Fibronectin and laminin in Achilles tendon

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Ten intact and 22 ruptured Achilles tendons were studied by immunohistochemical techniques. In intact tendons, both fibronectin and laminin were found in vascular walls, myotendineal junction, and endomysium, but not in the connective tissue of the tendons. In ruptured tendons the distribution of laminin was not changed. Fibronectin deposits, on the contrary, could be detected on the tear surface and in the collagen fibers of the ruptured tendons.

Fibronectin is a high molecular weight glycoprotein located in basement membranes and vascular walls, as well as around muscle and mesenchymal cells (Kurkinen et al. 1980, Sanes 1982, Mayne and Sanderson 1985). It is also present in soluble form in plasma and other body fluids (Chen et al. 1976, Kuusela et al. 1978). In human fetal skin and lung, fibronectin occurs in large amounts; but it has not been previously detected in mature, dense connective tissue, such as tendon or aponeurosis (Linder et al. 1978).

A possible function of fibronectin may be to serve as a link between the cell membrane or basal lamina and the intercellular matrix (Mayne and Sanderson 1985). Fibronectin shows an extensive codistribution with Type III collagen in connective tissue (Lindner et al. 1978) and Type IV collagen in the basal membrane (Mayne and Sanderson 1985).

Laminin is a large glycoprotein (mol. weight 900,000) possessing functionally distinct areas, which interact with other basement membrane components and cell surfaces (Timpl et al. 1983). Immunohistochemical and immuno-electron microscopic studies have indicated that laminin and Type IV collagen are abundant proteins of the basement membranes (Foidart et al. 1980, Weber et al. 1982).

We report the immunohistochemical location of fibronectin and laminin in different regions of normal human Achilles tendon and in pathologic conditions, such as tendon rupture, tendinitis, and intratendineal ganglia.

Material and methods

Normal Achilles tendons were excised at autopsy from 10 accident victims aged 21 to 40 years. Specimens from degenerated and ruptured tendons were obtained from 22 patients aged 25–47 years who were operated on at the National Institute of Traumatology, Budapest, usually for acute rupture, but in 1 case for an actinomycotic abscess and in 1 case for an intratendineal ganglion.

The tendon samples were fixed in buffered 6 percent formalin solution (pH 7.4), embedded in paraffin, and cut in 5-µm longitudinal and transversal sections. Enzyme pretreatment of the sections were performed as described by Burns et al. (1980).

Immunoperoxidase staining for laminin was performed according to Ekblom et al. (1982) and for fibronectin according to Burns et al. (1980). Control stainings with phosphate-buffered saline (PBS) and human serum were negative. After the peroxidase antiperoxidase (PAP) reaction, the sections were stained with hematoxylin.

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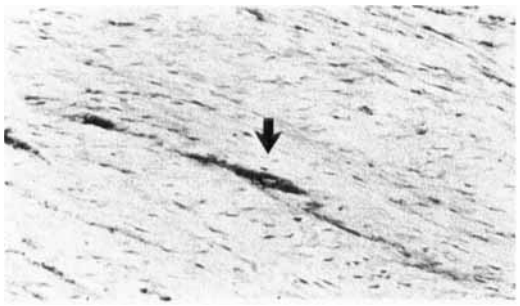

Figure 1. In the intact tendon, fibronectin is found in the walls of the vessels (arrow). PAP staining in Figures 1–6, x100.

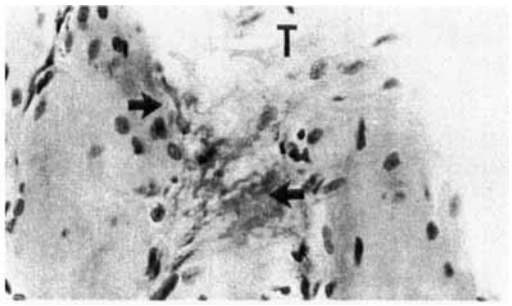

Figure 2. Marked laminin staining at the end of the muscle cells (arrows) in the myotendineal junction is visible, but not at the tendinous part (T) of the junction, x300.

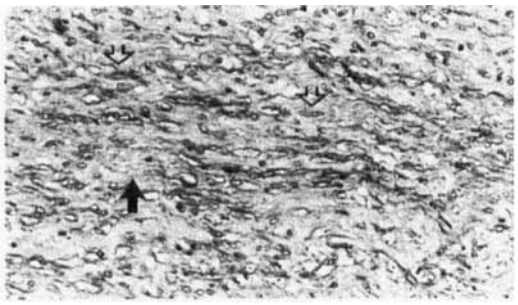

Figure 3. Fibronectin is found on the cell surface (open arrows) and collagen fibers (closed arrow), x150.

