Serum concentrations of cartilage oligomeric matrix protein, fibrinogen and hyaluronan distinguish inflammation and cartilage destruction in experimental arthritis in rats

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Abstract

Objectives. We investigated if changes in serum/plasma fibrinogen (FIB), hyaluronan (HA) and cartilage oligomeric matrix protein (COMP) levels can be used to differentiate between inflammation and cartilage involvement during arthritis.

Methods. Collagen-induced arthritis (CIA), oil-induced arthritis (OIA) and for comparison, experimental autoimmune encephalitis (EAE) induced in DA rats were investigated.

Results. Elevations of FIB concentrations were apparent at days 4–7 post-immunization in both arthritis models reaching a maximum on day 20–21, i.e. before peak arthritis. Elevations of HA in both models were seen shortly before macroscopically apparent arthritis, and peaked at or just before maximal arthritis, i.e. later in CIA than in OIA. COMP levels increased only after onset of arthritis and peaked late in disease (days 34–37), being significantly higher in the more destructive CIA compared with the less destructive OIA. During EAE flares, only FIB levels increased.

Conclusions. FIB is a general inflammation marker, HA appears to be a marker for synovitis and changes in COMP levels appear to reflect the cartilage destruction process.

KEY WORDS: Collagen-induced arthritis, Oil-induced arthritis, Experimental autoimmune encephalomyelitis, Cartilage oligomeric matrix protein, Fibrinogen, Hyaluronan.

Polyarthritis may vary in chronicity and severity. The magnitude of the overall inflammatory response, the intensity of local synovitis and the extent of cartilage destruction may vary independently of each other. For example, cartilage and bone destruction in equally inflamed joints is less pronounced in SLE as compared with rheumatoid arthritis (RA) [1]. Destruction of joint cartilage in RA may also occur in patients with little clinical sign of synovitis [2].

For evaluation of disease development and activity, for example when studying the effects of anti-arthritis treatment regimens, signs of inflammation as determined by quantification of serum levels of acute phase proteins are used. The magnitude of ongoing synovitis and cartilage and bone destruction is difficult to estimate. Indirect measures such as physical examination and radiography are used. Easily accessible serum markers for monitoring synovitis and joint destruction would represent valuable tools for evaluating arthritis development and treatment. Several such potential markers are being investigated with promising results [3].

Cartilage oligomeric matrix protein (COMP) is a pentameric protein, originally purified from cartilage [4]. It has also been shown to be present in other pressure loaded tissues, e.g. tendon [5, 6], and meniscus [7] (B. Månsson et al., unpublished results). COMP can also be produced by cells in the synovial membrane [8, 9], and the relative amounts of the protein in different tissues vary, being highest in cartilage (T. Saxne and D. Heinegård, unpublished results). Both in inflammatory arthritis and osteoarthritis, as well as in experimental arthritis, COMP has shown promise as a potential biomarker for monitoring progression of cartilage destruction, for evaluating cartilage effects of

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therapy, and as a prognostic tool reflecting cartilage damage [reviewed in 10]. Although being a promising cartilage marker, the lack of tissue specificity is a potential confounding factor for interpretations of changes in serum levels, especially in conditions with a marked inflammatory response in the synovium.

It has previously been demonstrated that serum COMP increases during arthritis development in collagen II-induced arthritis (CIA) and pristane-induced arthritis in rats [10, 11], and that this increase coincides with development of cartilage damage. Furthermore, therapeutic intervention which ameliorated cartilage destruction normalized serum COMP levels in murine CIA, whereas treatment which only reduced signs of inflammation did not affect COMP levels [12].

