MORPHOLOGY AND PATHOMORPHOLOGY

MORPHOLOGICAL ASPECTS OF CHONDROCLASIS IN RHEUMATOID ARTHRITIS

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KEY WORDS: rheumatoid arthritis; catabolism; reparative regeneration

The dominant role in destruction of the articular cartilage in rheumatoid arthritis (RA) is ascribed to the pannus of the synovial membrane. In the cartilage itself, no cells specialized in catabolism and which, by analogy with osteoclasts, could be called chondroclasts, have been found [3].

The aim of this investigation was to study the ultrastructural features of catabolism of the articular cartilage that are related to its reparative regeneration.

EXPERIMENTAL METHOD

An electron-microscopic investigation was undertaken of the articular cartilage of 23 patients with different clinical forms of RA: six had RA with typical roentgenologic changes in the joints, 12 had mild changes (delayed evolution of the articular process), and five had systemic manifestations. The study was carried out in the exudative phase of the disease, accompanied by accumulation of inflammatory exudate in the joint cavity of the knee. In six patients, on the basis of investigation of the synovial fluid, a low degree of local activity was established: the number of cells did not exceed 6000 in 1 mm^3 of synovia, the relative number of neutrophils did not exceed 10%, solitary RA cells were present, the mucin clot was firm, viscosity high, the fluid moderately turbid; six patients had a moderate cell count of up to 6000-12,000/mm^3, including up to 60% of neutrophils, up to 20% of RA cells, intensive turbidity of the fluid with readily fragmenting mucin clot, and particularly low viscosity. With activation of the disease elevation of the acid phosphatase level (from 6 to 170 μmoles) and nitrophenol level and an increase in the concentration of compounds of proteoglycan type from 6 to or a little over 20 mg% (Fil'chagin's rhizoquine test) were obtained. Cartilage was removed from the affected joint with the aid of a Soviet puncture needle (L. S. Artem'eva's modification). The site from which the biopsy material was taken was the lateral, parapatellar region of the knee joint. The material was fixed in 5% glutaraldehyde solution in phosphate buffer, pH 7.2-7.8, at +4°C for 1-1.5 h and in OsO_4 by Caulfield's method, at the same temperature for 2.5 h. The material was then washed for 10 min in 1 M phosphate buffer, and dehydrated in alcohols of increasing concentration, with uranyl acetate and 100% acetone: 50° for 30 min, 70° overnight, and 96° for 45 min (three changes). Embedding in Araldite was followed by polymerization in capsules for 12-48 h at 60°C. To locate the necessary region semithin sections about 1 μ thick were cut on an LKB 88c 2A microtome (Sweden), using glass knives. The sections were straightened out on slides covered with albumin, after removal of which with a 5-7% solution of KOH in absolute alcohol, the material was stained with a 5% aqueous solution of toluidine blue. Sections for ultramicroscopy were cut to a thickness of 50-70 nm and stained with uranyl acetate and lead citrate. Ultrathin sections were studied in the JMM-7 electron microscope (Japan) with accelerating voltage of 80 kV and magnification of between 2 and 40,000 times.