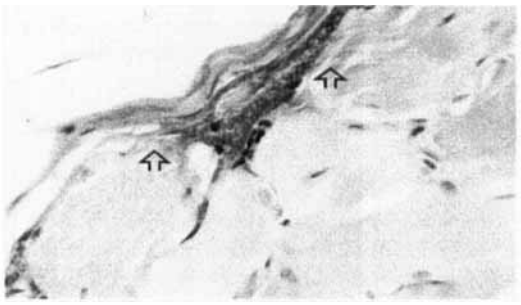

Figure 4. Fibronectin is seen at the ruptured myotendineal junction (arrows), x150.

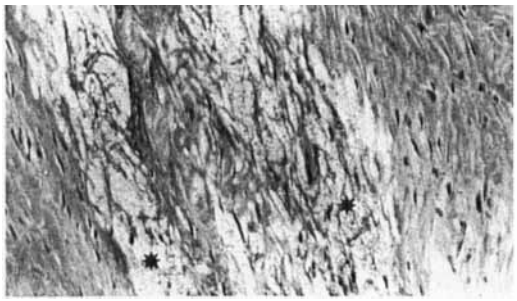

Figure 5. Marked fibronectin deposits in the degenerated mucous areas (asterisks) of the tendon, x200.

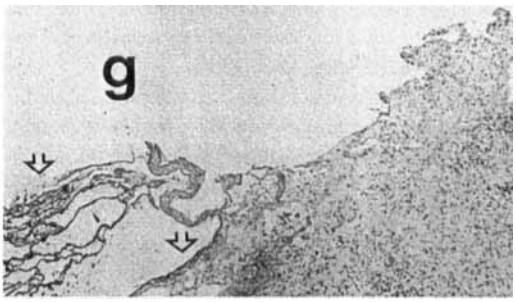

Figure 6. Fibronectin is observed on the internal surface (arrows) of the intratendineal ganglion (g), x100.

Results

In the normal Achilles tendon, fibronectin and laminin were found in the vascular walls (Figure 1). The anti-laminin antibodies produced intensive staining in the myotendineal junction (Figure 2). Laminin was also found in the endomysium, but not in the perimysium, which was clearly stained with the antifibronectin antibodies. The myotendineal junction showed fibronectin deposits.

On the tear surface of the ruptured tendons, fibronectin could be detected 6 hours after the rupture. In the other parts of the tendon, fibronectin was detected on the surface of the collagen fibers and tenocytes (Figure 3). When the tendon had ruptured at the myotendineal junction, fibronectin deposits were also seen in the neighboring areas (Figure 4). The degenerated mucous areas of tendons showed marked fibronectin deposits (Figure 5). The staining intensity of laminin and its location were similar in both the intact and the ruptured tendons.

No fibronectin was observed in the actinomycotic abscess of the Achilles tendon. However, around the abscess a moderate amount of fibronectin could be detected. In the case of rupture due to an intratendineal ganglion, the location of fibronectin was similar to that of the ruptured tendons (Figure 6).

Discussion

We suggest that the existence of fibronectin on the tear surface and on the collagen fibers in ruptured tendons may be due to extravasation of plasma proteins; a phenomenon we have previously demonstrated in Achilles paratendinitis (Kvist et al. 1988). In vitro experiments have shown that fibronectin binds more actively to denatured than to native collagen (Engvall et al. 1978, Kleinman et al. 1981). Recently, this has been demonstrated in vivo as well (Rucklidge et al. 1986). The similarity of staining patterns obtained by binding either fibronectin or its antibody to denatured Type I collagen provides further evidence that it is possible to localize partially degraded collagen (Rucklidge et al. 1986). The location of fibronectin in ruptured tendons in our study may thus indicate denaturation of the collagen fibers.

Our observation of a similar distribution of laminin in intact and ruptured tendons supports previous observations that laminin with Type IV collagen is an abundant protein in every basement membrane in the body (Foidart et al. 1980, Weber et al. 1982); laminin does not seem to play an important role in tendon pathology.

The existence of fibronectin in tendon tissue may be regarded as a sign of connective tissue degeneration.

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