

Age-related blood and lymph supply of the knee menisci

A cadaver study

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We investigated 20 human menisci (22 weeks of gestation-80 years) immunohistochemically with an antibody against laminine in the basement membrane. Differentiation of blood vessels and lymphatics was possible with histochemical proof of alkaline phosphatase and 5' nucleotidase in the endothelium.

Blood vessels entered the menisci from the joint capsule accompanied by loose connective tissue. In the 22nd gestational week, vessels could only be found in the lateral third. At the time of birth, nearly

the whole meniscus was vascularized. In the second year of life, an avascular area developed inside the inner circumference. In the second decennium, blood vessels occurred only in the lateral third. After 50 years of age, only the lateral quarter of the meniscal base was vascularized. The dense connective tissue of the insertion was vascularized, but not the fibrocartilage of the insertion. Blood vessels were accompanied by lymphatics in all areas.

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Submitted 94-10-22. Accepted 95-02-21

Observations on the vascularization of the menisci are based on light microscopy and injection techniques (Ishido 1923, Tobler 1933, Raszeja 1938, Reinbach 1954, Scapinelli 1968, Amoczky and Warren 1982, Danzig et al. 1982, Amoczky and Warren 1983, Clarke and Odgen 1983, Day et al. 1985, Amoczky 1992). Injection techniques visualize the vascularization only if the vessels are filled and the absence of vessels may be caused simply by insufficient filling. Detection of blood vessels inside dense connective tissue is difficult with a light microscope, if the material has been fixed in one piece, as collapsed vessels are hardly visible.

An indirect method has proved to be more successful for the detection of blood vessels. The immunohistochemical detection of the basement membrane components laminine and type IV collagen allows identification of blood vessels (Korn and Schünke 1989, Tillmann et al. 1991, Rudert and Tillmann 1993). We investigated the blood and lymphatic supply of fetal, juvenile and adult menisci.

Material and methods

20 medial and lateral human knee joint menisci were obtained at routine autopsies from subjects of different ages and sexes (Table 1). The menisci were dissected according to Figure 1.

Frozen sections (8-16 µm) from each specimen (Figure 1) were cut in a cryostat and mounted on gelatin-coated slides. Rabbit anti-laminine (6 mg/mL,

Table 1. Age-related blood and lymph supply of the menisci of the knee

No.	Age	Sex	Vascularization
1	22 weeks of gestation	f	a
2	newborn	m	b
3	11 months	m	b
4	18 months	m	a
5	18 years	f	a
6	50	m	c
7	54	f	c
8	55	m	c
9	60	m	c
10	62	f	c
11	63	f	c
12	65	f	c
13	65	m	c
14	67	m	c
15	72	f	c
16	75	f	d
17	77	f	d
18	80	m	d
19	80	f	d
20	80	f	d

a vessels in the outer third
b completely vascularized
c vessels in the outer quarter
d vessels in the outer margin

E-Y Laboratories, San Mateo, CA, U.S.A.) served as a specific antibody for the immunofluorescent technique. Sections were washed in phosphate-buffered saline (PBS, pH 7.2-7.4). Following incubation with the antibody (diluted 1:20, 45 min at room temperature, in a moist chamber), the sections were rinsed three times with PBS. Then they were incubated for 30 min with fluorescein isothiocyanate (FITC)-conjugated anti-rabbit IgG ([1:30], diluted with human serum [1:20]), rinsed again 3 times with PBS and mounted with PBS/glycerin (9.1 in 2.5% NaN₃, pH 8.6). Sections incubated with FITC-conjugated antibodies alone served as controls to exclude artefacts due to nonspecific binding of the antibody. Basement membranes of muscle fibers of skeletal muscle tissue that showed a positive immunoreaction served as tissue controls.

To demonstrate lymphatics with the 5' nucleotidase reaction (Vetter 1970), the sections were fixed in 4% formaldehyde for 2 min, incubated for 35 min at a temperature of 37 °C in a medium modified after Heusermann (1979) containing 5 mM MgSO₄, 2 mM Pb(NO₃)₂, 135 mM saccharose and 2 mM AMP in 0.2 mM trismaleate buffer, pH 7.2. To visualize blood vessels, the demonstration of 5' nucleotidase activity was followed by the alkaline phosphatase reaction. Histochemical visualization of alkaline phosphatase was achieved by incubation in a medium containing 25 mg naphthol AS-BI dissolved in 0.5 mL N,N-dimethylformamide added to 50 mL 0.1 M tris-HCL buffer, pH 9.2, containing 50 mg Fast Red TR for 60 min at a temperature of 37 °C (Werner et al. 1987). Lymphatics could be identified by their brown staining, which was caused by a dark precipitation of lead sulphide that indicates activity of 5' nucleotidase (Figure 3). Since blood capillaries and vessels showed no such activity, they could easily be distinguished from lymphatics by using this method. Blood vessels were distinguishable from lymphatics by the red color of alkaline phosphatase, which is the product of the chemical reaction.

