Collagen ultrastructure in ruptured cruciate ligaments

An electron microscopic investigation

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The ultrastructure of collagen fibrils was investigated in normal (n 39) and ruptured (n 23) human anterior cruciate ligaments. The normal ligament had a complex three-dimensional structure. Collagen fibrils predominantly had a unidirectional course with parallel arrangement and a mean diameter of 75 (20–185) nm. Four days after anterior cruciate ligament rupture, the mean fibril diameter was increased; it later decreased, probably due to synthesis of young, thin 30–40 nm fibrils. Interfibrillar dysplastic collagen fibrils were detected in the extracellular matrix of ruptured ligaments. They were more frequently found later than 3 days after rupture and were seen also at a distance of 2–3 cm from the rupture zone. The presence of dysplastic fibrils may explain the functional insufficiency of the repair tissue in ruptured cruciate ligaments.

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Extraarticular ligaments such as the medial collateral ligament can heal effectively after injury (Woo et al. 1990), whereas intrasynovial ligaments such as the anterior cruciate ligament fail to heal adequately (Amiel et al. 1990b, Arnoczky 1991, Hefti et al. 1991). It has been suggested that a poor collagen synthesis in the ruptured anterior cruciate ligament may contribute to these differences in healing (Wiig et al. 1991).

We have studied time-dependent changes in the collagenous fibril systems after anterior cruciate ligament rupture by scanning and transmission electron microscopy.

Material and methods

Specimens from 23 complete ruptured anterior cruciate ligaments (12 men, 11 women) were taken during reconstructive ligament surgery. The average age of the patients was 31 (17–53) years. There were 10 ruptures in the proximal third, 8 in the middle, and 5 in the distal third of the ligament. Samples were taken both directly and at a distance of 2 to 3 cm from the rupture zone. The mean size of the samples was 2 × 6 mm for transmission electron microscopy and 5 × 14 mm for scanning electron microscopy. A time-dependent evaluation based on the interval between rupture and ligament repair was performed. In Group A (n 11) the interval from injury was less than four days and in Group B (n 12) the interval was four days or more.

As controls, 39 anterior cruciate ligaments without known degenerative or inflammatory changes were dissected up to 6 hours post mortem. These control knees (16 men, 23 women) were obtained from the Department of Pathology (University of Marburg). The average age of the controls was 35 (15–60) years.

For transmission electron microscopy, specimens were fixed in osmium tetroxide (1%) sodium cacodylate buffer (0.2M; pH 7.4), at 4 °C for 1 hour. Dehydration was performed with graded ethanols (50, 70, 80, 90, 95, 98, and 100%), and samples were embedded in Spurr's Epon. 50–90-nm ultra-thin sections were contrasted with uranyl acetate and lead citrate. An EM 10 Zeiss microscope was used.

For scanning electron microscopy, after an extended wash in PBS, tissues were osmicated for 2 hours, dehydrated in a graded series of acetone, then critical point-dried and sputter coated (Edwards S150) before examination on the upper stage of a Stereoscan MK 250 (Cambridge) and an ISI-SX 30 (Zeiss).

For statistical analyses, 200 collagen fibril diameters and 150 distances between the major bands were measured in each specimen using a semiautomatic Morphomat 10-analysis system (adapted on an EM 10) at a final magnification of 100,000 on a tracing board. Statistical comparisons between the different groups were made separately for fibril macroperiodicity and diameters by use of Wilcoxon's *U*-tests. All fibril data were used for multivariance analyses with the SAS-system 6.03.

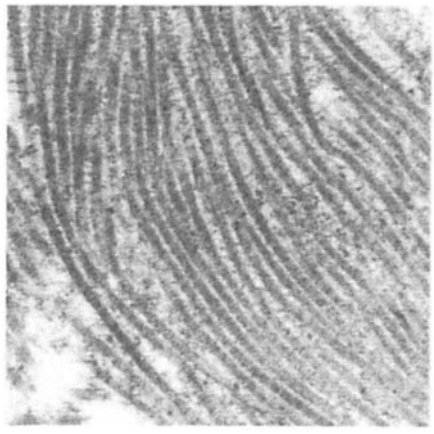

Figure 1. Densely packed collagenous fibrils with unidirectional course in a patient of the control group. Transmission electron microscopy, x17000.