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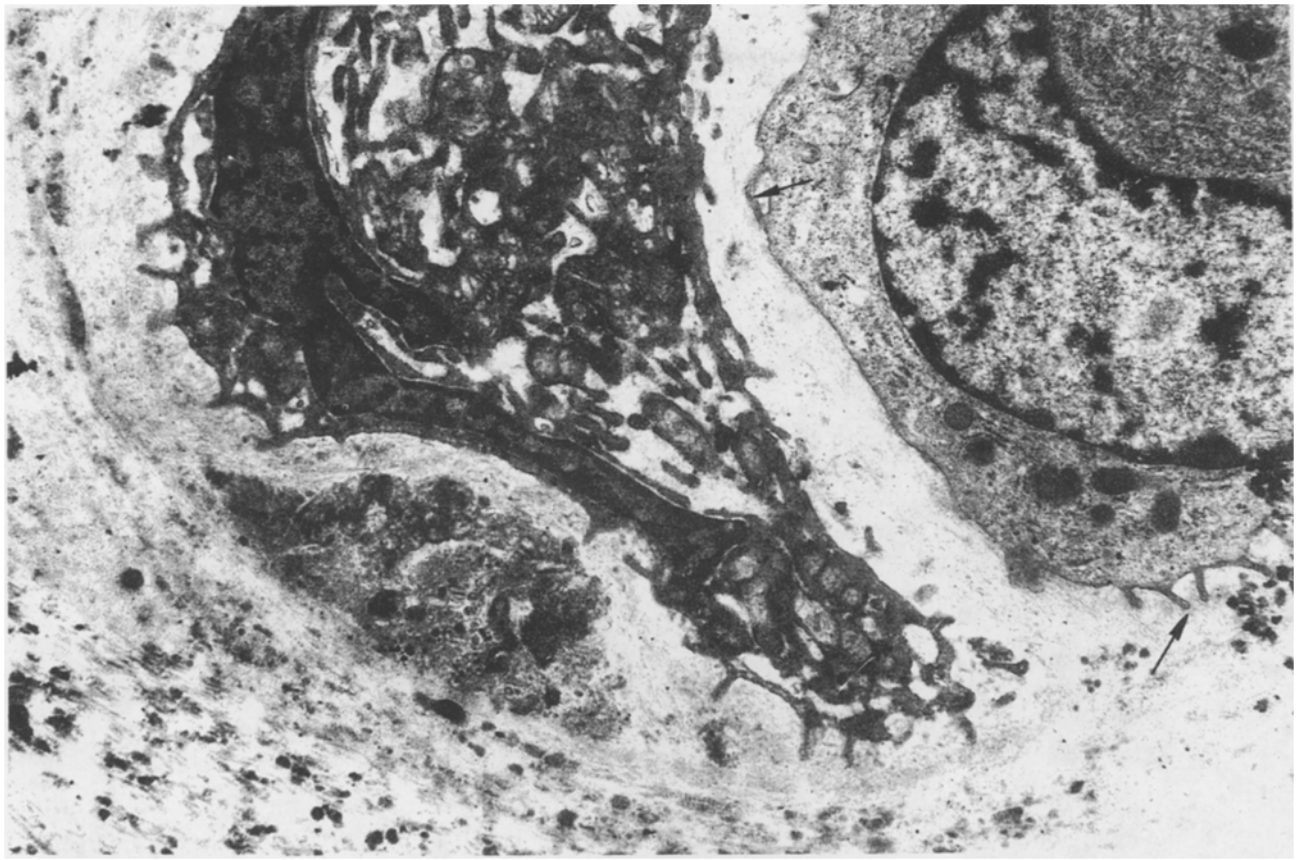

Fig. 1. Cartilage cells in lacuna of articular cartilage in rheumatoid arthritis. Left — modified cell: uneven surface with outgrowths mainly of pyramidal type, destruction of organelles, osmiophilia. On right — intact cell: even surface with solitary thin villi around concentrations of osmiophilic granules (top arrow). Obliteration of two villi with formation of phagocytic vacuole (bottom arrow). Elements of endoplasmic reticulum predominate in cytoplasm, with optimal uptake of osmium. 7500×.

