Postersession

Cartilage proteins in serum and synovial fluid as markers for cartilage degradation

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Cartilage oligomeric matrix protein (COMP) (Hedbom et al. 1992), a pentameric chondrocyte-binding glycoprotein, and cartilage matrix protein (CMP) (Paulsson and Heinegård 1982), a trimeric glycoprotein, have been characterised from different cartilages.

The structure of COMP, as revealed by electron microscopy, consists of five arms containing a peripheral glomerular domain, a flexible strand and a central assembly domain, where the five arms meet in a cylindrical structure (Mörgelin et al. 1992, DiCesare et al. 1994a). COMP can be isolated from human articular cartilage and is present in much lower amounts in other tissues, with exception of tendon (DiCesare et al. 1994b, 1995). The physiological role of COMP is unclear yet; however, evidence exists that COMP interacts with chondrocytes (DiCesare et al. 1992, 1994a).

In serum, rheumatoid arthritis (RA) patients with rapidly progressive joint destruction have initially increased levels of COMP, which subsequently decrease (Forslind et al. 1992). RA patients with more benign disease, and less extensive joint damage, have normal serum levels of COMP (Forslind et al. 1992, Saxne and Heinegård 1992).

In joint fluid, increased amounts of COMP are found after knee injury and in early stage osteoarthrosis (OA) (Lohmander et al. 1994). Similarly, high knee joint synovial fluid levels of COMP are found in reactive arthritis and low levels are observed in RA patients with advanced destruction of the joint. In patients with long-standing reactive synovitis, the concentration is decreased (Saxne and Heinegård 1992).

We evaluated whether COMP and/or CMP in serum and synovial fluid can be employed as markers for cartilage degradation in 60 patients with OA or RA. In addition, we determined whether these circulating matrix proteins are associated with the presence of specific autoantibodies, including IgG anti-COMP, IgM rheumatoid factors (IgM-RF) and IgG anti-type II collagen autoantibodies (IgG ACA II).

Material and methods

Human COMP was purified from articular cartilage (DiCesare et al. 1994a, 1995) and bovine CMP was isolated from rib cartilage (Hauser and Paulsson 1994). Polyclonal antibodies against human COMP and bovine CMP were raised in rabbits. They were used to detect protein levels in serum and in hyaluronidase-treated synovial fluid by competitive enzyme-linked immunosorbent assay (ELISA) as well as by qualitative immunoblots. IgG anti-COMP autoantibodies, IgM-RF and IgG ACA II were determined by ELISA.

Results and Discussion

The amount of COMP detected in serum correlated with ageing in healthy subjects ($P < 0.05$, $n = 24$), but not in patients. Increased serum levels of COMP (> mean plus two standard deviations of controls) occurred in 73 and 30 percent of patients with OA and RA, respectively. In RA, an elevated amount of COMP in serum seems to be associated with a longer morning stiffness ($P < 0.05$), but not with elevated C-reactive protein or erythrocyte sedimentation rate. At early stages, RA patients with aggressive diseases have increased serum levels of COMP (Forslind et al. 1992).

The levels of COMP in synovial fluid were always higher than in serum. However, on average, the synovial fluid levels of total COMP were not significantly different between the groups. Despite this, important qualitative differences can be observed in some patients with OA and most cases with RA (Figure 1). In synovial fluids of patients, some intact protein (about 500 kDa) and monomers (about 110 kDa) can be detected, but in addition there exist high amounts

of well defined fragments. Under non-reducing conditions, 25/30 (83%) synovial fluids taken from RA patients (but only 2/8 controls and 4/30 OA patients) showed an additional low molecular weight (MW) COMP fragment (50-60 kDa). Under reducing conditions, a pattern of two large (85–90, 75–80 kDa) and three small immunoreactive COMP molecules can be obtained (65–70, 55–60, < 55 kDa). In controls and OA patients, the two large molecules together constituted 90 and 86 percent of the synovial fluid COMP; they resembled to the intact monomers of purified human COMP. In RA, the relative amount of the largest COMP molecule (85–90 kDa) was reduced ($P < 0.01$); this was accompanied by increases in proportions of the three smallest fragments ($P < 0.01$). In serum, mostly fragments were present. Applying similar methods to CMP, there was, in contrast, no detectable protein present in serum or synovial fluid in controls as well as in the examined group of patients (detection level of < 0.1 µg/ml). This shows that this protein is much less abundant in the examined body fluids.

Furthermore, on average, the serum levels of IgG anti-COMP autoantibodies were similar in controls and patients. However, in all RA synovial fluids, high levels of IgG anti-COMP autoantibodies occurred ($P < 0.01$, compared with controls). In RA synovial fluid only, the correlation network was completed by the associations between IgG anti-COMP with both IgM-RF and IgG ACA II ($P < 0.01$ for both).