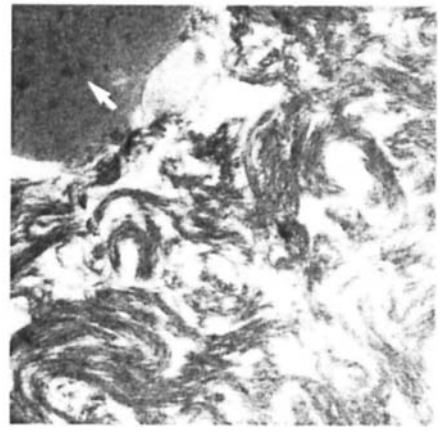

Figure 2. An erythrocyte (arrow) and abundant fibrin in a ruptured anterior cruciate ligament. Group A, x10000.

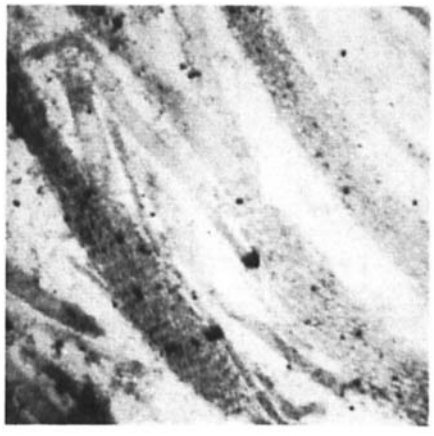

Figure 3. Three giant fibrils in group A near the rupture zone. Thin fibrils are visible between them, x32000.

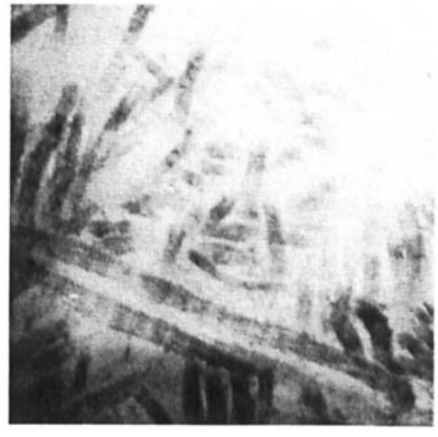

Figure 4. Dramatic changes of the interfibrillar collagen architecture directly near the rupture zone. Group A, x45000.

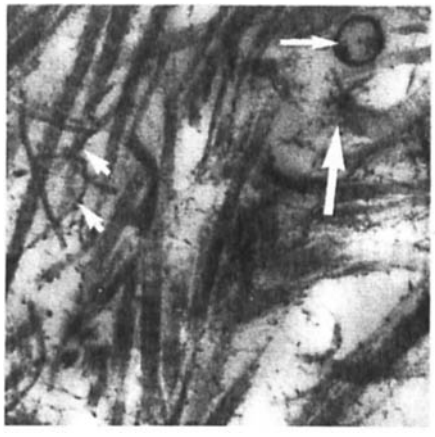

Figure 5. Interfibrillar dysplastic collagen fibrils (short arrows). Ruptured fibril (thick arrow) and a matrix vesicle (long thin arrow). 2cm distance to the rupture zone. Group B, x28000.

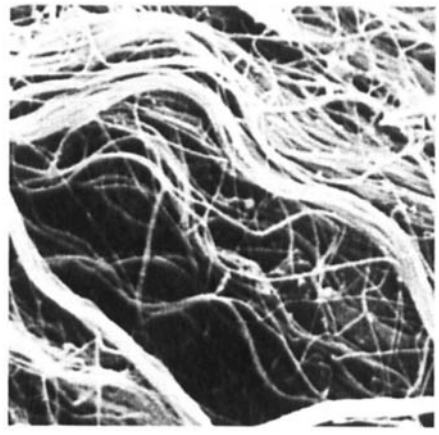

Figure 6. Loss of collagen density and more wavy course of the single fibrils. Scanning electron microscopic detection in a 3cm distance from the rupture zone. Group B, x5000.

