

Lymph and blood supply of the human intervertebral disc

Cadaver study of correlations to discitis

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Immunohistochemical (antibodies against laminin) and histochemical methods (Ulex europaeus lectin, 5'-nucleotidase activity) were used to describe the vascular pattern of human intervertebral discs and the surrounding tissue at different ages. Blood and lymph vessels were found in the connective tissue outside the annulus in all age groups. In the annulus blood vessels and lymphatics were detected up to 20

years of age, in the cartilage end-plate blood vessels appeared up to 7 years of age (cartilage canals). In the nucleus pulposus neither blood nor lymph vessels could be seen at any age. The occurrence of blood and lymph vessels in growing intervertebral discs help us to understand childhood discitis without simultaneous affection of the vertebral body.

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A consensus has still not been reached about the vasculature of the intervertebral disc and its adjacent tissues. Some authors describe the disc as being avascular throughout life (Ferguson 1950, Ratcliffe 1980, Whalen et al. 1985), others find a good vascular supply in fetal and neonatal discs that subsequently disappears after 2 years (Töndury 1958) and 4 years (Taylor et al. 1992) to 30 years (Übermuth 1929, Böhmig 1930). Since common histological techniques are not sufficiently accurate to recognize blood vessels up to the capillary regions, immunohistochemical and histochemical techniques were used. Antibodies against the basement membrane component laminin served as a marker for the identification of vessels (Timpl et al. 1979). This method was confirmed using Ulex europaeus (UEA I) lectin histochemistry to visualize sugar-binding sites in vascular endothelial cells (Lis and Sharon 1986).

Proper identification of lymph vessels and especially their distinction from small blood vessels is difficult. This might be the reason why lymphatics in the region of the intervertebral disc have been neglected, except in a few earlier studies (Brack 1929, Brettschneider 1952). To visualize and differentiate lymph vessels we used a modified histochemical method based on the 5'-nucleotidase activity of their endothelium (Werner et al. 1987).

Material and methods

Lumbar intervertebral discs L2-L4 were obtained from 23 autopsied subjects of different ages and sexes (females: week 27 of pregnancy to 90 years, n 12; males: 6 weeks to 84 years, n 11). All discs and vertebral bodies of the lumbar spine were removed en bloc and the discs were separated with their contiguous regions in following preparations. Each disc was bisected in the median sagittal plane. One half was prepared for paraffin embedding and the other one was frozen immediately in liquid nitrogen. The discs were dissected to obtain horizontal and sagittal sections (Figure 1). Investigations had to be restricted to the

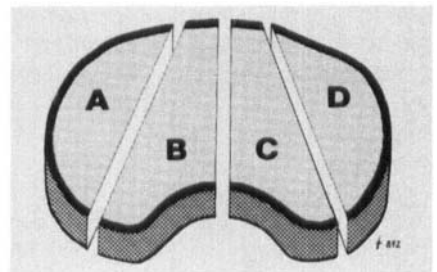


Figure 1. The macroscopic dissection of the disc in four specimens. Specimens A and C were cut horizontally, B and D were cut sagittally for microscopy.

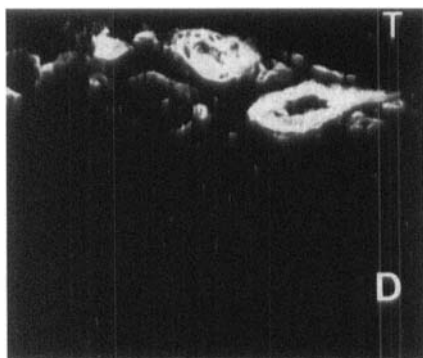


Figure 2. Connective tissue (T) adjacent to the intervertebral disc (D) of a 42-year-old individual (horizontal section, $\times 50$). The detection of laminin by antibodies labeled with FITC revealed blood vessels restricted to the connective tissue outside the annulus fibrosus.

