laminin in diabetic human eyes was determined by indirect immunofluorescent techniques. The intense fluorescence suggests increased amounts of fibronectin and laminin in the diabetic internal limiting membrane (ILM). A double laminated pattern of fluorescence for both glycoproteins suggests structural abnormalities of the ILM of the posterior retina. Preretinal and subretinal proliferative tissues fluoresced strongly and diffusely with antifibronectin. This study indicates that in diabetic patients, the ILM, especially in the posterior retina, is biochemically and morphologically abnormal. Invest Ophthalmol Vis Sci 28:515–521, 1987

Fibronectin and laminin, two extracellular glycoproteins, have been believed to mediate the attachment of cells to their substrata, and to be responsible for cell-cell adhesion. Fibronectin is produced by many different cell types; it is found on the surface of most cells, in most extracellular matrices, as well as in plasma and other body fluids. Fibronectin binds with high affinity to interstitial collagens, proteoglycans, hyaluronic acid, and mesenchymal cells. In contrast, the distribution of laminin is restricted primarily to basement membranes, and binds most strongly to basement membrane (type IV) collagen. Posterior vitreous detachment (PVD) occurs frequently in aged and diabetic patients, probably as a result of some fundamental structural changes in the vitreous due to aging or other pathological conditions.

Our interest in the distribution of these glycoproteins in ocular tissues is based on the hypothesis that (1) fibronectin and laminin play a role in the attachment of the vitreous to the ILM and of the ILM to the Mueller cell processes; and (2) abnormalities of one or both of these glycoproteins may be a feature of the diabetic eye. Previously we have demonstrated in human and bovine eyes that fibronectin is present in the ILM as well as in the vitreous, whereas laminin is restricted to the ILM.

This article reports the distribution of fibronectin and laminin at the vitreoretinal interface in diabetic human eyes.

Materials and Methods

Diabetic Human Eyes

Seven eyes from diabetic patients ranging from 59 to 89 years of age were examined. The eyes were obtained through the Lions Doheny Eye Bank within 17 hr after death.

The unfixed eyes were cut in half in the horizontal plane and carefully examined with a dissecting microscope for the presence of posterior vitreous detachment (PVD) or other macroscopic changes. Total PVD was found in four eyes; partial detachment was seen in one eye with retinitis proliferans. In four eyes we observed pathological changes, i.e., retinitis proliferans, disseminated chorioretinal degeneration following panretinal photocoagulation, a soft white patch in the peripapillary region, and a solitary white mass in the equatorial region.

The procedures used for the indirect immunofluorescence were reported in full earlier.

Results

In order to avoid affecting the antigenicity and distribution of fibronectin and/or laminin, we used only unfixed tissues for immunofluorescence even though the morphology was not always well preserved. Because the intensity of immunofluorescence was not necessarily an index of quantity, we did not attempt to evaluate the intensity of staining quantitatively. However, based on our extensive experience with immunofluorescence of more than 50 postmortem human eyes,
fibronectin and laminin in these diabetic eyes stained more intensely than in aged, nondiabetic or in young, normal human eyes.  

The Vitreoretinal Interface

Fibronectin and laminin distribution was thoroughly examined in the retina from the optic disc to the ora serrata regions in all specimens except for one eye with retinitis proliferans.

One of the most prominent findings was a double laminated pattern of fluorescence in the ILM of the posterior retina (Figs. 1, 2). This pattern was seen for both fibronectin (three of six cases examined) and laminin (four of five cases examined). Although one eye (62-year-old male) showed no such pattern for either glycoprotein, fluorescence of the posterior ILM was intense and band-like.

The laminated pattern was somewhat different for fibronectin than for laminin. Fibronectin stained as an intense band of fluorescence both surfaces of the ILM with weak fluorescence in between the two intense bands (Fig. 1, left); laminin exhibited intense fluorescence on the retinal face of the ILM and a weak fluorescent line at the vitreous face (Fig. 2, left).

In some cases, the ILM of the optic disc stained as a thick fluorescent band for fibronectin, and as a double laminated line for laminin (Fig. 3).

