



# S-100 protein in human articular cartilage

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We studied the immunohistochemical distribution of the alpha and beta subunits of S-100 protein in human articular cartilage by the Avidin-Biotin Complex method using monoclonal antibodies against each subunit. Immunostaining for both subunits was detected in chondrocytes in superficial, intermediate, and deep zones of normal articular cartilage. In the matrix, only the superficial zone was stained positively. In arthrotic joints, we detected intense immunostaining in clustered chondrocytes in the hypercellular area, and weak or no immunostaining in isolated chondrocytes in the hypocellular area of articular cartilage.

The acidic calcium-binding S-100 protein was isolated from the bovine brain (Moore et al. 1965). Isobe et al. (1978, 1981) demonstrated that S-100 protein is a dimer composed of two subunits (alpha, beta) and a mixture of three types of proteins (alpha-alpha, alpha-beta, beta-beta). Recently, the presence of S-100 protein in some cartilaginous tumors and human cartilaginous tissues has been demonstrated by several authors; but the function of S-100 protein in chondrocytes is controversial, and its distribution in human articular cartilage is unknown. We studied the distribution of alpha and beta subunits of S-100 protein in articular cartilage from normal and arthrotic human joints.

## Materials and methods

Twenty-five specimens from 23 patients were examined. Nine specimens were taken from seven normal joints (Table 1) and 16 specimens from 16 arthrotic joints: viz., 11 femoral heads and five lateral femoral condyles harvested during total joint replacement for arthrosis.

Small cartilage blocks were cut off with a razor from the thickest portion of the cartilage in each

specimen. Resected cartilage (noncalcified zone) were fixed in formalin and cut into 6- $\mu$ -thick sections. By hematoxylin and eosin staining, all the sections were confirmed as containing all the noncalcified zone close to the tidemark.

The Avidin-Biotin Complex method of Hsu et al. (1981) was used for immunostaining. As primary antibodies, commercial monoclonal mouse antibodies to alpha or beta protein subunit (Japan Immunoresearch Laboratories Co., Ltd. were used. Secondary antibodies and avidin biotin complex were from Vectastain ABC Kit for mouse antibody.

Table 1. Clinical information and the site of specimens on nonarthrotic group

Case	Disease	Age	Specimens
1	Recurrent dislocation of radial head	13	Radial head
2	Brachial plexus injury	21	Joint surface of glenoid and humerus
3	Brachial plexus injury	27	Joint surface of humerus
4	Tibial and peroneal nerve palsy	24	Talonavicular joint surface of talus
5	Chondrosarcoma of femur	49	Femoral head
6	Arteriosclerosis	65	Ankle joint surface of talus
7	Arteriosclerosis	78	Ankle joint surface of talus and tibia

All the incubations were performed at room temperature. The sections were washed with phosphate-buffered saline, treated with 0.35 percent hydrogen peroxide in methanol for 30 min, and then washed again with phosphate-buffered saline. After incubation with normal horse serum for 20 min, the sections were incubated with primary antibody for 30 min and washed with phosphate-buffered saline. The sections were treated with secondary antibody for 30 min, washed with phosphate-buffered saline, and incubated with avidin-biotin complex for 30 min. After rinsing with phosphate-buffered saline, freshly prepared diaminobenzidine tetrahydrochloride solution (containing 0.006 percent hydrogen peroxide) was applied for 10 min. The sections were washed well with phosphate-buffered saline and distilled water, and finally counterstained with hematoxylin.

Positive and negative control sections were cut and prepared in the same way from rabbit trachea, which is known to contain S-100 protein. Positive

control sections were treated in the same way. For negative control sections, primary antibody was simply omitted or replaced by nonimmune mouse serum; the procedures were otherwise unchanged. The distribution of yellow-brown staining products were examined by light microscope.

## Results

The pattern of immunostaining for the alpha subunit was nearly identical in all the cases in normal cartilage. Yellow-brown staining products were detected in the cytoplasm and nuclei of almost all the chondrocytes of superficial, intermediate, and deep zones (Figure 1). In the extracellular matrix, only the most superficial layer was stained positively. No immunostaining was detected in the matrix, except in this layer.