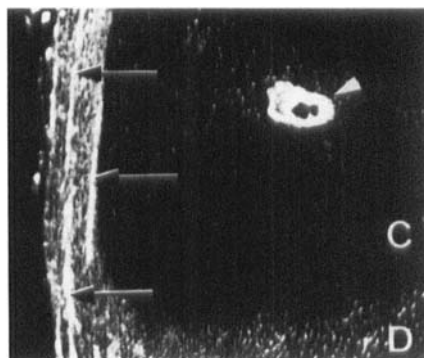


Figure 3. Sagittal section through the cartilage end-plate (C) and a part of the disc (D) of a fetus (36 wop, $\times 13$). Vascular structures were visualized by anti-laminin antibodies. Note the well outlined cartilage canal (arrowhead) and vascular structures in the perichondrium (arrows).

dorsal tissue parts of some specimens, since ventral parts of the disc were not available in necropsies of elderly individuals. For immuno- and histochemical methods, frozen samples were sectioned at 8–16 μm in a cryostat and mounted on gelatin-coated slides. To visualize the basement membrane component laminin, sections were incubated for 45 min with rabbit anti-laminin purchased from MEDAC, Hamburg/FRG (6 mg/mL diluted 1:20, Product of E-Y Laboratories, San Mateo, CA, U.S.A.) and labeled with fluorescein isothiocyanate (FITC)-conjugated anti-rabbit IgG (1:30, diluted with human serum (1:20)). To exclude artefacts due to nonspecific binding of the antibody, sections incubated with FITC-conjugated antibodies alone served as controls. Further controls using tissues with definite antigen sites (liver and skin) were conducted. For lectin histochemistry, sections were incubated with the lectin UEA I-FITC (*Ulex europaeus* agglutinin labeled with fluorescein isothiocyanate; gorse seed; 1 mg/mL; E-Y Laboratories) for 30–45 min in a moist, dark chamber, according to the method described by Schünke et al. (1988). Specific binding of the lectin was checked in control experiments by combining the lectin solution with the corresponding sugar L-fucose prior to incubation (Goldstein and Hayes 1978). Detection of lymphatics was conducted by applying the 5'-nucleotidase activity technique described by Werner et al. (1987).

Slides were examined with a Zeiss-fluorescence-microscope (Photomikroskop II). Micrographs were taken using Agfachrome 1000 RS films for immuno-histochemistry and Kodak Ektachrome 160 T films for histochemistry.

Results

Concerning the vascular supply of intervertebral discs, a typical, age-related pattern of vessels was revealed. The immunohistochemical detection of basement membrane with anti-laminin as well as the histochemical visualization of endothelial sugar-binding sites with UEA I showed a good vascularization, i.e., a broad fluorescent staining of vascular structures of the peridiscal tissue in all age groups (Figure 2).

The nucleus pulposus proved avascular throughout life. In young individuals up to 7 years of age, vessels penetrated into the cartilage end-plate emerging from the marrow space of the adjacent vertebral bodies (Figure 3). They accumulated in areas where the end-plate is thick, i.e., towards the margins of the cartilage. Cartilage canals did not stay in direct contact with the intervertebral disc itself. In our investigations the annulus fibrosus was supplied by vessels up to the age of 20 years (Figure 4). These vessels diminish from the inner parts to the outer annulus during aging. In older individuals from the fourth decade on, no vascular structures were detectable in the annulus.

Lymphatic endothelium presented as brown lead sulfide precipitates, applying the histochemical staining method for 5'-nucleotidase activity (Figure 5). They accompanied most of the small blood capillaries according to their age-related distribution.

