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

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

Abstract
Objective — To determine the influence of IL-1β on the presence and the distribution of tenascin 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 tenascin isoforms (BC-4), and then quantified by video imaging densitometry.

Results — Tenascin 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.

(H Ann Rheum Dis 1996;55:772-775)

Laboratoire de Biologie du tissu conjonctif, Faculté de médecine, the Department of Rheumatology of the Henri-Mondor hospital, Créteil, France
X Chevalier
P Claudepierre

National Institute for Cancer Research, Genoa, Italy
N Groult
G J Godeau

Correspondence to: Dr X Chevalier, Department of Rheumatology, Henri-Mondor hospital, Bd De Latre De Tassigny, 94010 Créteil, France.

Accepted for publication 6 June 1996

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 metalloproteases. In osteoarthritis, a decrease in the content of normal cartilage components is associated with an accumulation of adhesive glycoproteins such as fibronectin 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 conjugated streptavidin-biotin complex was from Dako (Paris, France). Testicular hyaluronidase was from Serve (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 ( Créteil, 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 lipd 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
Influence of IL-1 on Tenascin distribution in cartilage.

slices during sectioning so as to obtain superficial, middle, and deep zones. Slides were previously coated with L-polysine and fixed in acetone at +4°C for 10 min. Sections were pre-treated 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

Immunohistostaining 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...
showed some differences from normal cartilage (fig 1). First, the number of chondrocytes immunoreactive for tenascin 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.

**Presence of Tenascin in Normal and Osteoarthritic Cartilage Explants: Influence of IL-1β (10 ng ml⁻¹)**

In normal cartilage, treatment with IL-1β induced an increase in the number of positive cells as well as an increase in the intensity of pericellular staining (intensity score). Similarly, but to a lesser degree, IL-1 induced a four- to fivefold increase in the matrix score.

In osteoarthritic cartilage the number of immunoreactive chondrocytes did not increase significantly, but the intensity of staining increased in the pericellular area and in the interterritorial matrix (figs 2 and 3). This was mostly due to enhanced staining in the superficial layer.

When comparing normal and osteoarthritic cartilage, the effect of IL-1 appeared more pronounced in normal cartilage. In the superficial layer the intensity score increased threefold in normal cartilage and only 1.6-fold in osteoarthritic cartilage. Similarly, the matrix score in the superficial layer increased fourfold in normal cartilage and only twofold in osteoarthritic cartilage.

Otherwise distribution of tenascin was not strictly correlated to the proteoglycan loss.

**Discussion**

The main result of this study is to show that IL-1β induces an increase in tenascin content in both normal and osteoarthritic cartilage. We used an automated computerised technique which is a valid method to quantify tenascin 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. Tenascin 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 tenascin in damaged cartilage. In osteoarthritic cartilage, tenascin 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 tenascin in matrix distant from the chondrocyte may be due to a continuous increase in the production of tenascin by the chondrocyte.

However, this study does not address the mechanisms of tenascin accumulation. IL-1 can upregulate tenascin mRNA expression in synovitis.11 Alternatively, tenascin epitopes may become detectable during collagenase production or proteoglycan depletion induced by IL-1.
Influence of IL-1 on tenascin distribution in cartilage.

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.

The functions of tenasin in normal cartilage are not known. The presence of tenasin in normal cartilage indicates that it plays a physiological role. This protein has been implicated in the phenomenon of adhesion of cell on foreign substrates.

In osteoarthritic cartilage, tenasin might favour the migration of 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.


