

Turnover of Type II Collagen and Aggrecan in Cartilage Matrix at the Onset of Inflammatory Arthritis in Humans

Relationship to Mediators of Systemic and Local Inflammation

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Objective. To determine in vivo the extent of damage to, and changes in turnover of, articular cartilage type II collagen (CII) and the proteoglycan aggrecan following the onset of inflammatory arthritis in humans, and to examine the hypothesis that there are direct relationships between cartilage biomarkers of damage/turnover and clinical, histologic, and molecular markers of inflammation.

Methods. Synovial fluid (SF) and synovial membrane (SM) were obtained by arthroscopy, and a synovitis score was determined, in 32 patients with rheumatoid arthritis (RA) (13 with early untreated disease, 19 with established disease), 18 with psoriatic arthritis (PsA), and 10 with osteoarthritis (OA). Systemic disease activity markers were recorded, and SM CD3+ T cells, CD4+ T cells, CD68+ macrophages, and lining layer hyperplasia were quantified. SF levels of tumor necrosis factor α (TNF α), interleukin-10 (IL-10), matrix metalloproteinase 1 (MMP-1), MMP-3, Col2-3/4C_{Long} mono neopeptide (C2C) (reflecting collagenase cleavage of cartilage CII), C-propeptide of type II procollagen (PIICP) (a biosynthesis marker), keratan sulfate (KS), and the 846 epitope of aggrecan (turnover) were mea-

sured by enzyme-linked immunosorbent assay or radioimmunoassay.

Results. Levels of cartilage degradation products in early RA or early PsA were not elevated above levels in OA, although in early inflammatory arthritis, TNF α and MMP-1 levels were similar to those observed in late inflammatory disease and higher than those in OA. PIICP was reduced in early RA. Correlations were observed between the SF C2C neopeptide level and the Health Assessment Questionnaire score, C-reactive protein level, plasma viscosity, synovitis score, and SF TNF α and MMP-1 levels. KS epitope content was reduced in direct relation to SM macrophage infiltration in the sublining and lining layers and in the presence of elevated SF MMP-3. Both SF MMP-1 and SF MMP-3 levels correlated with CD4+ T cell infiltration and lining layer hyperplasia in the SM, and MMP-1 levels correlated with lining layer CD68 levels, but TNF α and IL-10 levels did not.

Conclusion. Except for CII synthesis, there were no significant changes in extracellular matrix turnover of aggrecan or CII in the early stages of human inflammatory arthritis. However, the direct correlation between the increases in TNF α and MMP-1 production and collagen degradation suggests that collagenase cleavage of cartilage collagen is related to the activities of TNF α and MMP-1. The reduction in CII synthesis in early RA may contribute to the developing pathology, since a lack of synthesis of this molecule would inhibit maintenance of cartilage matrix.

Bone and cartilage erosions in inflammatory arthritides, such as rheumatoid arthritis (RA), are believed

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to involve the actions of cytokines and matrix metalloproteinases (MMPs), generated by active synovitis (1,2). Levels of MMP-1 (interstitial collagenase) and MMP-3 (stromelysin 1) are elevated in the synovial fluid (SF) and serum of patients with arthritis (3–7). Arthroscopy and magnetic resonance imaging (MRI) studies have demonstrated the presence of subclinical synovitis and bone erosions in patients with early RA, suggesting that synovial inflammation and bone and cartilage damage are early events that may predate clinical symptoms (8).

The destruction and remodeling of articular cartilage in arthritis involves increased cartilage matrix lysis and synthesis (9,10). This can be monitored by measurement of cartilage-derived synthesis and degradation products of matrix molecules released into SF and serum (9–14). Studies of cartilage turnover using these biomarkers have indicated significant changes in the turnover of the structural macromolecules in cartilage, which relate to the erosion of articular cartilage in arthritis (13–25).

Type II collagen (CII), the principle component of human articular cartilage, is excessively degraded in RA and osteoarthritis (OA), an effect believed to result from its cleavage by collagenases (26–30). Cleavage of the triple helix of CII by collagenase is up-regulated in experimental models of RA and results in the production of a collagenase-generated cleavage neopeptide, Col2-3/4C_{Long mono} (C2C), which can be detected by immunoassay (31,32). Increased cleavage in arthritis is associated with increased synthesis of type II procollagen (10,14,19–23), which can be detected by immunoassay of the C-propeptide of this molecule (PIICP). Together these assays can be used to study cartilage CII turnover in body fluids.

The main cartilage proteoglycan is aggrecan, which is composed of a core protein to which the glycosaminoglycans chondroitin sulfate (CS) and keratan sulfate (KS) are attached. Aggrecan is susceptible to proteolysis by aggrecanases and MMPs (30). KS epitopes can be detected in body fluids, where they reflect aggrecan turnover (12–18,24,33). The 846 epitope on CS chains of aggrecan has been identified on the largest molecules in healthy cartilage (34), in a region adjacent to the G3 globular domain (Morgelin M, Poole AR, Heinegård D: unpublished observations). Recent work has revealed that the release of the 846 epitope from cartilage into body fluids correlates positively with altered aggrecan turnover in arthritis (10,12–14,16,21,25,35,36).

Recent studies of joint fluids in experimental models of early inflammatory arthritis such as RA

(31,32) and in chronic human RA (4,15,16,36) have demonstrated extensive changes in cartilage extracellular matrix turnover, involving aggrecan and collagens. These changes are accompanied by alterations in SF levels of cytokines (9) and MMPs and their inhibitors (12,36), which can be regulated by these cytokines and can mediate the destruction and turnover of structural macromolecules in articular cartilage.