Discussion

Authors who found that the vascular supply of the intervertebral disc had disappeared in early childhood, believed this period to be the beginning of disc degen-

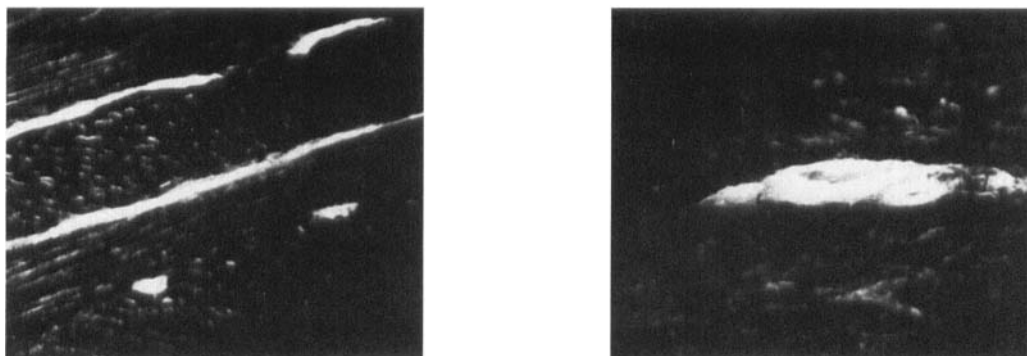


Figure 4. Horizontal section through the annulus fibrosus of a 6-week-old individual representing the dorsolateral region of the disc (left; $\times 25$). In between the obliquely criss-crossing laminae, vessels are marked by antibodies against laminin. Higher magnified view of vessels in the lateral aspect of an annulus fibrosus of a 1.5-year-old individual (right; laminin, $\times 50$).



Figure 5. Typical endothelial lining of collapsed lymphatics detected by the 5'-nucleotidase activity method in a horizontal section of an annulus fibrosus of a 1.5-year-old individual. Lymph vessels marked by dark lead sulfide precipitates (arrows) accompany small blood vessels not visualized by this method ($\times 25$).

eration (Töndury 1958, Taylor et al. 1992). It is generally accepted that adult discs are mainly nourished by diffusion through the cartilage end-plates, and some investigators could demonstrate a decrease in permeability of aging end-plate cartilage (Brodén 1954-55, Nachemson et al. 1970). In addition, Bernick and Caillet (1982) described age changes in the vasculature of the bone adjacent to the hyaline cartilaginous plates and disc. Those age changes would impede the passage of nutrients to the intervertebral disc and lead to degenerative processes.

In the cartilaginous end-plates from individuals between the 27th week of pregnancy to 7 years, blood vessels were detectable with immunohistochemical and histochemical methods. These cartilage canals predominated during a period of time when the growing end-plate is considerably thicker than the adult one, therefore representing a longer diffusion distance from bone to disc tissue. In the same age group, ves-

sels were found in the outer annulus leading to the assumption that a higher consumption of nutrients and oxygen during maturation necessitates a better vascular supply. On the other hand, the supply of adult intervertebral discs seems to be adequate until degenerative changes in the cartilage end-plates occur.

Lymphatics were detected in areas where small blood vessels supplied the disc and adjacent tissues. Their intercellular junctions permit rapid passage of lymph from the surrounding connective tissue due to an incomplete or absent basal lamina. Whether this mechanism contributes to the plastic deformation of the disc during the day under load, with recovery during the night or at rest still needs to be elucidated.

Disc space infection (discitis) is an inflammatory lesion of the intervertebral disc in children and adolescents. The infection begins in one of the contiguous vertebral bodies or end-plates, and the disc is infected secondarily, or the disc is infected primarily by blood-born bacteria (Scoles and Quinn 1982). Thus, the persisting presence of vessels in the outer annulus fibrosus and in the cartilage end-plates in infants and children as a site of entry for septic microemboli could be an important factor in the pathophysiology of disc infection.

Whalen et al. (1985) support these considerations, at least regarding the cartilaginous end-plate. With the exception of degenerated discs, the term discitis should, therefore, only be used in children when the intervertebral disc still has an intrinsic vascularization. Ratcliffe (1985), who believed the disc to be avascular, mixed the terms discitis and vertebral osteomyelitis caused by a metaphyseal infection. Even when the disc itself is involved during vertebral osteomyelitis at an early stage, the term spondylitis should be preferred in this case. Although the outcome of both kinds of infection may be the same, one has to regard both diseases to be different concerning their origin.

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