Y. Mitani · A. Honda · H. E. Jasins

Polymorphonuclear leukocyte adhesion to articular cartilage is inhibited by cartilage surface macromolecules

Received: 10 April 2000 / Accepted: 15 November 2000 / Published online: 19 April 2001 © Springer-Verlag 2001

Abstract The present studies deal with polymorphonuclear neutrophil (PMN) adhesion inhibitory properties of cartilage surface proteoglycans. Normal human PMN were used in adhesion experiments with bovine cartilage surfaces exposed to neutrophil elastase and reconstituted with fibronectin (Fn) or on plastic-bound Fn. An extract of cartilage surface small proteoglycans (SE) and purified fibromodulin (FM), decorin (DCN), biglycan (BGN), and aggrecan (AGN) on the surface of normal cartilage were used to test for inhibition of Fn-dependent cell adhesion. The PMN did not adhere to intact articular cartilage surfaces, whereas significant adhesion was measured using cartilage explants digested with elastase and reconstituted with Fn. Incubation of elastase-treated, Fn-reconstituted cartilage with 45 µg/ml SE inhibited PMN adhesion by 50.7 ± 5.8% (P < 0.0001). Addition of 50 µg/ml purified FM to the reconstituted articular surfaces inhibited cell adhesion by 71.2 ± 13.9% (P < 0.0001). Inhibition of PMN adhesion to plastic-bound Fn was seen with 1.7 µg/ml SE (20.4 ± 8.0%). Maximal inhibition of 67.4 ± 14.8% (P < 0.01) was obtained with 17.0 µg/ml SE. With FM, concentrations of 4.3 µg/ml resulted in 34.7 ± 25.2 inhibition (P < 0.001), and maximal inhibition of 66.3 ± 16.2% (P < 0.01) was obtained with 43.0 µg/ml. Similar results were obtained with purified bovine DCN and BGN. The main component of cartilage matrix, AGN, failed to inhibit cell adhesion significantly. The results indicate that macromolecules normally present on articular cartilage surfaces act as a barrier to PMN adhesion. Since cartilage surface proteins are susceptible to breakdown by proteases from synovial fluid inflammatory cells, we postulate that the degradation of this barrier may be responsible for increasing PMN adhesion and subsequent cartilage damage in inflammatory arthritis.

Keywords Cell adhesion · Articular cartilage · Nitric oxide · Polymorphonuclear neutrophils · Fibronectin

Introduction

The articular surface of intact cartilage is a highly organized structure [1, 2, 3] containing a variety of macromolecules such as collagen type II, fibronectin (Fn) [3], and the nonaggregating proteoglycans fibromodulin (FM), decorin (DCN), and biglycan (BGN) [4, 5]. We showed previously that one of these protease-susceptible macromolecules, FM, mask collagen type II epitopes so that anticollagen antibodies are partially hindered from binding to the articular surface [2, 6]. In addition, the intact superficial macromolecular layer is organized in a fashion that prevents cell adhesion to the cartilage surface [7, 8]. Of particular interest was the observation that although Fn was detectable at the cartilage surface, its cell-binding domain was masked by other surface macromolecules, particularly FM [7]. Pertinent to the phenomenon of cell adhesion to cartilage are the demonstration that nitric oxide (NO) inhibits cell adhesion to endothelium [9] and our recent observation that activated superficial chondrocytes secrete much larger amounts of this gas than the deeper cells [10]. Since polymorphonuclear leukocytes (PMN) make up a significant proportion of the synovial fluid cells in rheumatoid arthritis and since there is abundant evidence that these cells or their products, particularly proteolytic enzymes, are present at the cartilage-pannus junction and may be responsible for cartilage damage [11, 12, 13, 14, 15], it was of interest to identify the cartilage surface macromolecules responsible for the adhesion/inhibitory
properties of the intact articular surface of cartilage. The present studies show that the small, nonaggregating cartilage proteoglycans present in the articular surface inhibit Fn-dependent PMN adhesion.

Materials and methods

Reagents

Hanks’ balanced salt solution (HBSS) was purchased from Sigma Chemical (St. Louis, Mo., USA), human plasma fibronectin (Fn) from Life Technologies (Gaithersburg, Md., USA), one-step polymorphs from Accurate Chemical and Scientific (Westbury, N.Y., USA), human leukocyte elastase from Elastin (Owensville, Mo., USA), and sepharose CL 6-B, sephacryl S-300 HR, and the monoQ 5/5 column from Pharmacia (Uppsala, Sweden). The bovine Fn was prepared as previously described [2] by Plaa et al. [16]. Bovine DCN was purified by ion exchange, hydrophobic interaction chromatography according to Choi et al. [17]. Bovine BGN and aggrecan were purchased from Sigma. Rabbit antibovine fibromodulin (FM) antisera were the generous gift of Dr. Anna Plaa (Tampa, Fl., USA) and antihuman DCN monoclonal antibody of Dr. Paul Scott (Edmonton, Canada). The purity of all proteoglycans was checked by polyacrylamide gel electrophoresis (PAGE).