EXPERIMENTAL RESULTS

Electron microscopic investigation revealed cell-free debris in the superficial parts of the articular cartilage of patients with high local activity, and with typical systemic RA. Collagen fibers were not always visible in the amorphous masses. The only ultrastructural component which could be definitely identified was lipid drops. The intensity of necrosis correlated with the neutrophil and RA cell count, with the level of acid phosphatase activity, and the concentration of proteoglycans in the synovial fluid. Synovial destruction of the articular cartilage also depends on generation of free oxygen radicals by neutrophils [7]. An increase in the content of glycosaminoglycans, reflecting the intensity of destruction of the cartilaginous matrix, correlated with the synovial C3d level [6]. If local activity was low (predominantly in patients with delayed progression of arthritis) areas of cell-free matrix more often contained fragments of collagen fibers. Single chondrocytes at different stages of degeneration and necrobiosis were found in the deeper sections. As a result of their ultrastructural analysis a definite idea could be obtained of the three-phase pathology of the chondrocytes: 1) swelling of the mitochondria, reduction of the number of elements of the rough endoplasmic reticulum and Golgi's lamellar complex (dystrophic phase); 2) accumulation of lysosomelike granules, lipid vacuoles, and the formation of foci of destruction of the cytoplasm (destructive phase); 3) lysis of the plasmalemma, contact between the cellular debris and collagen fibrils and proteoglycans of the cartilaginous matrix (phase of severe destruction of the intercellular matrix). These data are in agreement with other investigations [4].

Consequently, accumulation of lipids reflects the intensity of cellular destruction. They can pass from the cartilage into the synovial fluid and undergo phagocytosis by neutrophils and macrophages (RA cells) [2].