At the light and electron microscopic level, all specimens showed pathological changes in the ILM, such as condensed peripheral vitreous that looked like laminated ILM, cells on the ILM, or epiretinal membrane, although in age-matched nondiabetic eyes such findings are infrequent. 17

In the equatorial and peripheral retina, a laminated pattern was never seen in the ILM. Occasionally the ILM showed a band-like pattern with intense fluorescence for fibronectin and laminin, especially in regions with subretinal proliferation (Fig. 4). In the peripheral retina, laminin exhibited an intermittent staining pattern at the ILM in the vitreous base (Fig. 5) such as is observed in the aged eye. 17

In the pars plana of the ciliary body, no significant differences were evident in diabetic eyes as compared with the normal aged eye: fibronectin was distributed on the cell surface of the nonpigmented epithelium.
Fig. 3. Fluorescent micrographs of the optic disc stained with antisera to fibronectin (FN) and to laminin (LM) in the eye of an 86-year-old diabetic male. The internal limiting membrane (opposed arrows) of the optic disc exhibits an intense fluorescent band for fibronectin (left) and a double laminated pattern for laminin (right). The walls of the central retinal artery (A) and vein (V) and the pial septa of the optic disc show positive staining for both fibronectin and laminin; the distribution pattern, however, is different. (Calibration bar = 20 µm).

Fig. 4. Fluorescent micrographs of the equatorial retina with subretinal proliferation stained with antisera to fibronectin (FN) or to laminin (LM) in the eye of an 86-year-old diabetic male. The internal limiting membrane (ILM) shows intense fluorescence for both fibronectin (left) and laminin (right). v: retinal vessel. (Calibration bar = 20 µm).

Fig. 5. Fluorescent micrograph of the peripheral retina and the vitreous base stained with antilaminin (LM) and a phase contrast micrograph of the same field (ph) in the eye of an 86-year-old diabetic male. The retina (R) is not well preserved due to peripheral cystic degeneration. The internal limiting membrane (ILM) shows an intermittent pattern of fluorescence for laminin; the peripheral vitreous (vit), is negative. (Calibration bar = 20 µm).
and laminin was restricted to the basement membrane of the nonpigmented ciliary epithelium. The basement membrane of the pigment epithelium reacted strongly with both antisera.

The vitreous did not stain with antilaminin (Fig. 5). The intensity of fluorescence for fibronectin was stronger than in the normal eyes (Fig. 6).

There was no definite difference in the pattern and distribution of fluorescence between eyes with and without vitreous detachment.

**Preretinal Proliferation**

In the eye with retinitis proliferans, preretinal proliferation, subretinal proliferation, and partial posterior vitreous detachment were observed. Only fibronectin distribution was examined in this eye.

Proliferative tissues showed bright fluorescence for fibronectin, which was as intense as that of the retinal vessel walls (Figs. 7, 8). Proliferative tissues partly adhered to the ILM (Figs. 7, 8), proliferated on the vitreous surface (Fig. 8), and appeared to be membranous or connective tissue-like. The ILM and the remaining peripheral vitreous to which proliferative tissues attached also fluoresced with antifibronectin (Fig. 8).

**Subretinal Proliferation**

Subretinal proliferation was observed in three eyes. Fibronectin was diffusely distributed in the proliferative tissues and bordered the vessel walls. Laminin stained as a fluorescent line bordering vessel walls and proliferative tissues (Fig. 9). The retinal pigment epithelial cells with granular autofluorescence were present in a tubular pattern in the proliferative tissues.
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Fig. 8. Fluorescent micrograph of the posterior retina with preretinal proliferation stained with antifibronectin (FN) and a phase contrast micrograph of the same field (ph) in the eye of a 69-year-old diabetic female. The internal limiting membrane (ILM) with strong fluorescence is partially detached from the retina (R). Preretinal proliferative tissues (PRP) and remaining vitreous (vit) also have positive fluorescence for fibronectin. (Calibration bar = 20 µm).

