Influence of interleukin 1β on tenasin distribution in human normal and osteoarthritic cartilage: a quantitative immunohistochemical study

Xavier Chevalier, Pascal Claudepierre, Nicole Grout, Gaston-Jacques Godeau

Abstract

Objective — To determine the influence of IL-1β on the presence and the distribution of tenasin in matrix of human normal and osteoarthritic cartilage explants.

Methods — Cartilage was grown in organotypic culture with or without IL-1β (10 ng ml⁻¹). Tenasin antigen was detected on cryopreserved cartilage sections by immunohistochemical techniques with a monoclonal antibody directed against all tenasin isoforms (BC-4), and then quantified by video imaging densitometry.

Results — Tenasin was present in normal cartilage explants and increased in osteoarthritic cartilage explants. Treatment of normal and osteoarthritic cartilage explants with IL-1β (10 ng ml⁻¹) induced an increase in tenasin content, which was particularly high in normal cartilage and predominated in the superficial layers of damaged cartilage. There was no obvious correlation between proteoglycan loss and presence of tenasin.

Conclusions — In human normal and osteoarthritic cartilage explants, the presence and the distribution of tenasin are influenced by IL-1β.


Interleukin-1 (IL-1) has been implicated in degradation of the extracellular matrix of cartilage during the osteoarthritic process through an increase in the expression of metalloproteinases. In osteoarthritis, a decrease in the content of normal cartilage components is associated with an accumulation of adhesive glycoproteins such as fibronectins and tenasin. Tenasin is a large extracellular matrix glycoprotein (1.900 kDa), comprising six similar subunits. Each subunit contains epidermal growth factor-like (EGF-L) repeats and Fn type III homology repeats, and a domain showing sequence similarities with fibrinogen. Tenasin has been isolated from various tissues undergoing remodelling.

Factors that influence the accumulation of tenasin in osteoarthritic cartilage are unknown. The purpose of this study was to determine whether tenasin distribution in normal and osteoarthritic cartilage can be influenced by IL-1β.

Methods

MATERIALS

All materials, unless otherwise specified were from Sigma (St Louis, MO, USA). OCT was from Miles (Napierville, IL, USA). Recombinant human IL1β was from Tebu Co (Paris, France). Alkaline phosphatase was from Serva (Heidelberg, Germany). Dishes for explant cultures were from Falcon (USA). Material for electrophoresis was from Biorad SA (Paris, France).

MONOCLONAL ANTIBODIES

A specific monoclonal (MAb) antibody (BC-4) against human tenasin was used to study the distribution of tenasin in cartilage.

HUMAN CARTILAGE EXPLANT CULTURE

Cartilage tissues (from femoral head) were obtained from the Department of Orthopaedic Surgery, Hôpital Henri-Mondor (Creteil, France) at the time of joint replacement in patients with hip osteoarthritis (n = 10) (five females and five males, mean age: 70, SD 4, years). Normal cartilage was obtained from three patients (femoral head cartilage at the time of joint replacement for femoral neck fracture) (two females and one male; mean age 72, SD 2, years). Each cartilage sample was immediately cut into small cartilage explants under aseptic conditions (25 mg average wet weight) and precautions were taken to select explants including deep to superficial layers. Normal and osteoarthritic cartilage explants were cultured in serum-free Iscove medium supplemented with bovine serum albumin, transferrin and lipid emulsion, and glutamine (2 mM). Recombinant IL1β (10 ng ml⁻¹) was added to the medium and left for 24 h. Media were then withdrawn and cartilage explants were frozen in liquid nitrogen, cryoprotected with OCT, and stored at −80°C until used.

IMMUNOHISTOCHEMISTRY

Immunohistochemical localisation of tenasin was performed on 6 μm frozen cartilage sections. Care was taken to orient cartilage
slices during sectioning so as to obtain superficial, middle, and deep zones. Slides were previously coated with L-polylysine and fixed in acetone at +4°C for 10 min. Sections were pretreated with bovine testicular hyaluronidase (0.5 mg ml⁻¹; 1500 U ml⁻¹) at 37°C for 60 min in Tris-NaCl, pH 5.8, as this procedure has been shown to increase tenascin detection. To prevent non-specific binding of the primary antibody, sections were incubated with Tris-NaCl buffer containing 5% BSA for 45 min. Then anti-tenascin MAb (1:10) diluted in 5% bovine serum albumin was added for 1 h. After washing in TBS buffer (Tris-HCl 0.05 M, NaCl 0.15 M, pH 7.4), the slides were incubated with biotinylated goat anti-mouse immunoglobulin (diluted 1/1000) for 30 min. Sections were then washed and incubated with a 1:50 dilution of soluble complex of alkaline phosphatase conjugated streptavidin (Dako kit), for 30 min at 22°C. Immunoreactivity was revealed by addition of naphthol and fast-red solution. Levamisole was added to block endogenous alkaline phosphatase.

Appropriate controls were performed with another MAb from a hybridoma cell culture (anti-Ed-A fibronectin; IST-9), showing no staining in normal cartilage.

AUTOMATED IMAGE ANALYSIS OF TENASCIN STAINING

Immunopositivity for tenascin appears as a red to brown staining. The colourless background was averaged from one section to another, so that the sections could be compared. Computerised morphometric analysis of tenascin staining was carried out in the pericellular area (staining in and/or around the cells) and in the interterritorial matrix as previously described (Zeiss microscope, at ×40 magnification, equipped with a Sophretec CF 126 PH R video camera). Superficial, middle, and deep layers of the cartilage were tested. The volume of each area was automatically calculated by the computerised program and the number of cells was divided by the surface area. Each slide was analysed by two independent observers.