Slides were examined with a Zeiss fluorescence microscope. Micrographs were taken using Agfachrome 1000 RS films and Kodak T-MAX 400 films for immunohistochemistry and histochemistry, respectively.

Results

The immunohistochemical analysis of laminine inside the wall of meniscal blood vessels was positive in all investigated cases (Figure 2). Blood vessels run from the joint capsule via connective tissue septa into

Figure 1. Site of the investigated areas in the medial and lateral meniscus. Cornu anterior (a), cornu posterior (p), middle segment (m) and area of the subpopliteal recess (sp).

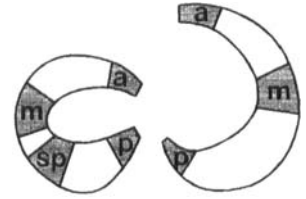


Figure 2. Demonstration of blood vessels with an antibody against the glycoprotein laminine as a component of the basement membrane (x 360).

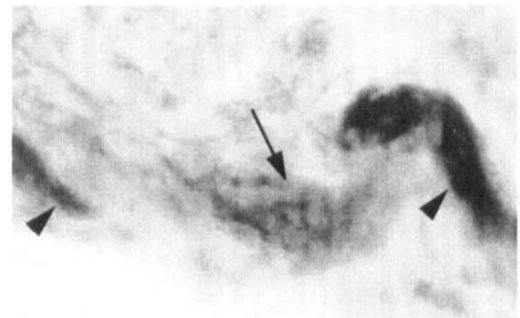


Figure 3. Selective histochemical demonstration of blood vessels and lymphatics. Lymphatics (arrow) can be distinguished by the brown appearance of the reaction product of 5' nucleotidase. Blood vessels (arrowheads) are stained red, due to the reaction product of alkaline phosphatase (x 360).



Figure 4. Immunohistochemical demonstration of blood vessels in a fetal meniscus (22nd week). Vessels can be detected only in the lateral third.

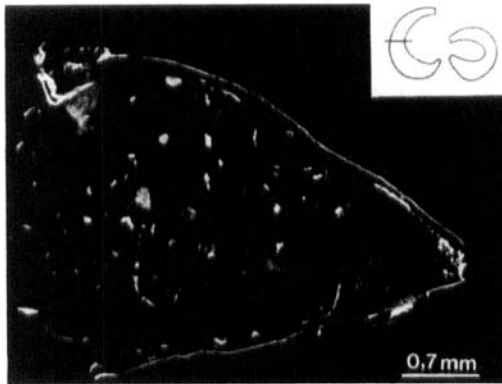


Figure 5. In the meniscus of a newborn, vessels can be demonstrated in all areas with positive immunoreactivity with an antibody against laminine (montage). The meniscal surface is demonstrated by nonspecific fluorescence on the cutting edges (see Figures 6 and 8).

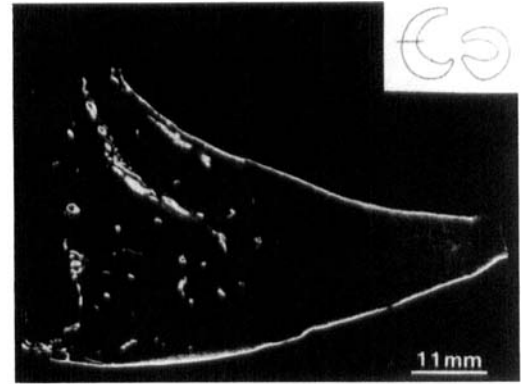


Figure 6. Immunohistochemical demonstration of blood vessels in a 1.5-year-old child with an antibody against laminine (montage). The inner circumference is avascular.