Better elucidation of the processes involved in the onset of joint damage would improve our understanding of the pathogenesis of early inflammatory joint disease. Toward this end, it would be desirable to study the relationships of joint cartilage matrix turnover not only to MMPs but also to cytokines such as tumor necrosis factor α (TNF α), which have been shown to play an important role in this disease. In this study, we measured some of these molecules during the early and later stages of the synovitis of inflammatory joint disease, and we compared the findings with regard to intraarticular synovitis, results of SF analysis, and histologic features of the synovial membrane (SM) in patients with inflammatory arthritis versus patients with OA.

PATIENTS AND METHODS

Patients. Patients with symptoms of inflammatory arthritis of <12 months' duration were classified as having early arthritis; those with a disease duration of >12 months were considered to have established or late arthritis. Patients with OA and patients with RA satisfied the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for diagnosis (37,38), and those with psoriatic arthritis (PsA) satisfied criteria previously defined by Veale et al (39).

A total of 60 patients with early or established arthritis were studied. Thirty-two of the patients had RA (13 with early untreated disease, 19 with established disease), 18 had PsA (10 with early untreated disease, 8 with established disease), and 10 had knee OA. The cohort consisted of 27 men and 33 women. The mean age was 52.6 years (range 20–93 years). Among patients with inflammatory arthritis, the mean disease duration since clinical onset was 38.7 months (range 1–312 months) (7.1 months [range 1–12 months] in the group with early inflammatory arthritis, 6.2 years [range 1–26 years] in the group with established inflammatory arthritis). Patients with OA were examined >12 months after the onset of clinical symptoms.

The study was approved by the local ethics committee. Written informed consent was obtained from all patients.

Assessment of systemic inflammation and disability. The following clinical and laboratory assessments were performed in patients with inflammatory arthritis: tender joint count (64 joints), swollen joint count (62 joints), modified Health Assessment Questionnaire (HAQ) (40) (range 0–24), and determination of C-reactive protein (CRP) level (normal

0–10 ng/ml) and plasma viscosity (PV) (a measure of plasma acute-phase protein equivalent to erythrocyte sedimentation rate) (normal 1.50–1.72 mPa).

Arthroscopy. All patients underwent needle arthroscopy of a symptomatic knee joint, at which time SF was obtained. Synovitis was assessed based on the results of a thorough examination and was scored on a 0–100-mm visual analog scale (VAS), which provided a global measure of inflammation based on the observers' assessment of vascularity and villous hypertrophy in all areas (41). Interobserver reliability between the arthroscopists (DJV and RR) for macroscopic synovitis has been described previously ($\kappa \geq 0.8$) (42). SF was centrifuged at 3,000 revolutions per minute for 10 minutes, and the supernatants removed and frozen at -70°C . SM biopsy samples from the cartilage–pannus junction were obtained under direct visualization and snap frozen in liquid nitrogen, foil wrapped, and stored at -70°C until processed.

Antibodies and immunohistologic analysis. Cryostat sections (6 μm thick) were cut, dried overnight, and fixed in acetone for 10 minutes. Prior to staining, endogenous peroxidase was quenched by treatment with 3% H_2O_2 for 5 minutes, followed by pretreatment in 3% normal serum for 10 minutes. Sections were incubated for 1 hour at 37°C with mouse monoclonal antibodies (mAb) to CD3, CD4, and CD68 (all at 1:100 dilution; Dako, Glostrup, Denmark) as T cell and macrophage markers. A routine 3-stage immunoperoxidase staining technique using avidin–biotin–immunoperoxidase complex (Dako) was performed. Between incubations, SM sections were washed for 5 minutes in Tris buffer. Color was developed in solution containing diaminobenzidine tetrahydrochloride (Sigma, Dorset, UK), 0.5% H_2O_2 in Tris buffer without saline (pH 7.6). SM sections were also incubated with an irrelevant isotype-specific IgG mAb (Dako), as a negative control. Slides were counterstained with hematoxylin and mounted. Positive cells were assessed under blinded conditions by 2 observers using an established and validated semiquantitative scoring method (43) (0 = no stained cells; 1 = $\leq 24\%$ stained cells; 2 = 25–50% stained cells; 3 = 51–75% stained cells; 4 = 76–100% stained cells). Lining layer thickness was measured as the number of cells in depth at 3 points in each high-power field, expressed as the mean value.

Immunoassays for SF cytokines and MMPs. SF samples were assayed for $\text{TNF}\alpha$, interleukin-10 (IL-10), collagenase 1 (MMP-1), and stromelysin 1 (MMP-3) using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Oxford, UK). MMP-1 concentrations were determined with a quantitative sandwich enzyme immunoassay technique that measures recombinant and natural human proMMP-1 but not recombinant human active MMP-1 or tissue inhibitor of metalloproteinases (TIMP)–bound MMP-1. Antibodies utilized in the assay exhibit no significant cross-reactivity or interference with recombinant human MMP-2, MMP-3, MMP-9, TIMP-1, or TIMP-2. The mean minimum detectable dose of proMMP-1 is reported as 0.021 ng/ml. MMP-3 concentrations were determined with a quantitative sandwich enzyme immunoassay technique using a polyclonal antibody specific for MMP-3. This assay recognizes recombinant and natural human total MMP-3, and the antibodies used exhibit no significant cross-reactivity or interference with re-

combinant human MMP-1, MMP-2, MMP-7, MMP-8, MMP-9, MMP-13, TIMP-1, or TIMP-2. The mean minimum detectable dose of MMP-3 is reported as 0.009 ng/ml.

