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Comparison of joint degeneration models

Surgical instability and repetitive impulsive loading

We used surgical instability and repetitive impulsive loading in rabbits to initiate degenerative changes in knee joints. Synovial membrane and cartilage samples were examined by light and electron microscopy. Early synovial inflammation at three days postoperatively preceeded cartilage destruction in the instability model. Synovial inflammation was only apparent after eight weeks in the loading model and increased subsequently to cartilage destruction. Cartilage breakdown was focal and limited to the weight-bearing area. Comparison of the histological data of the two arthrosis models suggests that different inductive mechanisms may be involved in cartilage degeneration, but in both models the inflammatory changes appeared to be secondary to mechanical factors.

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Introduction

Several experimental models are used to study osteoarthrotic change in joints (Hulth 1982). However, the etiology of the disease is still obscure. There are conflicting views about the role of the synovial membrane in the onset of degenerative cartilage destruction (Arnoldi et al. 1980, Goldenberg et al. 1982, Griesen et al. 1982, Pelletier et al. 1985). This paper contains observations of histological changes in knee joints subjected to instability using the method described by Hulth et al. (1970) (Hulth-model) and those subjected to repetitive impulsive loading (Simon et al. 1972) (RIL-model) in order to clarify the extent and role of the synovial membrane in these two joint degeneration models.

Materials and Methods

Twelve healthy, skeletally mature white New Zealand rabbits underwent surgical destabilization of their right knee joints by cutting the cruciate ligaments, the medial collateral ligament, and by removal of the medial meniscus (Hulth et al. 1970). The left knees served as controls. The animals were exercised by being allowed to hop around in a wide room for twenty minutes per day after the third day postoperatively. They were killed in groups of two at three days and one, two, four and twelve weeks.

An additional ten rabbits received repetitive impulsive loading of their right knees (1 Hertz for 60 minutes/day and five days a week), using a method described previously (Simon et al. 1972). In contrast to the previously described protocol, loads in the proximal tibiae were measured at only one times body weight, due to no stabilization of the hip and flexion of the knee during the loading cycle. The left legs of these animals were held in sham splints and received no impulsive load. Four animals were killed after four weeks; the remainder were killed in groups of two at six, eight and twelve weeks.

Synovial membrane samples were taken from two different sites in the knee joint, supra- and lateral patellar. From the medial tibial plateau and the underlying subchondral bone plate, a rectangle of six millimeters by total width of the medial plateau was removed and divided into five sample blocks. Synovial membrane and cartilage specimens were fixed for four hours in 4% glutaraldehyde and embedded conventionally in Epon. Semithin sections of one micrometer thickness were stained with 1% toluidin blue for light microscopy. Thin sections were stained with uranyl acetate and lead citrate for electron microscopy.

Cartilage samples were examined by light and electron microscopy for evidence of degenerative changes. Synovial membrane samples were viewed both qualitatively and semiquantitatively. Four slides for each sample location were evaluated. Intimal hyperplasia, evidenced by cell layers, cell shape and size was graded from + to ++++. The following grading scale was used: + = normal appearing synovial membrane, mostly monolayered intima; ++ = moderate hypertrophy of the intima, increase in subsynovial fibrous tissue; +++ = multilayered intima, increase in cell size and in subsynovial fibrosis; ++++ = pronounced hyperplasia with increase in synovial cell size and number, bottleneck shaped synoviocytes, increase in subsynovial fibrosis.