In the present study, we wanted to analyse further the relationship between changes in serum COMP concentrations and the process in cartilage and the inflammatory process both locally and systemically. To accomplish this we studied changes in serum concentrations of COMP as a potential cartilage marker, hyaluronan (HA) as a putative marker for synovial inflammation [13, 14] and fibrinogen (FIB) as a marker for generalized inflammation [15] in the rat. Fibrinogen was chosen as the marker of preference for general inflammation in the rat, as serum levels of FIB have shown a more dynamic response to inflammation than C-reactive protein (CRP), and as there were no available tests for amyloid A [15]. HA was chosen as a marker of preference for synovitis on the basis of previous rat studies [13] as well as on human studies in RA where serum levels of HA correlated to synovitis as estimated by the Ritchie articular index [14].

We utilized two models of arthritis with different disease courses (CIA [16, 17] and oil-induced arthritis (OIA) [18]), and experimental autoimmune encephalitis (EAE) as a non-arthritic inflammatory control. CIA in the DA rat is a chronic and heavily destructive disease [17], while OIA in the same rat strain is transient and has a less destructive disease course [18]. The serum concentrations of the different markers were related to clinical signs of disease.

Materials and methods

Animals

Male DA rats aged 3.5–4 months at the start of the experiments were used. The animals were health monitored according to guidelines from the Swedish Veterinary Board, and found to be free of pathogens. The Ethical Board (Djurförsöks etisk nämnd) at the Karolinska Institute, Stockholm, approved all animal procedures performed.

Induction and clinical monitoring of experimental diseases

For CIA, collagen II was prepared from rat chondrosarcoma as previously described [19, 20]. The collagen was dissolved in 0.1 M acetic acid and emulsified 1:1 with Freund’s incomplete adjuvant (FIA) (Difco, Detroit, MI, USA). A 150 μg quantity of collagen II in 200 μl emulsion, was injected intradermally at the base of the tail. OIA was induced by injection of 200 μl of FIA intradermally at the base of the tail. EAE was induced by intradermal injection at the base of the tail of 200 μl with homogenized DA rat spinal cord emulsified 1:1 in FIA.

Arthritis was quantified by a clinical scoring system, scaled 0–16. Each paw was scored as follows: 0 = no arthritis, 1 = swelling in one type of joint, 2 = swelling in two types of joints, 3 = swelling in three types of joints and 4 = swelling of the entire paw. A total score for an animal was calculated by summing up the scores for each of the four paws [18].

EAE was evaluated using a clinical scoring system scaled 0–3 where 0 = no illness, 1 = dropping tail, 2 = unsteady walk and 3 = inability to walk [21].

Blood samples were taken by retroorbital puncture before immunization and at selected time points after immunization.

Immunoassays

Serum concentrations of COMP were determined by ELISA, using similar conditions as described for the assay for human COMP [22]. The assay was modified by using rat COMP for coating microtitre plates and for the standard curve included in each plate as well as by using a polyclonal antiserum raised against rat COMP [10].

Plasma levels of FIB were measured with nephelometry as previously described by Larsson et al. [15]. Results are presented as per cents of a reference sample consisting of pooled plasma from healthy rats.

Hyaluronan was analysed using a previously described radiometric technique according to the manufacturer’s instructions (Pharmacia HA test, Pharmacia Diagnostica, Uppsala, Sweden) [23]. The feasibility of the assay technique for rat serum samples has previously been documented [13].

Statistical calculations

Wilcoxon’s matched pairs test (two-tailed) was used for comparing concentrations of FIB, HA, COMP and scores at different time points. The Mann–Whitney U-test was used for comparing differences between groups. A P-value <0.05 was considered significant. Only animals developing disease after immunization were included in the calculations.

Results

Development of disease

The disease scores are presented in Table 1 and Fig. 1A. In the CIA group 58% (7/12) and in the OIA group 80% (8/10) exhibited clinical signs of arthritis. Ninety per cent (9/10) of immunized animals in the EAE group exhibited clinical signs of encephalitis (and no signs of arthritis).
The onset of disease demonstrated by the arthritis score occurred between days 13 and 19 post-immunization (p.i.) in CIA as well as in OIA. For CIA, the maximum arthritis score was found between days 27 and 34 p.i. The arthritis score remained elevated during the whole observation period ($P < 0.05$ vs baseline day 16–113 p.i.).