Immunoassays for cartilage matrix molecules/processes. *C2C assay for CII cleavage by collagenase.* A solution-phase inhibition ELISA was used to detect the reactivity of antibodies to the carboxy-terminal neopeptide generated by the cleavage of human CII by collagenase MMPs. This immunoassay (HDM Diagnostics and Imaging, Toronto, Ontario, Canada), which is closely related to that described previously for the C2C_{Short} neopeptide (29), uses an mAb that is specific for collagenase cleavage of CII (29,31,32). A peptide encoding the epitope is used as a standard.

PIICP. A solution-phase inhibition radioimmunoassay (RIA) was used to detect PIICP, which is a measure of type II procollagen synthesis. This RIA utilizes bovine PIICP isolated from fetal cartilage. The assay was used as described previously (23).

846 epitope of the proteoglycan aggrecan. Aggrecan fragments in joint fluid bearing the 846 CS epitope were assayed by RIA using an mAb as previously described (16,25). Results are expressed as weight equivalents of intact fetal human cartilage aggrecan.

KS epitope of aggrecan. The concentration of KS epitope (aggrecan equivalents) was measured by RIA using an mAb (AN9P1) directed against a highly sulfated epitope on KS, as previously described (16). Results are expressed as weight equivalents of adult human cartilage aggrecan. These assays have all been validated for use in human SF (36).

Statistical analysis. Data were stored on Microsoft Excel spreadsheets and analyzed using SPSS for Windows, version 9.0. Correlations were identified by Spearman's rank correlation test, and data were corrected for multiple testing, using the Bonferroni method. The Kruskal-Wallis and the Mann-Whitney tests were used as appropriate, to assess the statistical significance of differences between groups. *P* values less than 0.05 were considered significant.

RESULTS

Systemic and intraarticular inflammation and disability in the patient groups (Figure 1). Levels of the acute-phase reactant CRP were above the normal range in both the early RA group (mean \pm SD 13.5 ± 12.7 ng/ml) and the early PsA group (12.2 ± 6.2 ng/ml), and both CRP and PV levels were significantly higher in patients with established RA than in those with early RA (both $P < 0.001$). CRP levels were also higher in patients with established RA than in those with early PsA (established PsA ($P < 0.001$ and $P < 0.05$, respectively). The tender joint count tended to be higher in the early RA group than in the early PsA group, but the difference was not significant. Both the tender joint count and the swollen joint count were higher in patients with established RA than in those with early RA ($P < 0.05$ and $P < 0.01$, respectively), and the swollen joint count

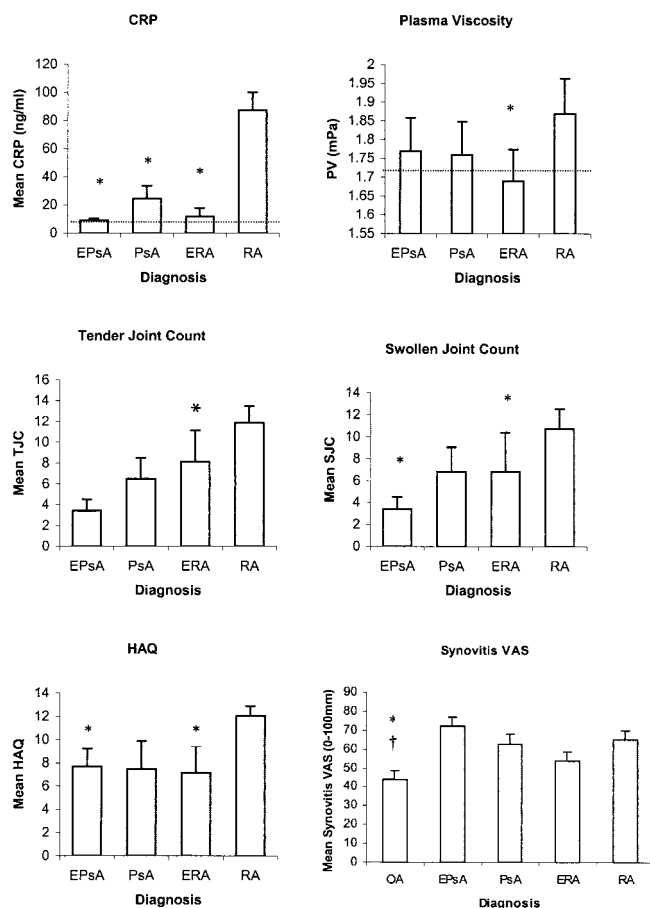


Figure 1. Parameters of systemic and local disease activity and severity in patients with early psoriatic arthritis (EPsA), established PsA (PsA), early rheumatoid arthritis (ERA), and established RA (RA). Broken lines indicate the upper level of the normal ranges for C-reactive protein (CRP) (<10 ng/ml) and plasma viscosity (PV) (<1.72 mPa). TJC = tender joint count (0–64); SJC = swollen joint count (0–62); HAQ = Health Assessment Questionnaire (0–24); VAS = visual analog scale (also determined in patients with osteoarthritis [OA]). Values are the mean and SEM. * = significantly less than established RA group; † = significantly less than early PsA group (both $P < 0.05$).

was higher in patients with established RA than in those with early PsA ($P < 0.01$). The HAQ scores in patients with early RA and those with early PsA were equivalent, and were significantly lower ($P < 0.05$) than in patients with established RA.