Tabel 1. Grading of synovial hyperplasia

	HULTH - MODEL				
	3 Days	1 Week	2 Weeks	4 Weeks	12 Weeks
Experiment	++	+++	++++	++++	+++
Control	+	+	+	+	+

	RIL - MODEL			
	4 Weeks	6 Weeks	8 Weeks	12 Weeks
Experiment	++	++	+++	+++
Control	++	++	++	++

Results

The control joints of the Hulth-model showed no macroscopic or histological changes during the experimental time period. In the knee joints of the experimental side, a synovial inflammation, evidenced by capillary injection, was obvious in macroscopic examination after the third day postoperatively. After two weeks the synovial membrane was grossly thickened and firmly attached to the fibrous capsule. Histologically, the previously monolayered synovial intima appeared multilayered and thickened, reaching its greatest extent at four weeks postoperatively. The semiquantitative evaluation of the hyperplasia of the synovial membrane demonstrated increased intimal thickness after the third day postoperatively, progressing to maximum thickness at two and four weeks (Table 1). Increasing subsynovial fibrosis was seen during the entire experimental time period. Increased synovial cell size, columnar-like orientation of synoviocytes and the presence of plasma cells, polymorphonuclear cells and monocytes were observed electron microscopically (Figure 1).

Macroscopic examination of the cartilage of the Hulth-model knees showed loss of the shiny appearance of normal wet cartilage at four weeks. Histologically, the cartilage surface destruction was gradually increasing and extending over the medial plateau. Deep fibrillation was observed at twelve weeks. Chondrocytes were extensively loaded with lipid at three days postoperatively (Figure 2), but lipid content of the cells was decreased at four and twelve weeks. After two weeks the chondrocytes exhibited an enlarged ergastoplasma and dilated Golgi zones with pleomorphic nucleoli, which were occasionally doubled (Figure 3). Clone formation was evident at four weeks and increased at twelve weeks, with clones of ten or more chondrocytes visible in one broad capsule section.

The control knees of the RIL-model appeared normal on macroscopic examination. Microscopically moderate synovial intima thickening was seen in all joints. The cartilage showed tangential splits in the superficial zone, but no fibrillation and no clone formation. The knees of the experimental side appeared macroscopically normal; however, the joint capsule was markedly thickened. The synovial membrane showed no capillary injection over the experimental time period, but its firm attachment to the fibrous capsule was atypical. Histologically, intimal hyperplasia and thickening was obvious after eight weeks. Moderate hyperplasia at four weeks progressed to a multilayered intima at eight and twelve weeks (Tabel 1). No plasma cells or polymorphonuclear cells were seen. The subsynovium was filled by large amounts of loosely arranged collagen fibers. At twelve weeks the intimal matrix showed dense clumps of fibrin (Figure 4) and increased synovial cell size (Figure 5).

On histological examination, the cartilage of the RIL-joints showed small focal surface changes at eight weeks. These were limited to weight-bearing areas of the medial plateau not