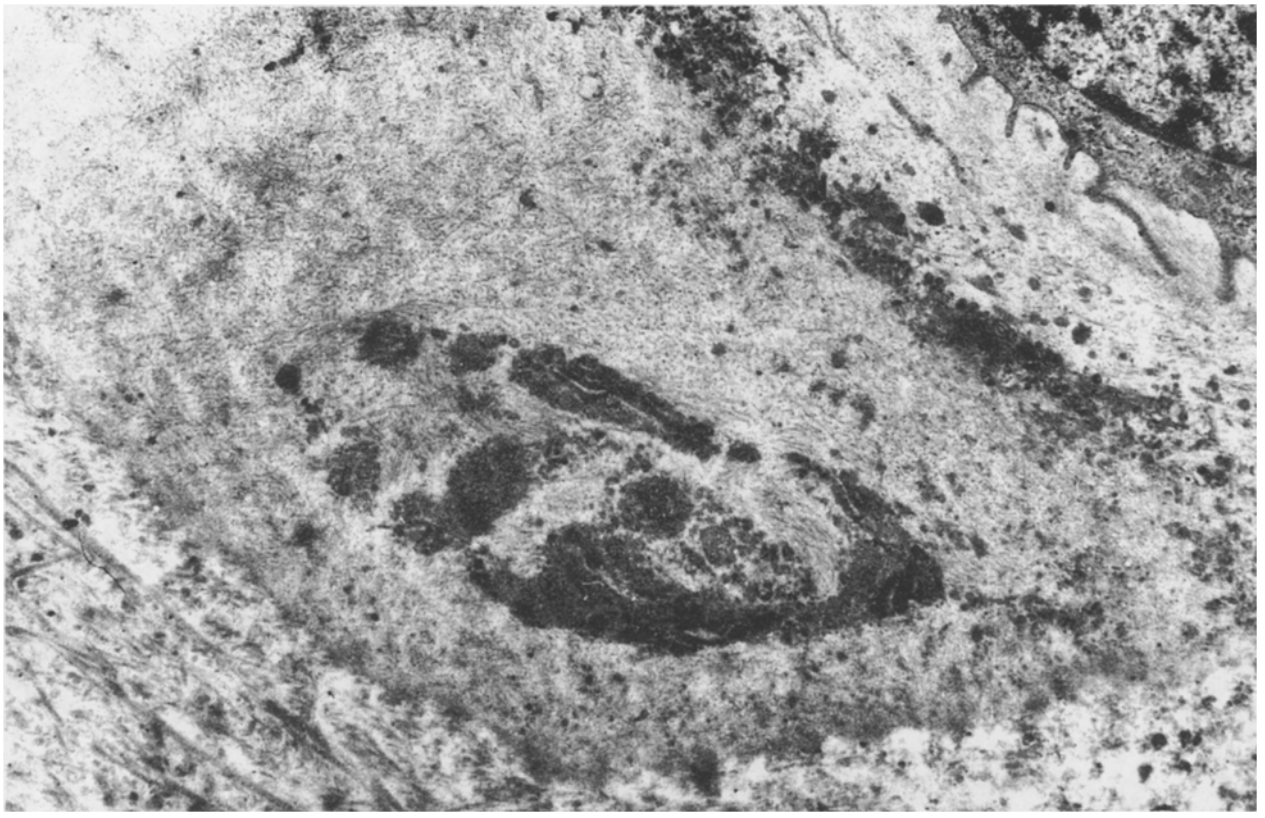

Fig. 2. Mainly compact osmiophilic remnants of necrotic cell of articular cartilage in rheumatoid arthritis. Some enlargement of filamentous villi on surface of cell adjacent to pathological substrate. 7500×.

Forms of cellular necrosis varied and entirely pycnotic osmiophilic chondrocytes ("mummies") were found. The presence of a necrotic and an intact cell in the same lacuna afforded the rare possibility of tracing the cellular response of the living to the dead actually in the articular cartilage of patients with RA. The cytoplasmic membrane of the pathologically unchanged cell usually had a smooth surface, but when pericellular areas of necrosis were present, threadlike formations differing from pyramidal outgrowths in their ability to join together to form phagocytic vacuoles (Fig. 1) appeared on it. The view that the existence of pyramidal outgrowths on the surface of the chondrocytes is a phenomenon due to the living state is disputed, and their artefactual nature has been suggested [5]. In the course of destruction of the necrotic cell the number of villuslike structures on the surface of the active cell increased (Fig. 2). With intensive dissociation of the cell residues, villi covered the cell surface uninterruptedly (Fig. 3). These outgrowths of the plasma membrane can hardly be called pseudopodia or filopodia by analogy with macrophages, for in this particular situation the cells are nonmotile. In cells possessing villi the ultrastructural profile of the cytoplasm is modified: an increase in the number of mitochondria and of various granules and vacuoles (Fig. 4).

The ultrastructural changes described above can be interpreted as a process of transformation of chondrocytes synthesizing components of the cartilaginous matrix into cells whose function is aimed at resorption of pathological substrates. Morphologically these cells have no common features with osteoclasts: they have few nuclei and do not reach giant size. Definite similarity can be suggested with macrophages, possessing moderate ability to carry out phagocytosis. Among phagocytic vacuoles containing small components of the cartilaginous matrix, no residual cell bodies were found in the present investigation. The cells with villi may perhaps be more adapted to exocytic destruction of pathological foci with the aid of lysosomal enzymes, of which interleukin 1 is an activator [5].

The abundance of mitochondria [1], which is uncharacteristic of typical chondrocytes, is evidence of the large energy expenditure of cells resorbing the articular cartilage. Some workers have found polymorphonuclear leukocytes in cartilaginous lacunae in patients with RA [3]. We did not observe the presence of blood cells in the cartilaginous lacunae. Migration of hematogenous phagocytes into very dense cartilage containing lacunae is extremely difficult and almost impossible. It can be tentatively suggested that the resorptive function is taken over by local cells, adequately transformed