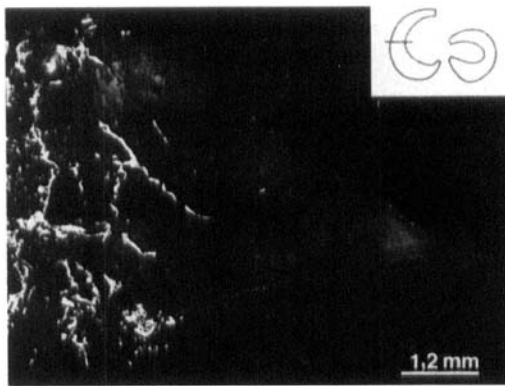


Figure 7. Immunohistochemical demonstration of blood vessels in an 18-year-old woman with an antibody against laminine (montage). Vessels enter the outer meniscal third from the joint capsule.

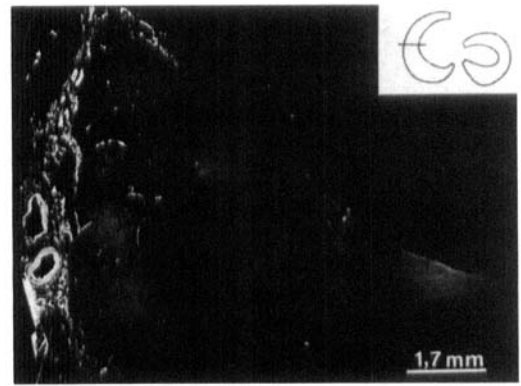


Figure 8. Immunohistochemical demonstration of blood vessels in a 50-year-old man with an antibody against laminine (montage). Nearly a quarter of the meniscus is vascularized.

the menisci in a centripetal direction.

In a fetus (22 weeks), blood vessels could only be detected in the outer third of the meniscus (Figure 4). In a newborn and in an 11-month-old child the immunohistochemical proof was positive in all areas (Figure 5). In a 1.5-year-old child, the inner circumference was avascular (Figure 6). In an 18-year-old woman, vessels could be detected only in the outer third of the menisci (Figure 7). Vessels were found only in the area close to the joint capsule in all individuals examined after the age of 50 (Figure 8). The part of the lateral meniscus facing the popliteal recessus showed fewer vessels than areas adjacent to the joint capsule. The tendon-like collagenous connective tissue of the attachment sites of the anterior and posterior cornu was vascularized regardless of age. At the insertion of the menisci on the bony tibial plateau, the fibrocartilage inside the inserting structures was avascular (Figure 9).

Histochemical investigations showed that lymphatics generally occurred together with blood vessels (Figure 3). The distribution of lymphatics inside the meniscus is subjected to the same age dependency as are blood vessels. In the first year of life, lymphatics reached the inner meniscal circumference, whereas supply of the outer meniscal third was observed only in adults.

Discussion

Controversy about the blood supply of human menisci is caused by the different methods used. Conventional light microscopy and injection techniques are inadequate because of false negative or false positive results (Korn and Schünke 1989, Tillmann et al. 1991, Rudert and Tillmann 1993, Petersen and Tillmann 1994), but immunohistochem-

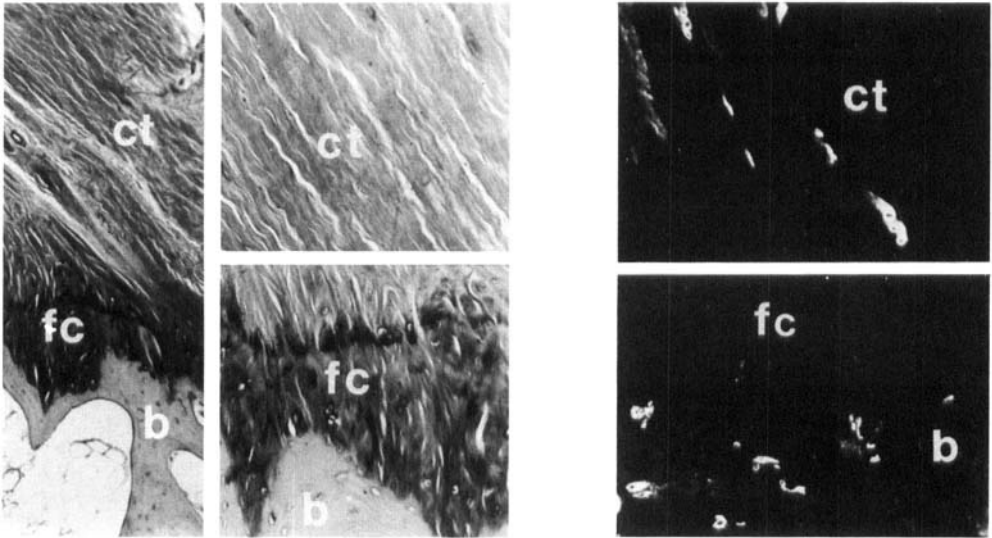


Figure 9. Case 8, 55-year-old man.