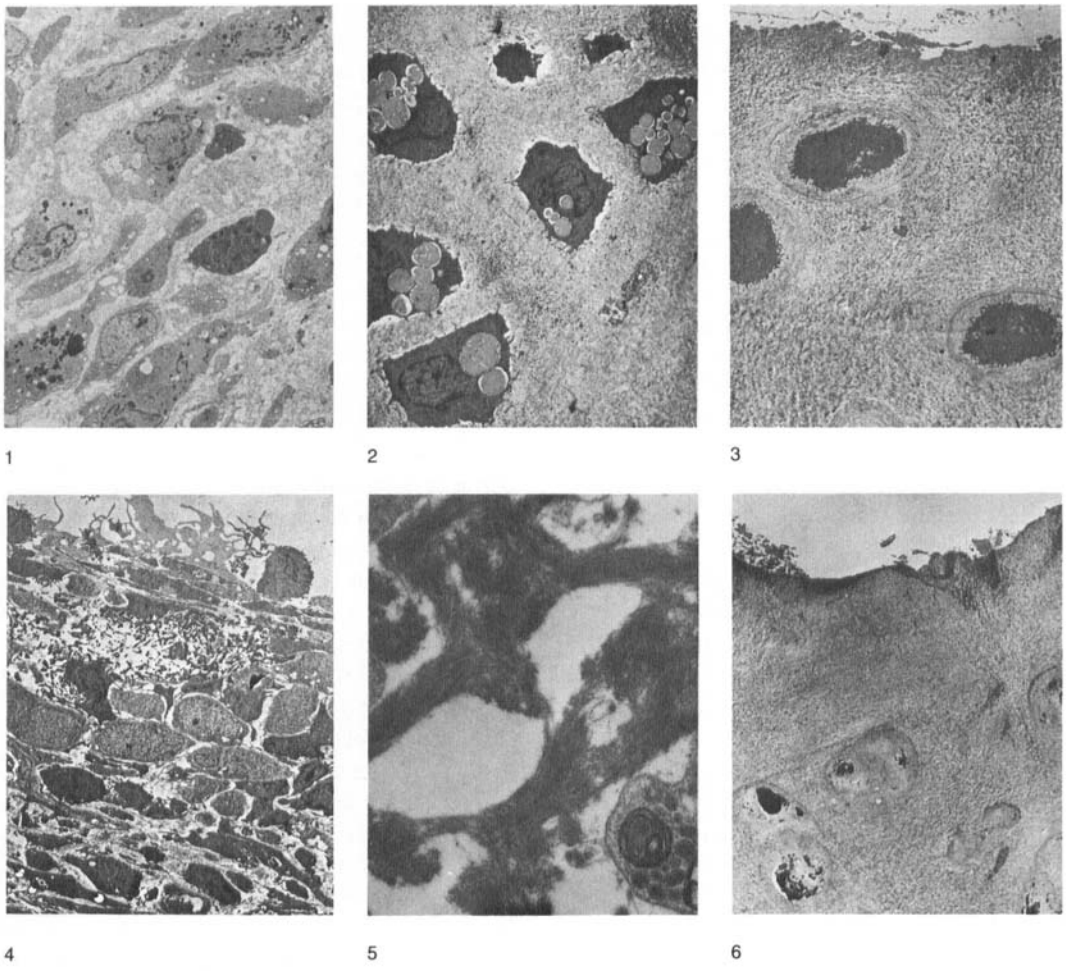

Figure 1. Synovial membrane of Hulth model knee four weeks postoperatively shows signs of acute inflammation. Note the increased fibrillar matrix substance. Erythrophagosomes are seen in almost every synovial cell. Polymorphonuclear cell is seen in the middle of picture. (860×).

Figure 2. Cartilage of Hulth model joint three days postoperatively; chondrocytes of superficial and intermediate zone exhibit lipid loading in their cytoplasms suggesting chondrocyte damage due to surgical intervention. (1320×).

Figure 3. Cartilage of Hulth model knee four weeks postoperatively with surface destruction extending into the intermediate zone. Chondrocytes display an enlarged Golgi zone and a dilated ergastoplasma. (1320×).

Figure 4. Synovial membrane of RIL-model knee at twelve weeks of loading shows intima hyperplasia with clumps of matrix material. (725×).

Figure 5. Enlargement of matrix material demonstrates fibrin in the synovial membrane of RIL-model joint at twelve weeks. (15800×).

Figure 6. Cartilage of a loaded knee at twelve weeks shows surface destruction and empty lacunae. Cartilage destruction of RIL-model is supposedly of mechanical origin. (725×).

covered by the meniscus. Electron microscopic examination revealed fraying of superficial collagen fibers with occasional fibrillation extending to the intermediate zone (Figure 6). Around the affected areas the chondrocytes were loaded with lipid, and an increased number of fibrous-material filled lacunae were seen in the superficial and intermediate zones. The chondrocytes exhibited large variations in their shape with irregular cytoplasmatic pro-

jections into the territorial matrix. Small clone formations were seen at twelve weeks, not exhibiting more than five chondrocytes in any one clone section.