Preparation of PMN

Heparinized venous blood was obtained from normal human adult donors. PMN were separated from heparinized blood by isopycnic centrifugation in Ficoll-Isoaque gradients using one-step polymorphs. After hypotonic lysis of red blood cells with 0.2% NaCl for 1 min at 4°C, the cell suspension was washed twice in HBSS. Cell viability as assessed by trypan blue exclusion was greater than 95%. The PMN were resuspended in HBSS, quantitated, and adjusted to 50,000/ml for cell adhesion assay.

Purification of cartilage surface extract

Cartilage surface extract (SE) was obtained by extraction of the articular surface of bovine metacarpal bones with 4 M guanidine solution for 5 min as described previously [2].

Cell adhesion to cartilage

Bovine cartilage slices were cut with a scalpel into 4x4-mm squares, taking care not to damage the articular surface. After washing with HBSS, some explants were incubated with 1.0 µg/ml elastase in HBSS for 1 h at 37°C. After two washes with Dulbecco PBS (DPBS) containing 0.01% PMSF and 0.01% EDTA at 4°C, they were incubated with 5 µg/ml Fn or HBSS at 37°C for 90 min, and washed twice with HBSS at 4°C. After incubation with 50 µg FM or 45 µg/mL SE for 15 min, they were incubated for 1 h with 5x10^4 PMN. Finally, the tissue explants were washed briefly with DPBS containing 0.9 mM CaCl2 and 0.5 mM MgCl2, and fixed in buffered formalin. After fixation, the cartilage was cut into 2x2-mm pieces and stained for leukocyte myeloperoxidase with a commercial kit (Sigma). Controls consisted of PMN incubated with intact cartilage pieces. Cell attachment to the articular surfaces of quadruplicate explants was determined by counting all cells attached to the 4-mm² explants using an inverted microscope. The results were expressed as cells per mm². Percentage of adhesion was calculated by assuming the mean cell number attached to elastase-treated, Fn-reconstituted explants from four separate experiments as 100%. The average cell adhesion was 0.15 ± 0.08 mm² for intact cartilage and 6.5 ± 1.4 mm² for elastase-treated, Fn-reconstituted cartilage.

Cell adhesion assay on microtiter plates

Ninety-six-well polystyrene microtiter plates were coated with 5 g Fn per well in 0.1 M NaHCO3 at 37°C for 2 h. The remaining binding sites were blocked with 1% BSA for 30 min. Between each incubation step, the wells were washed twice with DPBS containing 1% BSA, 0.9 mM CaCl2, and 0.5 mM MgCl2. Triplicate wells were incubated with several concentrations of purified proteoglycans, SE, or DPBS for 15 min. PMN (50,000/ml) were added, the plate was incubated for 1 h at 37°C, and the nonadherent cells were removed with two gentle washes with 0.9 mM CaCl2 and 0.5 mM MgCl2. Adherent cells were quantified by the biocytin-chrome acid (BCA) protein assay (Pierce, Rockford, Ill., USA) as previously described [7]. The numbers of attached cells were determined from a standard curve constructed with six different cell concentrations in triplicate ranging from 0 to 50,000 PMN/well. Optical absorbance at 562 nm and 490 nm was measured in an EL 310 microtiter reader (Bio-Tek, Winooski, Vt., USA). Positive controls consisted of cells incubated on wells coated with Fn alone, and negative controls consisted of wells coated with BSA alone. The mean cell adhesion to control, BSA-coated wells was 7.2 ± 1.4% of cell input, and the mean for Fn-coated wells was 40.4 ± 4.5% of input. Incubation of the cells with the different proteoglycans followed by washing did not result in inhibition of adhesion.

Statistical analysis

The data obtained were expressed in percentage of cell adhesion compared to positive controls, which were taken as 100%. As a rule, the results are expressed as mean ± SEM. Analysis of significance was performed using Student’s two-tailed t-test.

Results

Previous studies from our laboratory had shown that neither fibroblasts [7] nor PMN [8] adhered to the intact surface of articular cartilage. In the present studies, PMN did not attach significantly to the surface of intact cartilage or elastase-treated cartilage not reconstituted with Fn (Fig. 1a), confirming our previous observations. However, reconstitution of the articular surface with Fn resulted in a significant increase in PMN adhesion (Fig. 1b). Incubation of elastase-treated, Fn-reconstituted cartilage with 45 µg/ml SE inhibited PMN adhesion by 50.7 ± 5.8% (P < 0.0001) (Fig. 2). Adding 50 µg/ml of purified FM to the reconstituted articular surfaces inhibited cell adhesion by 71.2 ± 13.9% (P < 0.0001) (Fig. 1c, Fig. 2). Incubation of elastase-treated cartilage not reconstituted with Fn, with addition of 50 µg/ml of FM alone, did not result in PMN adhesion (results not shown).

The inhibitory effects of SE and FM on PMN adhesion were also tested on Fn-coated plastic surfaces. Significant inhibitory activity of PMN FN-dependent adhesion of 20.4 ± 8.0% was seen with protein concentrations as low as 1.7 µg/ml SE. Maximal inhibitory activity (67.4 ± 14.8%, P < 0.01) was obtained with 17.0 µg/ml SE (Fig. 3). Similar results were obtained using purified FM as the inhibitory protein. Concentrations of 4.3 µg/ml resulted in 34.7 ± 25.2% inhibition of cell adhesion (P < 0.001), reaching 66.3 ± 16.2% inhibition with 43.0 µg/ml FM (P < 0.01).