The pattern of immunostaining for the alpha subunit was not identical in all the cases in arthrotic cartilage, and the intensity of staining

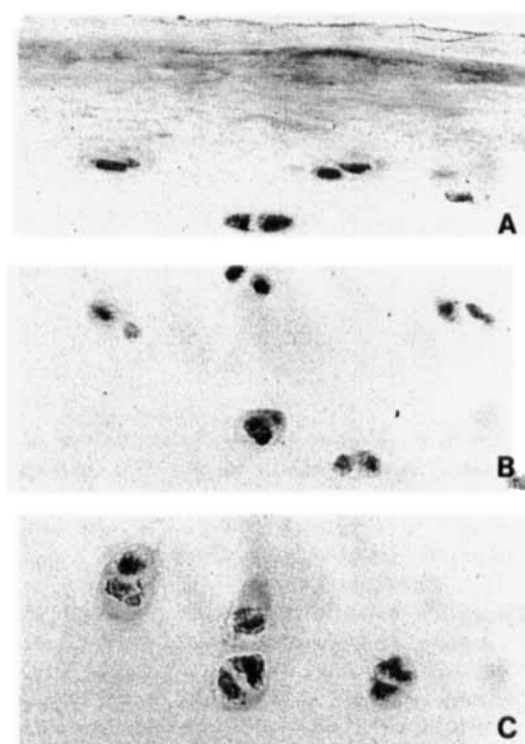


Figure 1. Articular cartilage of talus from a 65-year-old female with arteriosclerosis. A superficial zone, B intermediate zone, C deep zone. Yellow-brown immunostaining for alpha subunit was seen in chondrocytes in all three zones. ( $\times 200$ )

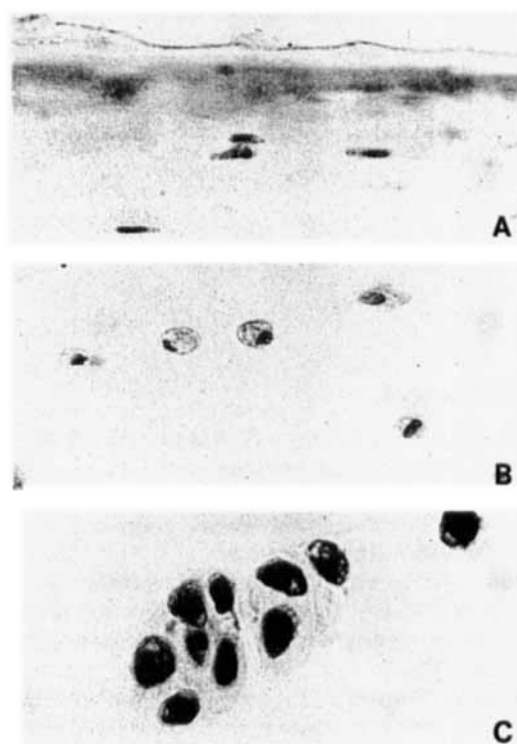


Figure 2. Articular cartilage of the lateral femoral condyle from a 72-year-old male with arthrosis. A, B, C, as in Figure 1. Intense immunostaining for alpha subunit was seen in chondrocytes forming clusters in C. Weak staining was seen in isolated chondrocytes in B. ( $\times 200$ )

was different from one area to another in the same section. In arthrotic cartilage the pattern of cell distribution was changed completely from that of normal cartilage. Intense immunostaining was detected in clustered chondrocytes in the hypercellular area. On the other hand, no staining or weak staining was detected in isolated chondrocytes in the hypocellular area (Figure 2). In the matrix, immunostaining in the most superficial zone was detected in sections of six specimens, but not in the other 10 specimens.

Weak immunostaining for the beta subunit was detected in accordance with alpha subunit distribution in both normal and arthrotic specimens.

No immunostaining was observed in the negative control sections. In the positive control sections, intense immunostaining for both alpha and beta subunits was observed in chondrocytes of rabbit trachea.

## Discussion

In order to detect immunoreactivity in the matrix of small and soluble proteins such as S-100 protein, it is better to avoid decalcification of the tissue, because it might lead to false negative results. This is why we selected a method without decalcification.

As regards the positive staining in the matrix of the most superficial zone, two possible interpretations should be considered: one is that this staining is nonspecific diffusion as a result of destruction of superficial chondrocytes, and the other is that this staining could be the result of active secretion of S-100 protein by chondrocytes in the superficial zone. This controversy needs further investigation.

The function of S-100 protein in chondrocytes remains unknown. In human cartilage, S-100 protein has been demonstrated in chondrocytes of adult larynx and xiphoid process (Stefansson et al. 1982), adult trachea (Takahashi et al. 1984), cartilaginous tumors, and epiphyseal growth plates (Weiss et al. 1986). Weiss et al. (1986) found strong immunostaining for S-100 protein in hypertrophic chondrocytes adjacent to zones of matrix mineralization, and suggested that this protein might be related to mechanism of this calcification. This does not accord with our results; we found S-100 protein in almost all the chondrocytes in noncalcified zones obtained from normal cartilage; and in arthrotic joints, intense staining was found in clustered cells and slight or no staining was seen in isolated cells. This means that the amount of S-100 protein in the articular cartilage is not related to the calcification of the matrix.

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