Discussion

Comparison of histological changes seen in two osteoarthrosis models, the surgical instability model (Hulth-model) and the repetitive impulsive load model (RIL-model), shows that two different pathways can lead to cartilage degeneration. Early acute inflammatory synovial membrane changes were found in the Hulth-model joints and appeared to be directly attributable to the surgical procedure (Figure 1). The inflamed synovial membrane and the invading cells are likely to produce enzymes and messengers, which are able to destroy or mediate the destruction of cartilage (Crossly et al. 1984, Dingle et al. 1979, Meats et al. 1980, Steinberg and Sledge 1983). Maximal synovial hypertrophy of the synovium was seen at four weeks. This coincided with maximal synovial enzymatic activities that are involved in cartilage destruction, measured by Swiersta (1982) using the same model. In the Hulth-model the initial, acute inflammatory synovial response appears to rapidly affect the articular cartilage, evidenced by the lipid loading of chondrocytes during the early period of the experiment. The observed progressive destruction of the cartilage surface is consistent with enzymatic action following the synovial inflammation. In contrast, in the RIL-model a severe initial synovial reaction is not seen, and significant synovial changes are not developed until the late period of the experiment (Tabel 1). The focal changes in the cartilage of the RIL-model make synovial enzymatic activities unlikely causative factors in this model. The focal pattern of cartilage destruction in the weight bearing area of the RIL-model could be related to subchondral bone stiffening, as this bone stiffening occurs early in the RIL-model (Simon et al. 1972, Radin et al. 1984). The stiffened subchondral bone raises stress concentrations in the cartilage, making the cartilage more susceptible to mechanical breakdown (Abernethey et al. 1978, Radin 1982, Brown et al. 1983). However, the chondrocyte damage apparent from lipid loading, after three days in the Hulth-model (Figure 2) and focally after eight weeks in the RIL-model, suggest a similar chondrocyte reaction induced by apparently different damaging influences.

The late synovial derangement in the RIL-model could be triggered by cartilage derived debris (Chrisman 1969) and therefore be secondary to cartilage destruction. The fibrin material seen in the synovial matrix at twelve weeks demonstrates synovial inflammation that will unquestionably influence the further destruction of the cartilage. It is also possible that the late synovitis seen in the RIL-model is not triggered by cartilage debris but by the reaction to soft tissue irritation. That this soft tissue reaction occurs is evidenced by marked fibrosis of the capsule of the loaded joints. The increase in capsule fibrosis and the observed fibrosis of the subsynovium that attaches the intima to the fibrous capsule are the earliest changes seen in the RIL-joints. Whether, and to what extent, capsular and synovial fibrotic changes are related to the development of arthrotic changes in this experimental model requires further investigation.

No fibrin material was found in the synovial membrane of control joints of the RIL-model. The moderate synovial hyperplasia and cartilage splits in the control knees are changes that are attributable to relative immobilization of the joint, caused by cage holding without exercise, and immobilization of control joints during the loading cycle in sham splints. Immobilization has been shown to create degenerative joint changes (Langenskiöld et al. 1979).

The Hulth- model represents a reliable arthrosis model with fast cartilage destruction that extends over the medial plateau at two weeks postoperatively. The initial synovial inflammation in this model, evident at three days postoperatively, may induce the cartilage changes. Because the control knees in the Hulth model showed no early gross inflammatory changes, it would appear that the early synovitis cannot be attributable to the surgical procedure alone; the crucial factor must be the altered stress on the synovium from joint instability. That inflammation-provoking sulfated proteoglycan fragments are not produced

by the peak stress created by the instability and subsequently escape into the synovial crevices cannot be ruled out. The RIL-model is a non-invasive osteoarthrosis model with slowly developing, focal cartilage destruction by eight weeks. Late synovial inflammatory changes after eight weeks probably affect the development of subsequent cartilage destruction in the RIL-model.

Joint degeneration can be associated with differing early changes. In both models studied, the inflammatory changes appear to be secondary to mechanical factors.

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