VAS scores for synovitis were higher in patients with inflammatory arthritis than in those with OA ($P < 0.01$), and significant subgroup differences were noted between the OA and early PsA groups and between the OA and established RA groups (both $P < 0.05$). The VAS score for synovitis tended to be higher in patients

with established disease compared with those with early disease, whereas it tended to decrease between the early stage and the established stage of PsA. Indeed, the synovitis score was higher in the early PsA group than in any other group.

Synovial fluid analysis (Figure 2). Levels of the proinflammatory cytokine TNF α were higher in the SF of patients with early RA or early PsA than in those with OA (both $P < 0.05$), and levels were further increased in patients with established disease, although these increases were not significant compared with the levels in patients with early disease. The antiinflammatory cytokine IL-10 exhibited a very different relationship to disease. IL-10 was highly expressed in early PsA, but levels were markedly and significantly reduced in established PsA ($P < 0.01$), being even lower in PsA at this stage than in OA ($P < 0.05$). In RA, IL-10 levels in patients with early disease were similar to those found in OA patients, but also decreased during established disease ($P < 0.05$, established RA versus OA and versus early PsA).

Concentrations of MMP-1 were significantly higher in all early and established inflammatory arthritis groups than they were in the group with OA (all $P < 0.05$). Among the early or established PsA and RA groups, MMP-1 levels were similar. Similarly, MMP-3 levels were significantly higher in all inflammatory arthritis groups than in the OA group (all $P < 0.001$), but, in contrast to MMP-1, MMP-3 levels reflected in part the synovitis VAS by increasing from early to established RA ($P = 0.01$) and being higher in established RA than in established PsA ($P < 0.05$).

In spite of these variations in MMP levels, the C2C neoepitope (collagenase cleavage of CII) was similar in content in SF from patients with early and those with established inflammatory arthritis (both PsA and RA), and in OA patients. Among RA patients and among PsA patients, the median C2C level was higher in those with established disease, but the difference compared with patients with early disease did not reach statistical significance. Conversely, levels of the PIICP biomarker were higher in patients with OA, early PsA, and established RA than in those with early RA (all $P < 0.05$). Compared with levels in OA patients, levels of the KS biomarker were increased, but not significantly, in patients with early PsA and established PsA, but not in those with early or established RA. Concentrations of the 846 epitope in all of the groups with inflammatory arthritis were similar to those in the group with OA.

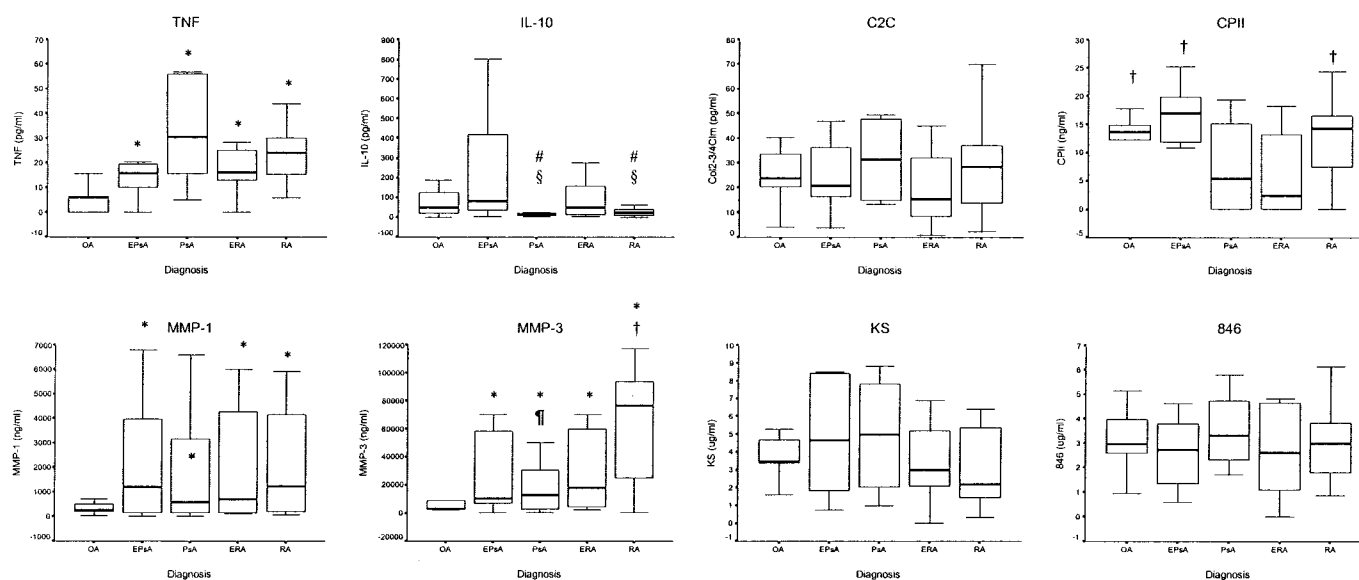


Figure 2. Synovial fluid concentrations of proinflammatory cytokine tumor necrosis factor α (TNF), antiinflammatory cytokine interleukin-10 (IL-10), matrix metalloproteinase 1 (MMP-1), and MMP-3 and of biomarkers of cartilage turnover, including the collagenase cleavage (Col2-3/4_{Long mono}) neopeptide of type II collagen (C2C), the C-propeptide of cartilage type II procollagen (CPII), and the keratan sulfate (KS) and 846 epitopes of aggrecan, in patients with OA, early PsA, established PsA, early RA, and established RA. Boxes represent the 25th through 75th percentiles; vertical lines outside the boxes represent the 10th and 90th percentiles; horizontal lines within the boxes represent the median. * = significantly greater than OA group; # = significantly less than OA group; § = significantly less than early PsA group; † = significantly greater than early RA group; ¶ = significantly less than established RA group (all $P < 0.05$). See Figure 1 for other definitions.