OIA was most pronounced at day 25 p.i. ($P < 0.05$ vs baseline day 17–28 p.i.), hereafter the rats gradually improved and clinical signs of arthritis had disappeared completely at day 54 p.i. in all animals.

Serum concentrations of FIB, HA and COMP in CIA

Serum concentrations of FIB were increased at day 7 p.i. and peaked at day 21 p.i. ($P < 0.05$ vs baseline at the respective time points). The levels of FIB thereafter decreased, and were down to baseline after day 42 p.i. in both models. Elevations of HA levels appeared shortly before macroscopically apparent arthritis, and peaked at maximal arthritis, i.e. day 34 p.i. and decreased rather rapidly thereafter. In contrast, COMP levels started to increase after arthritis onset, i.e. at day 20 p.i., peaked at day 34–42 p.i. and remained elevated until day 77 p.i. ($P < 0.05$ vs baseline at the respective time points) (Table 1 and Fig. 1B–D).

Serum concentrations of FIB, HA and COMP in OIA

Serum levels of FIB were increased at day 4 p.i., peaked at day 20 p.i. ($P < 0.05$ vs baseline), and then rapidly declined ($P > 0.05$ vs baseline at day 25 p.i.). Levels of HA were increased before arthritis onset (days 8 and 12 p.i.), peaked at day 20, and then decreased rapidly. COMP levels were seen only after onset of arthritis (day 25 p.i.) and peaked at day 37 p.i. ($P < 0.05$ vs baseline at the respective time points). At day 46 p.i. COMP had returned to baseline levels.

The COMP increase was less pronounced ($P < 0.002$ for peak values) and less prolonged in OIA as compared with CIA (Table 1 and Fig. 1B–D).

Discussion

The main findings in the present study are that serum/plasma levels of FIB, HA and COMP show different patterns of changes in CIA, a chronic and...
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Fig. 1. (A) Disease scores for CIA, OIA and EAE. The maximal score in arthritic models is 16 points whereas the maximal score in EAE is 3 points. (B) Plasma levels of FIB in CIA, OIA and EAE. (C) Serum levels of HA in CIA, OIA and EAE. (D) Serum levels of COMP in CIA, OIA and EAE. All values in the figure are medians.

severely destructive arthritis, in OIA, a transient and less destructive arthritis and in EAE, a demyelinating encephalomyelitis.

In the arthritis models, FIB and HA increased before onset of clinical disease. This indicates that levels of these markers reflect inflammation. However, no significant change in HA levels could be detected in EAE. Thus, HA did not seem to reflect inflammation per se but rather inflammation in relation to arthritis. This is further emphasized by the observation that HA levels declined more rapidly in the transient OIA as compared with the more long-standing CIA. In contrast, COMP levels increased after onset of clinical arthritis. This observation indicates that the COMP levels reflect cartilage involvement. COMP did not only seem to reflect cartilage involvement but possibly also the extent of the involvement because the increase was more pronounced and more extended in time in the more chronic and more destructive CIA as compared with the transient, less destructive OIA. In conclusion, this study, which has investigated potential markers for inflammation, synovitis, and cartilage involvement in experimental arthritis, provides support for the discriminative value of these markers. Thus, FIB is a marker of inflammation in both the arthritis models and in EAE. We suggest that HA could be a marker preferentially reflecting local inflammation in the joint, i.e. synovitis. Taken together the experiments indicate that changes in serum COMP concentrations reflect the cartilage process. Thus, this study with experimental models supports the feasibility of COMP as a serum marker for cartilage involvement in arthritis. It also strengthens its potential as a tool, both in studies of mechanisms of cartilage damage in arthritis, and in studies examining effects of therapeutic interventions aimed at modifying the destructive process.

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