In summary, our preliminary results suggest that, at least in RA: 1) important proteolytic processes occur affecting the COMP molecule, probably as many other proteins released into the synovial fluid; and 2) an autoimmune reaction occurs against COMP and its degradation products, probably a physiological process in serum, but with pathological significances in synovial fluid. In view of these data, the possibility of a T-cell reaction against COMP should be investigated. An important question for the future is the clinical significance of COMP and its fragments in various types of arthritis. They could be of prognostic value and/or allow to monitoring the disease activity. Thus, longitudinal studies need to be done.

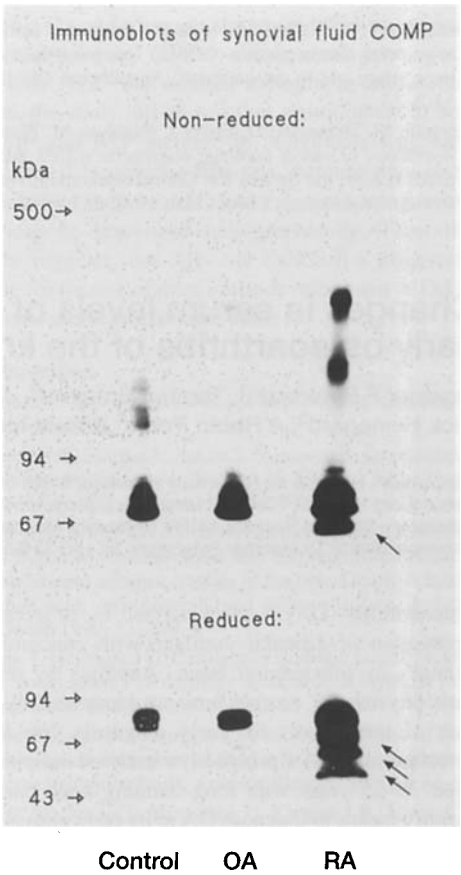

Figure 1. Representative immunoblots of synovial fluid COMP in controls, osteoarthrosis (OA) and rheumatoid arthritis (RA) patients, under non-reducing or reducing conditions. The immunoreactive COMP molecules in controls and OA resembled to the intact monomers of purified human COMP. In RA, the arrows show the additional low molecular weight COMP fragment under non-reducing conditions, which in fact correspond to three small COMP fragments under reducing conditions.

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Changes in serum levels of cartilage and bone markers in early osteoarthritis of the knee

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Osteoarthritis (OA) is characterized by progressive destruction of articular cartilage with concomitant changes in subchondral bone. Attempts to reveal pathophysiologic mechanisms are hampered by the lack of instruments for early diagnosis. We have therefore initiated a prospective study of individuals aged 35–55 years with long-standing knee pain to identify means to diagnose OA in its early phases and to monitor the progression of the disease by using novel biochemical and imaging techniques (Petersson et al. 1993).

In an attempt to delineate differences in tissue specific molecular fragments in serum between individuals with or without radiographic evidence of OA, sequential measurements of cartilage and bone markers in sera of individuals with knee pain were performed. The serum concentrations were correlated to presence of radiographic and bone scintigraphic changes in the knee joints at the 3-year follow-up.

Materials and methods

Individuals with chronic knee pain (>3 months during the last 12 months) were identified using a questionnaire sent to a random sample of the population in a community in southern Sweden (Petersson et al. 1993). Initially 279 out of 2000 individuals were identified as having chronic knee pain. Of these, 204 accepted clinical and radiographic examination. In this report, 38 individuals were randomly selected for follow-up. Baseline radiographs and serum samples were available. Serum samples were drawn at follow-up 3 years after inclusion. Plain radiographs were taken under weight-bearing conditions and bone scintigraphic examination using technetium labeled diphosphonate was performed at follow-up on both knee joints on the same day as serum was drawn. The radiographs were graded according to Ahlbäck for tibiofemoral OA (Ahlbäck 1968) and by an arbitrary scale for patellofemoral OA. The scintigraphy was visually evaluated using an arbitrary scale. All evaluations were done without knowledge of clinical data.

Serum samples were analyzed for cartilage oligomeric matrix protein (COMP), bone sialoprotein (BSP), and the keratan sulfate (KS) found primarily in the proteoglycan aggrecan by specific immunoassays (Saxne and Heinegård 1992, Saxne et al. 1995, Poole et al. 1990). The Spearman correlation coefficient was used for calculating correlations and the Wilcoxon matched pair's test for comparisons between serum levels at baseline and at follow-up. P-values < 0.05 were considered significant.

Results

Twenty-three subjects had radiographic signs of OA at the 3-year follow-up (6 in the tibiofemoral compartment, 14 in the patellofemoral compartment and 3 in both compartments). Fifteen subjects had normal radiographs after 3 years. Scintigraphic abnormalities were detected in 25 cases. In 7 of these no radiographic changes were seen.

The serum concentrations of COMP at follow-up correlated significantly ($p < 0.001$) with the grades of the scintigraphy findings at follow-up ($r_s=0.56$ for all subjects, $r_s=0.65$ for the subjects with radiographic OA). The serum concentrations of BSP or KS did not correlate with the scintigraphic findings.

The serum concentrations of the tissue specific molecular markers at baseline or at follow-up did not