Histologic features of synovial membrane (Figure 3). SM T cell infiltration (CD3+ and CD4+), macrophage infiltration (CD68+) at the sublining layer, and lining layer hyperplasia tended to be greater in PsA (early or established) than in OA, and greater in RA (early or established) than in PsA or OA. No significant differences were noted between early and established disease in PsA or RA.

The proportion of SM CD3+ cells was significantly greater in early RA than in early PsA, and CD4+ cell infiltration was significantly greater in early PsA, established PsA, early RA, and established RA than in OA (all $P < 0.05$). CD68+ macrophage infiltration at the sublining layer was greater in early RA and established RA than in OA or established PsA (all $P < 0.05$), although CD68+ lining layer infiltration was not significantly different between any of the groups. Lining layer hyperplasia was significantly lower in OA than in early PsA, established PsA, early RA, or established RA (all $P < 0.05$) and lower in early PsA than in either early RA or established RA (both $P < 0.05$).

Interrelationships between clinical, biochemical, and histologic measures of local and systemic disease and inflammation (Table 1). Measures of systemic disease were examined to determine whether there were

relationships between any of these measures, or between these measures and changes in an individual joint. These comparisons were made using the data from all patients. The CRP level and PV correlated strongly with one another ($P < 0.01$). Both correlated with the swollen joint count ($P < 0.01$ for CRP, $P < 0.05$ for PV) and the tender joint count ($P < 0.05$ for CRP, $P < 0.01$ for PV), and the swollen joint count and tender joint count correlated with one another ($P < 0.01$). The HAQ score correlated with the CRP level, PV, swollen joint count, and tender joint count (all $P < 0.01$). Serum CRP levels closely correlated with SF MMP-3 levels, and the synovitis VAS score correlated with the SF MMP-1 concentration (both $P < 0.01$). The MMP-1 level and MMP-3 level correlated with one another ($P < 0.01$). Neither TNF α nor IL-10 showed significant correlations with other clinical, biochemical, or histologic measures.

SM cellular infiltrates were all strongly interrelated, except that neither CD3+ nor CD4+ T cells demonstrated a relationship to CD68+ macrophage lining layer infiltration. Lining layer hyperplasia also correlated with each of the other histologic parameters assessed ($P < 0.01$ for CD3+ cells, CD4+ cells, and CD68+ macrophage sublining layer infiltration; $P < 0.05$ for CD68+ macrophage sublining layer infiltra-

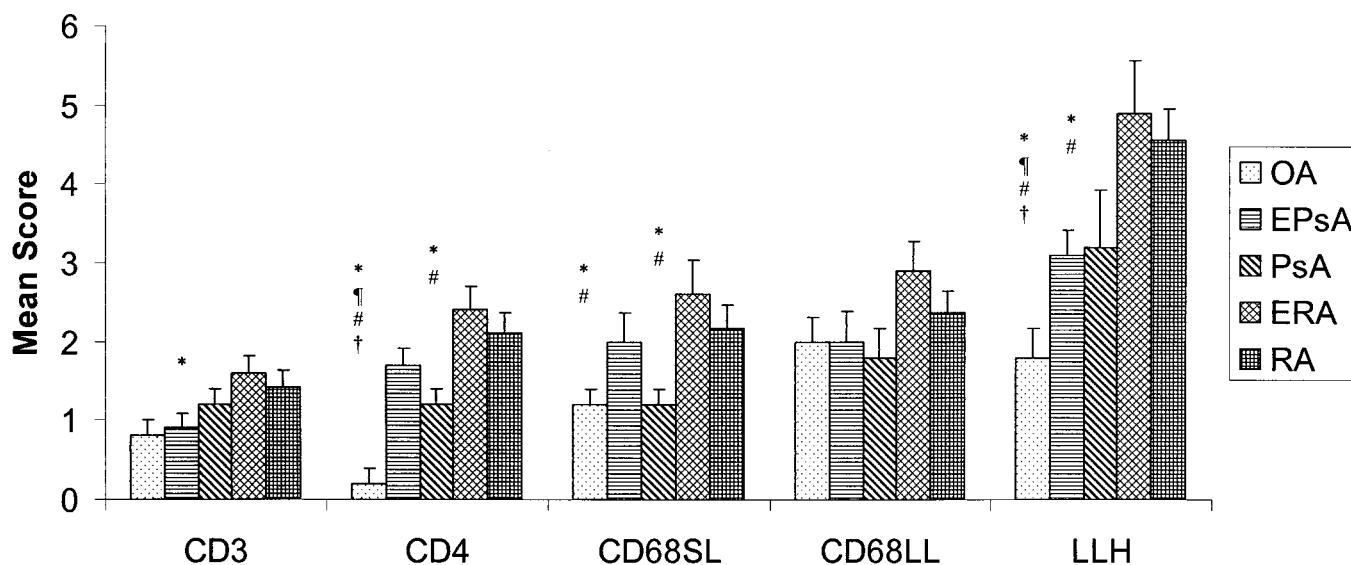


Figure 3. Synovial membrane histologic features in patients with OA, early PsA, established PsA, early RA, and established RA. T cell markers CD3 and CD4 and the macrophage marker CD68 at the sublining layer (CD68SL) and the lining layer (CD68LL) were measured using a semiquantitative scale of 0–4, and lining layer hyperplasia (LLH) was measured using a scale of 0–7. Values are the mean and SEM. * = significantly less than early RA group; † = significantly less than established PsA group; # = significantly less than established RA group; ‡ = significantly less than early PsA group (all $P < 0.05$). See Figure 1 for other definitions.