A. Histological section through the insertion of the anterior cornu, toluidine blue. Fibrocartilage (fc) is located at the insertion in the bone (b) of the tibial plateau (left). The fibrocartilage located next to the bone is mineralized ($\times 45$). Detail from the area of the dense connective tissue (ct) of the insertion (upper right; $\times 180$) and from the insertion close to the bone (lower right; $\times 180$).

B. Immunohistochemical demonstration of blood vessels in the area of the dense connective tissue (ct) of the insertion (upper; $\times 180$) and in the attachment zone close to the bone (lower; $\times 180$) with an antibody against laminine. Vessels are observed only in the dense connective tissue and in the bone (b). The fibrocartilage (fc) is avascular.

ical staining of basement membrane components with antibodies against laminine or collagen type IV is a reliable method to detect blood vessels (Timpl et al. 1979).

Contrary to what Ishido (1923) reported in the newborn, the whole meniscus was supplied by blood vessels. Our observations in the adult meniscus agree with most reports (Ishido 1923, Tobler 1933, Raszeja 1938, Reinbach 1954, Scapinelli 1968, Arnoczky and Warren 1982, 1983, Danzig et al. 1982, Clarke and Odgen 1983, Day et al. 1985, Arnoczky 1992). It is clinically important that in the widest area of the medial meniscus 90 percent of the tissue is avascular. Slany (1942) observed mucoid degeneration most often in the posterior part of the medial meniscus; Müller (1982) held a particularly poor supply of nutrients responsible for this. We confirmed the hypovascularized area at the meniscal base (Arnoczky and Warren 1982, 1983, Danzig et al. 1982, Day et al. 1985, Arnoczky 1992) in the area of the popliteal recessus. Using arterial injection techniques, a complete blood supply of the insertions of the anterior and posterior horn has been shown (Arnoczky and Warren 1982, 1983, Danzig et al. 1982, Arnoczky 1992). These results are not in agreement with ours, which showed an avascular fibrocartilaginous area at the attachment site. This may be

clinically important for the development of degenerative changes and for the healing of meniscal transplants (Kohn and Moreno 1994). Body weight and knee joint motion have been suggested as the cause of this absence of vessels in the inner and middle aspects of the menisci (Arnoczky 1992). Nutrition of meniscal tissue occurs via diffusion from blood vessels and via convection from synovial fluid of the joint cavity. Conditions for the nutrition via convection is the intermittent load and release of the articular surfaces which are stressed by the resultant of body weight and muscle force (Pauwels 1976). The mechanism is comparable to the nutrition of articular cartilage (Benninghoff 1925). In the early fetal period, meniscal tissue seems to be nourished via diffusion from vessels of the meniscal base. A marked increase of meniscal volume between the late fetal period and birth requires a spread of blood vessels, as nutrition via convection from the synovial fluid is missing because the knee is unloaded. The avascular zone in the inner area of the meniscus that develops in the second year of life may be caused by the increasing load that acts on the knee joint due to bipedal gait. Nutrition of these areas then occurs via convection.

Detection of lymphatics or their differentiation from blood vessels inside connective tissues is often impossible with a light microscope. A simple and

selective test showing lymphatics can be performed histochemically by using the activity of 5'-nucleotidase in lymphatic endothelium (Vetter 1970, Werner et al. 1987). In accordance with reports by Rudert and Tillmann (1993) about human intervertebral discs, lymphatics occur only together with blood vessels. Lymphatics carry away interstitial fluid, macromolecules and cells. Apart from a nourishing function, structured water inside the menisci also serves a biomechanical purpose. Meniscal deformation due to stress causes a shift of fluids inside the tissue (Mow et al. 1992). A part of the interstitial fluid is pressed into the joint cavity and lubricates the joint (Mow et al. 1992). Due to the wedge form of the meniscus, compression causes a decrease in resistance and a shift of fluids to the meniscal base. In this area a transportation of fluids via the lymphatics is possible.

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