Data obtained were: (1) percentage of positive cells (cell score); (2) intensity of cell staining (intensity score); (3) the average matrix staining (matrix score).

DETECTION OF PROTEOGLYCAN

Proteoglycan staining was assayed by toluidine blue dye and compared with tenascin staining on the same sections.

STATISTICAL ANALYSIS

Significance of differences between groups was evaluated by one way analysis of variance (Bonferroni test) and was considered significant when P < 0.05.

Results

Immunohistolocalisation is shown in fig 1. Scores and statistical analysis are summarised in figs 2 and 3.

PRESENCE OF TENASCIN IN NORMAL AND OSTEOARTHRITIC CARTILAGE EXPLANTS

In normal cartilage, tenascin was present mainly around the cells; in the interterritorial matrix it was very low (eightfold less when considering the full thickness of the cartilage) (fig 1). The staining was the most intense in the superficial layer while it was milder in the middle and deep layers.

In osteoarthritic cartilage, staining intensity in the pericellular area was 1.6-fold higher than in the interterritorial matrix when the full thickness of the cartilage is considered. Tenascin distribution in osteoarthritic cartilage

Figure 1 Presence of tenascin in normal and osteoarthritic cartilage explants. Immunohistological studies were performed using an avidin-biotin amplification system (see Methods). The monoclonal antibody (BC-4) used against tenascin recognizes all isoforms of the molecule. Top: Normal cartilage. (A) Tenascin staining without added mediators (×35). (B) Tenascin staining with IL-1β (10 ng ml⁻¹). Middle: Osteoarthritic cartilage. (A) Tenascin staining without added mediators (×35). (B) Tenascin staining with IL-1β (10 ng ml⁻¹) (×35). Bottom: Osteoarthritic cartilage; magnification. (A) Tenascin staining without added mediators (×70). (B) Tenascin staining with IL-1β (10 ng ml⁻¹) (×70).
showed some differences from normal cartilage (fig 1). First, the number of chondrocytes immunoreactive for tenasin was only slightly higher in osteoarthritic cartilage (68% of positive cells in osteoarthritis v 62% in normal cartilage). Second, the intensity (pericellular area) and the matrix scores were higher in osteoarthritic than in normal cartilage, whatever the layer (fig 1, fig 3). Third, in osteoarthritic cartilage there was intense staining on the cartilage surface in contact with synovial fluid.

**Discussion**

The main result of this study is to show that IL-1β induces an increase in tenasin content in both normal and osteoarthritic cartilage. We used an automated computerised technique which is a valid method to quantify tenasin because the staining appeared as a distinct structure on a colourless background. This technique would not be effective for other macromolecules with diffuse distribution, such as collagen or fibronectin.

The effect of IL-1β was more pronounced in normal than in osteoarthritic cartilage. Tenasin intensity score in IL-1β treated normal cartilage was close to the level observed in osteoarthritic cartilage, suggesting that IL-1 might contribute to the accumulation of tenasin in damaged cartilage. In osteoarthritic cartilage, tenasin diffusion from synovial fluid may account for the observed staining, notably on the cartilage surface. Under IL-1 stimulation there is an increased staining intensity in both the pericellular zone and its contingent interterritorial matrix, suggesting that the presence of tenasin in matrix distant from the chondrocyte may be due to a continuous increase in the production of tenasin by the chondrocyte.

However, this study does not address the mechanisms of tenasin accumulation. IL-1 can upregulate tenasin mRNA expression in synovitis. Alternatively, tenasin epitopes may become detectable during collagenase production or proteoglycan depletion induced by IL-1.
Our results suggest that accumulation of tenascin may be part of the effects of IL-1 action, and do not imply that IL-1 is effective in vivo. Similarly IL-1 can modulate the production of other non-collagenous proteins such as thrombospondin.12

The functions of tenascin in damaged cartilage are not known. The presence of tenascin in normal cartilage suggests that it plays a physiological role. This protein has been implicated in the phenomenon of adhesion migration of cells. Because tenascin can impede cell adhesion to fibronectin substrate,14 it has been suggested that it might promote cell migration rather than cell adhesion.15 In osteoarthritic cartilage, tenascin might favour the migration of the chondrocytes towards the lesions, a hypothesis which fits well with its accumulation in the superficial layer.

We thank professor L Zardi PhD, the National Institute for Cancer Research, Genoa, Italy, for providing the antibodies.

Figure 3 Immunohistological scores of tenascin staining in matrix distant from chondrocytes (matrix score) in normal and osteoarthritic cartilage explants with no added mediators or in presence of 10 ng ml⁻¹ of IL-1β. Matrix score corresponds to interterritorial matrix: full thickness cartilage; superficial layer; middle layer; deep layer. Results are expressed as average grey scale value and represent the mean average of 30 randomly chosen microscopic fields. This score was calculated in reference to unstained control sections (see Methods for description). P values (significance of the hypothesised trend between controls IL-1β treated cartilage explants, determined using one way analysis of variance): *P < 0.05; †P < 0.01; ‡P < 0.001.

10 Godeau GJ, Connord G, Joviet V, Hornebeck W, Robert L. A selective histochémical method for the quantitative esti-
Influence of interleukin 1 beta on tenascin distribution in human normal and osteoarthritic cartilage: a quantitative immunohistochemical study.

X Chevalier, P Claudepierre, N Groult and G J Godeau

Ann Rheum Dis 1996 55: 772-775
doi: 10.1136/ard.55.10.772

Updated information and services can be found at:
http://ard.bmj.com/content/55/10/772

These include:

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/