Retinal Vessels

Occasionally, the walls of retinal arterioles with arteriosclerotic changes were markedly thickened, and exhibited a lamellar arrangement of intense fluorescent lines with antisera to fibronectin (Fig. 10) and to laminin.

Discussion

Fibronectin and laminin distribution were increased in the skin and kidneys of diabetic patients. This is the first report on the distribution of fibronectin and laminin in diabetic human eyes. Although we did not use quantitative methods, fibronectin and laminin in the diabetic eyes stained more intensely than in aged nondiabetic or normal young human eyes, suggesting increased amounts of fibronectin and laminin in diabetic human eyes.

Aside from the more intense fluorescence, a double laminated pattern of fluorescence at the ILM of the posterior retina was a prominent finding not usually observed in normal young individuals. This double

Fig. 9. Fluorescent micrographs of subretinal proliferation stained with antiserum to fibronectin (FN) or to laminin (LM) in the eye of a 63-year-old diabetic male. Left, strong diffuse fluorescence for fibronectin is evident in the subretinal proliferative tissues (SRP), which are located on Bruch's membrane (Br). Right, the vessel walls (small arrows) in the subretinal proliferative tissues (SRP) are stained with antilaminin; however, the connective tissues do not fluoresce. Although Bruch's membrane (Br) does not fluoresce in this section, an intense fluorescent line for laminin is evident (large arrow) over the subretinal proliferative tissues, which may be a newly-formed basement membrane of the retinal pigment epithelium. The walls of choriocapillaris and choroidal vessels are positive for fibronectin and laminin. Ch: choroid. (Calibration bar = 20 µm).
by these cells and deposited between the ILM and these changes in diabetes mellitus, but there are few reports of such changes.25,26

Turbulence of the biosynthesis of extracellular matrix and basement membrane changes can be a part of more general disturbances unrelated to the capillary walls, and basement membrane thickening is known to take place in diabetic microangiopathy.22 This occurs in places unrelated to the capillary walls, and basement membrane changes can be a part of more general disturbance of the biosynthesis of extracellular matrix macromolecules in diabetes mellitus.23,24 The ILM, the basement membrane of Mueller cells, would be expected to show some morphological and biochemical changes in diabetes mellitus, but there are few reports of such changes.25,26

Fibronectin was diffusely distributed in preretinal and subretinal proliferations, while laminin was restricted to bordering the subretinal proliferative tissues and the vessel walls, a staining pattern very similar to that observed in the choroid. However, the significance of large amounts of fibronectin and laminin in these proliferative tissues remains unknown.

This study has shown that the posterior ILM of the diabetic retina has features similar to the senescent ILM,17 but much more prominent. Thus the results support the suggestion that changes which occur in the course of aging are accelerated in diabetics.27

Key words: fibronectin, laminin, internal limiting membrane, subretinal proliferation, preretinal proliferation, diabetes mellitus, indirect immunofluorescence

Fig. 10. Fluorescent micrograph of the posterior retina stained with antifibronectin (FN) in the eye of a 63-year-old diabetic male. The walls of a retinal arteriole (large arrow) are markedly thickened and show a lamellar arrangement of an intense fluorescent band. The internal limiting membrane also shows a laminated pattern (small arrow). (Calibration bar = 20 μm).

Laminated pattern of fluorescence at the posterior ILM was also found in some aged human eyes,17 but was more common and pronounced in diabetic eyes. However, in aged eyes the double laminated pattern was less prominent and was more frequently observed when the tissue was stained with antifibronectin than with antilaminin antibodies.

Immunoelectron microscopic studies have shown that both fibronectin and laminin are localized in the lamina lucida (or rara) of basement membranes9,20; however, the distribution of these glycoproteins in the ILM of the retina is not known. In the ILM of the posterior retina, remaining condensed peripheral vitreous, epiretinal membranes, and cells were observed. Fibronectin and laminin may have been synthesized by these cells and deposited between the ILM and these pathological tissues. On the other hand, this pattern may represent reduplication of this basement membrane, similar to what occurs in the walls of blood vessels.21

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