tion). The only clinical parameter to demonstrate a relationship with a histologic measure was the tender joint count, which correlated with macrophage infiltration at the lining layer ($P < 0.05$). Levels of the SF

cytokines TNF α and IL-10 did not correlate with cellular infiltration or lining layer hyperplasia. The most notable and persistent correlations with histologic measures were those observed for SF MMP-1 levels, which corre-

Table 1. Correlations between systemic and local markers of disease activity and severity, synovial fluid concentrations of cytokines, and matrix metalloproteinases, and synovial membrane histologic features*

| | Synovitis | | | | | | | | | | | | | |
|----------------|-----------|--------|--------|--------|--------|--------------|--------|--------|--------|--------|--------|--------|--------|--------|
| | PV | SJC | TJC | VAS | HAQ | TNF α | IL-10 | MMP-1 | MMP-3 | CD3 | CD4 | CD68SL | CD68LL | LLH |
| CRP | 0.675† | 0.396† | 0.387‡ | 0.170 | 0.505† | 0.108 | -0.086 | -0.029 | 0.376† | 0.277 | 0.227 | 0.038 | 0.064 | 0.230 |
| PV | | 0.510‡ | 0.444† | 0.315 | 0.622† | 0.091 | 0.184 | -0.062 | 0.012 | 0.259 | 0.256 | 0.075 | 0.070 | 0.115 |
| SJC | | | 0.880† | 0.017 | 0.527† | 0.289 | 0.051 | -0.131 | -0.126 | 0.182 | 0.116 | 0.197 | 0.197 | -0.116 |
| TJC | | | | -0.040 | 0.638† | 0.304 | -0.038 | -0.219 | -0.030 | 0.270 | 0.062 | 0.370 | 0.429‡ | -0.014 |
| Synovitis, VAS | | | | | 0.128 | 0.227 | 0.197 | 0.456† | 0.301 | 0.285 | 0.222 | 0.131 | 0.220 | 0.191 |
| HAQ | | | | | | 0.187 | -0.042 | -0.131 | 0.087 | 0.242 | 0.301 | 0.068 | 0.086 | 0.172 |
| TNF- α | | | | | | | -0.226 | -0.014 | 0.157 | 0.014 | -0.016 | 0.019 | 0.050 | -0.019 |
| IL-10 | | | | | | | | -0.079 | -0.006 | -0.011 | -0.038 | 0.194 | 0.091 | 0.054 |
| MMP-1 | | | | | | | | | 0.567† | 0.302 | 0.478† | 0.379‡ | 0.447† | 0.449† |
| MMP-3 | | | | | | | | | | 0.317 | 0.379‡ | 0.299 | 0.343 | 0.522† |
| CD3 | | | | | | | | | | | 0.661† | 0.394† | 0.207 | 0.434† |
| CD4 | | | | | | | | | | | | 0.478† | 0.287 | 0.521† |
| CD68SL | | | | | | | | | | | | | 0.753† | 0.522† |
| CD68LL | | | | | | | | | | | | | | 0.361‡ |

* Values are correlation coefficients calculated using Spearman's rank correlation. PV = plasma viscosity; SJC = swollen joint count; TJC = tender joint count; HAQ = Health Assessment Questionnaire; VAS = visual analog score; TNF α = tumor necrosis factor α ; IL-10 = interleukin-10; MMP-1 = matrix metalloproteinase I; CD68SL = macrophage marker CD68 at the sublining layer; CD68LL = macrophage marker CD68 at the lining layer; LLH = lining layer hyperplasia; CRP = C-reactive protein.

† $P < 0.01$.

‡ $P < 0.05$.

Table 2. Correlations between biomarkers of cartilage turnover and systemic and local markers of disease activity and severity*

| | C2C | PIICP | KS | 846 |
|--------------|--------|--------|---------|--------|
| CRP | 0.372† | 0.157 | -0.333 | 0.169 |
| PV | 0.397† | 0.267 | -0.026 | 0.397† |
| SJC | 0.215 | 0.006 | -0.107 | 0.289 |
| TJC | 0.068 | -0.063 | -0.107 | -0.185 |
| Synovitis | 0.375† | 0.203 | -0.178 | 0.080 |
| HAQ | 0.479‡ | -0.180 | -0.207 | 0.049 |
| TNF α | 0.330† | -0.080 | 0.000 | 0.029 |
| IL-10 | -0.025 | 0.051 | -0.004 | 0.029 |
| MMP-1 | 0.464† | 0.097 | -0.172 | -0.014 |
| MMP-3 | 0.006 | 0.118 | -0.297† | 0.008 |
| CD3 | -0.024 | -0.050 | -0.244 | 0.037 |
| CD4 | -0.044 | -0.003 | -0.143 | -0.055 |
| CD68SL | 0.137 | -0.118 | -0.510† | -0.090 |
| CD68LL | 0.296 | -0.203 | -0.416† | 0.095 |
| LLH | 0.045 | 0.030 | -0.137 | 0.273 |

* Values are correlation coefficients calculated using Spearman's rank correlation. C2C = Col2-3/4_{Long mono} neoepitope (reflecting collagenase cleavage of type II collagen); PIICP = C-propeptide of type II procollagen; KS = keratan sulfate epitope of aggrecan (aggrecan equivalents); 846 = 846 epitope of aggrecan (see Table 1 for other definitions).