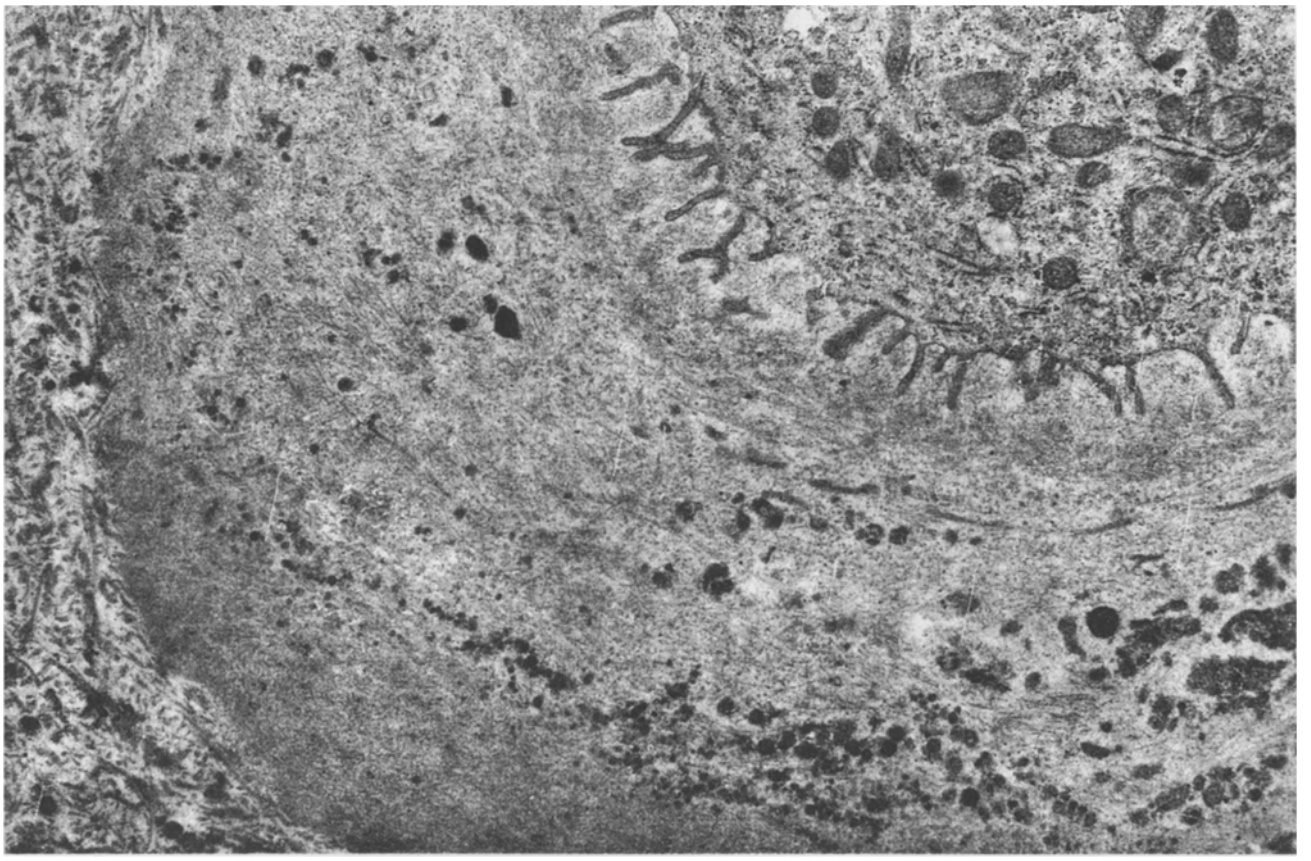

Fig. 3. Dispersion of cellular residues in articular cartilage in rheumatoid arthritis: induction of multiple villi and organelles. 7500×.