Results

The normal ligaments, obtained from cadavers, consisted of twisted collagenous fascicles and fiber bundles. The small fibrils had predominantly a unidirectional course with parallel arrangement (Figure 1). The average fibril diameter was 75 (20–185) nm. The fibrils had a regular macroperiodicity; the mean distance between the major bands was 58 (54–64) nm.

In the injured ligaments, fibrin and erythrocytes were abundant at the site of the injury (Figure 2). In all the cases, the matrix substructure was markedly altered. In Group A, atypical giant fibrils were detected near the rupture (Figure 3). Whereas the fibrillar macroperiodicity was normal, the mean fibril diameter was 88 (20–310) nm and therefore increased ($P < 0.01$) in comparison with the control group. In Group B, 22 percent of all fibrils were young, 30–40 nm thick with significantly reduced ($P < 0.01$) diameters—mean 71 (20–290) nm. The distances between the major bands in Group B were nearly unchanged.

Dramatic changes in the arrangement between the single collagenous fibrils occurred in the matrix, also called interfibrillar dysplastic collagen fibrils (Figure 4). They were predominantly located in vesicle-rich areas and were seen not only exactly in the rupture zone but also at a distance of 2 to 3 cm from the rupture (Figure 5). The content of dysplastic fibrils in Group B was higher than in Group A. However, in both groups these severe changes in collagen substructure were also observed on the surfaces of the ligaments (Figure 6). The multivariance analyses for fibril macroperiodicity and fibril diameters confirmed differences between the control group, Group A, and Group B (Wilks lambda < 0.1).

Discussion

Collagenous systems of the cruciate ligaments may be classified into "guiding" and "limiting bundles" (Fuss 1989, 1991). They are subdivided into fascicles, subfascicular units, fibers and fibrils (Haus and Refior 1987, Dye and Cannon 1988, Yahia and Drouin 1989, Hart et al. 1992). The anterior cruciate ligament has thinner collagen fibrils (Neurath et al. 1991b) in comparison with the posterior ligament. This finding means that the posterior ligament is stronger than the anterior (Kennedy et al. 1976), because thicker collagen fibrils withstand greater tensional forces than thinner fibrils probably because of the increased intrafibrillar, covalent cross links (Craig and Parry 1981). After ligament rupture, a time-dependent pattern of the collagen fibril diameters was observed. The increase in the average fibril diameter within the first three days after rupture is caused by atypical giant fibrils near the rupture zone. This period is followed by synthesis of thin, young collagen fibrils with reduction in the average fibril diameter. The 30–40 nm diameter of these fibrils is similar to the fibrils observed near Dacron prostheses (Salvi et al. 1991) in the anterior cruciate ligament.

The most striking alterations in the overall architecture of the ligaments after rupture were changes in the interfibrillar collagen arrangement. Interfibrillar dysplastic collagen fibrils have been described in degenerative vessel diseases (Staubesand and Fischer 1979), chronic pancreatitis (Neurath et al. 1991a), osteoarthritis (Neurath et al. 1992), rheumatoid arthritis (Neurath et al. 1991b), and in tendons after treatment with an anabolic steroid hormone (Michna 1986, 1987). It is suggested that collagen dysplasia may be due to the appearance of calcifying matrix vesicles released from fibroblasts (Bonucci 1981), and that dysplastic fibrils may not withstand great tensional forces. The appearance of dysplastic fibrils may therefore be due to collagenase-enzyme production of the ligament fibroblast itself (Wilhelm et al. 1986). Another possibility concerning their origin is that the injury to the protecting synovial sheath exposes ligamentous substance to the degradative effects of synovial proteases (Konttinen et al. 1991, Amiel et al. 1990a). However, these fibrils lead to a functional insufficiency in the regenerating tissue after the anterior ligament ruptures. This hypothesis explains the poor healing capacities of the anterior cruciate ligament.

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