† $P < 0.05$.

‡ $P < 0.01$.

lated with CD4+ cells ($P < 0.01$), CD68+ macrophage sublining layer infiltration ($P < 0.05$), CD68+ macrophage lining layer infiltration ($P < 0.01$), and lining layer hyperplasia ($P < 0.01$), and for SF MMP-3 levels, which correlated with CD4+ cells ($P < 0.05$) and lining layer hyperplasia ($P < 0.01$).

Interrelationships between cartilage biomarkers and clinical, biochemical, and histologic measures of local and systemic disease and inflammation (Table 2). SF C2C neoepitope levels correlated with CRP levels ($P < 0.05$), PV ($P < 0.05$), synovitis VAS scores ($P < 0.05$), and HAQ scores ($P < 0.01$). C2C levels also correlated with SF TNF α and MMP-1 levels (both $P < 0.05$). Joint counts did not correlate with any of the cartilage biomarkers, whereas MMP-3 concentrations were inversely correlated with KS epitope levels (aggrecan turnover) ($P < 0.05$); PV also correlated with levels of the 846 epitope ($P < 0.05$). C2C demonstrated no correlations with CII synthesis (PIICP) or with aggrecan turnover (KS and 846 epitopes) (data not shown). PIICP levels also did not correlate with levels of the 846 or KS epitopes (data not shown), indicating no linkages between aggrecan and CII turnover. However, KS and 846 epitope levels were well correlated with one another ($r = 0.561$, $P < 0.01$). Biomarkers of cartilage turnover did not demonstrate any specific relationships to SM histologic features, apart from an inverse relationship of KS epitope levels to CD68+ macrophage infiltration

both at the sublining layer and the lining layer (both $P < 0.05$).

DISCUSSION

The natural history of joint inflammation and its relationship to joint destruction continue to be the subject of much debate. There is mounting evidence from both MRI and arthroscopy studies that synovitis is well established in patients with early inflammatory arthritis and that this is associated with early joint damage (8). The study of articular cartilage at the molecular level has resulted in a new understanding of the processes involved in the destruction and remodeling of cartilage in the diseased joint. SF and serum biomarkers of cartilage assembly, degradation, and turnover have been identified and examined, primarily in animal models and human OA. Yet little is known about early damage to cartilage, proteoglycans, and collagens at the onset of inflammatory arthritis in humans. Studies of intraarticular changes in an experimental model of antigen-induced arthritis have revealed rapid onset of extracellular matrix degradation involving both proteoglycan and collagen (9,32). One recent study examined cartilage turnover in established RA (mean disease duration 10.1 years), but the degree of disease activity and inflammation was characterized only by SF polymorphonuclear cell (PMN) elastase levels (36). To our knowledge, the present investigation is the first to examine changes in human cartilage at the time of onset of inflammation, and the relationship between these changes and inflammation in a well-characterized clinical cohort of patients with different types of early and established inflammatory arthritis.

We used a number of biomarkers to investigate interrelationships between inflammation, cartilage degradation, and matrix turnover, and the results have provided valuable insights into molecular processes, their possible relationships in vivo, and the development and cause of inflammatory arthritis. Experiments with biomarkers are based on the specificities of the antibodies used to detect them. We must recognize, however, that any structural changes in these molecules detected by the antibodies, such as posttranslational alterations in glycosylation or hydroxylation of proteins, may lead to altered epitope recognition by antibodies. It is possible that such changes may have contributed to the findings observed.

Patients with early RA and those with early PsA both demonstrated a systemic acute-phase response as would be expected, and this was more marked in patients

with established PsA and established RA. The tender and swollen joint counts also were increased in established RA and PsA. The HAQ scores suggested a greater degree of disability in established RA than in established PsA, and this may reflect the frequency of polyarticular involvement in RA as opposed to PsA. These systemic changes were, however, not reflected at the single-joint level by the arthroscopically observed synovitis (VAS score), which was comparable in early and late inflammatory disease and indeed was highest in the group with early PsA.

The pattern of IL-10 and TNF α expression demonstrated in our patients with PsA and RA is characteristic of these arthropathies, with higher IL-10 levels and lower TNF α levels in the patients with early PsA compared with those with early RA. Previous comparative analyses have revealed the ratio of immunosuppressive (IL-10) to proinflammatory (TNF α) cytokines to be substantially higher in spondylarthropathy than in RA (44–46). Analysis of peripheral blood T cell TNF α secretion at the onset of reactive arthritis demonstrates that low secretors are at higher risk of developing a chronic pattern of disease than are high secretors, that reactive arthritis T cell clones will readily secrete IL-10 upon stimulation with specific bacteria, and that IL-10 down-regulates TNF α production (47,48). It is suggested that this impaired Th1 cytokine response may be partially responsible for a failure to clear intraarticular bacteria, resulting in chronic inflammation. Established disease in our cohort was further characterized by rising TNF α levels together with a marked reduction in IL-10 levels in both arthropathies, most notably in PsA.