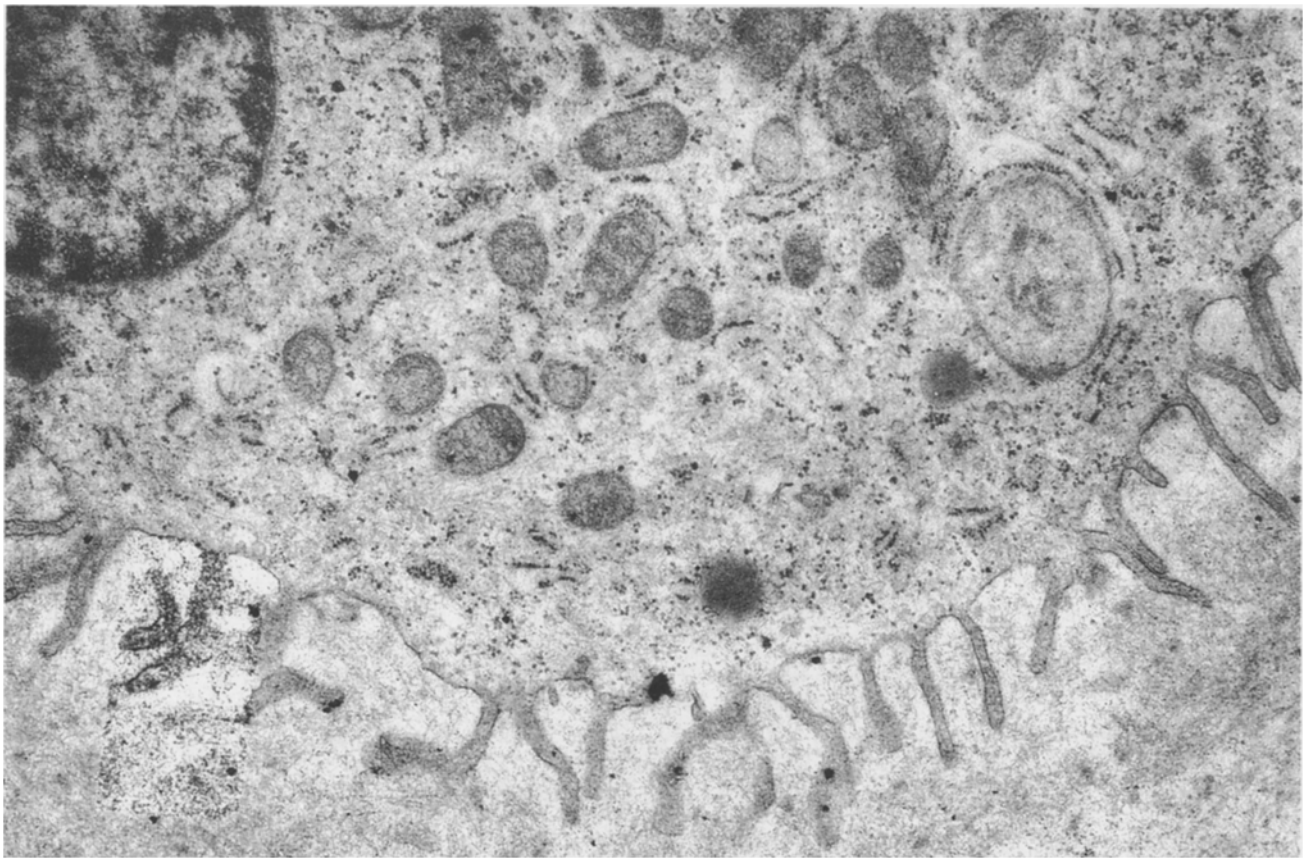

Fig. 4. Organelles in a cell of chondroplastic type in articular cartilage of patient with rheumatoid arthritis are represented by mitochondria, various granules, vacuoles, and villi. 10,000×.

from chondrocytes into chondroclasts. The latter prepare the conditions for reparative regeneration of the cartilage. Such catabolic reactions are perhaps to a certain extent characteristic of the bradytrophic cartilaginous tissue under normal conditions, and they thus fall into the general biological category.

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POST-TRAUMATIC MICROANGIOPATHIES

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In the late stages after injury to the limbs foci of recent destructive changes appear in the muscle fibers and nerve structures [2]. Since the basic processes of activity of tissue components are largely determined by the state of the microcirculation [1, 3] it was decided to assess the role of the vascular factor in progression of these dystrophic changes. This paper describes the results of an ultrastructural study of the microvessels of a skeletal muscle in the late stages after trauma.

EXPERIMENTAL METHOD

Muscles of the injured limb served as the experimental material. Mechanical trauma was inflicted on the sural muscles of five noninbred male rats by Cannon's method. An operation to replant the hind limb was performed on three rats. A particular feature of the model was the absence of prolonged thermal ischemia (during the operation the blood flow along the main vessels was preserved), and the effect of mechanical injury was excluded by studying the muscles below the level of replantation. Pieces of muscles were fixed consecutively in a cold solution of formol-sucrose and a 1% buffered solution of OSO_4, and embedded in Araldite. Ultrathin sections were examined in the JEM 7A electron microscope.

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