Synovial fluid concentrations of MMP-1 and MMP-3 in early inflammatory disease were markedly elevated, to levels similar to those in established inflammatory disease. Using a proMMP-1 assay similar to the one used in the present study but with a lower reported sensitivity, Ishiguro et al (36) found SF levels of MMP-1 to be lower in RA patients than in OA patients, although the difference was not significant. This finding is not in accordance with previously reported values for SF MMP-1, which show, as in the present study, significantly higher levels in RA than in OA (6,49). MMP-1 production is stimulated by many proinflammatory cytokines known to be active in inflammatory arthritis, including oncostatin M, IL-1, and TNF α , and it correlates with the degree of histologic inflammation of the SM (50,51). The report by Ishiguro and colleagues does not describe the degree of disease activity in the RA cohort, although the low PMN elastase levels (and hence low SF MMP-1 levels) reported may indicate less active

disease and a low degree of tissue inflammation in the group studied (34,52). The lower sensitivity of the proMMP-1 assay could also explain the low MMP-1 concentrations observed, although this would not explain why higher levels were found in OA than in RA patients. The SF MMP-1 and MMP-3 levels we report here are similar to those previously observed in patients with rheumatic disease (52,53).

The SM histologic differences noted between OA, PsA, and RA patients reflect previously published data (54,55). Specifically, the T cell and macrophage infiltration of SM and the degree of lining layer hyperplasia were less marked in PsA than in RA but greater in either PsA or RA than in OA, and these differences were already present in early disease. Since MMP levels and activity are up-regulated by the activities of these cells, it is not surprising that both MMP-1 and MMP-3 levels correlated with the degree of histologic infiltration of the SM, although the lack of a relationship of SF TNF α and IL-10 levels with either histologic inflammation or MMP-1 and MMP-3 levels was surprising.

A striking finding was that levels of biomarkers of collagen and aggrecan turnover were similar in early compared with late inflammatory arthritis, except in the case of PIICP, where clear differences were seen. The analyses of synovitis also reflected no differences between early and established disease. The increase in synovitis compared with OA clearly does not contribute to variations in biomarker levels. Previous studies using arthroscopy and MRI have highlighted the presence of more widespread and aggressive synovitis and bone erosion in early inflammatory arthritis than is evident with physical examination or plain radiography (8). Our findings, however, reveal that at a molecular level, compared with OA, altered matrix turnover in articular cartilage is not apparent in early inflammatory arthritis, except in the case of PIICP in early RA. Suppression of type II procollagen synthesis, which tends to be more marked in the presence of SM macrophage infiltration, is observed prior to the onset of more severe clinical and radiologic evidence of damage. This indicates the value of this biomarker for the early detection of disease and associated skeletal changes which are associated with cellular infiltration involving macrophages.

The C2C neoepitope generated at the primary collagenase cleavage site in CII is recognized by an mAb that sees a collagenase-generated conformational epitope with sequence specificity for CII (Poole AR, Ionescu M: unpublished observations). This offers a clear opportunity to detect and measure cartilage degradation. Results of studies using experimental models

have been promising (31,32), but this is the first clinical study to comprehensively examine the relevance of SF C2C to joint changes in vivo. This biomarker showed clear correlations with systemic inflammation (CRP and PV) and disability (HAQ) and, at a local level, correlated with synovitis and was the only biomarker to correlate with SF TNF α . This suggests that C2C is reflective of inflammation, both systemic and intraarticular, in arthritis and that TNF α is involved directly or indirectly in collagen cleavage. Interestingly, however, and perhaps of even greater importance, we noted a close correlation between levels of MMP-1 (but not MMP-3) and C2C in the SF. This directly links the C2C neoepitope in vivo to levels of one of the collagenases that can generate this epitope, lending support to the notion that the C2C epitope measured in this study reflects CII triple-helical cleavage by collagenases such as MMP-1.

Levels of the KS epitope in serum have previously been noted to be inversely related to TNF α levels (24) and serum acute-phase protein levels (15) in RA. We have now demonstrated, at an intraarticular level, an inverse correlation between SF KS epitope and MMP-3 concentrations. KS epitope concentrations have been shown previously to be inversely related to SF PMN numbers in OA (16). We showed that KS epitope concentrations in the SF were strongly and inversely correlated with CD68+ macrophages in the SM sublining and lining layers. KS epitopes also reside on newly synthesized aggrecan. Therefore, if synthesis of newly synthesized aggrecan is inhibited by mediators of inflammation, an inverse relationship between these molecules would be expected. Our observations regarding MMP-3 and its correlation with CRP and synovitis also provide evidence that it is reflective of inflammation as well as proteolysis.

The 846 epitope is almost absent in normal human adult articular cartilage; however, it appears during disease states (34,56). Increased levels of serum 846 epitope in patients with longstanding RA have been described previously (16), although in the serum of patients with early, rapidly progressive RA 846 levels appear to be suppressed (21). In this study, SF levels of 846 correlated only with PV, despite the direct relationship of 846 levels to levels of the KS epitope. Clearly, the 846 epitope measures a different subset of aggrecan molecules, as is also reflected by the results of the cross-sectional studies illustrated in Figure 2.

In conclusion, this study identifies linkages between systemic inflammation, local joint inflammation, and increased cartilage turnover in early human inflam-

matory arthritis. There is evidence of extensive changes in extracellular matrix turnover involving both proteoglycan and CII. Levels of the C2C collagen II cleavage neoepitope correlate with measures of local and systemic inflammation and with SF MMP-1 and TNF α levels, directly implicating these molecules in CII cleavage in vivo in inflammatory arthritis. The C2C neoepitope, like PIICP as a measure of CII synthesis, may be of use as a clinical prognostic biomarker for disease onset and activity in the arthritides. Future prospective analysis of SF C2C in arthritis, with cartilage assessment by arthroscopy and MRI, may help determine the value of this biomarker in clinical practice.

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