C reactive protein and immunoglobulin G in synovial fluid and serum in joint disease

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Abstract

C reactive protein (CRP) and immunoglobulin G (IgG) were measured in synovial fluid and serum of 72 patients (29 with rheumatoid arthritis (RA), 17 with osteoarthritis, 11 with crystal synovitis, seven with undifferentiated arthritis, and eight with seronegative arthritis). The synovial fluid:serum (SF:S) ratios were compared with those calculated from the SF:S ratios of transferrin, caeruloplasmin, and α_2 macroglobulin, using the binomial test within groups and the Mann-Whitney test between groups.

In RA synovial fluid CRP concentrations were lower than expected and IgG concentrations higher than expected. In osteoarthritis CRP concentrations were higher than expected. In seronegative arthritis IgG concentrations were raised. The ratio of CRP:IgG was depressed in RA. These findings are consistent with a role for CRP in the inflammatory process of RA, while the CRP:IgG ratio may be of value in the differential diagnosis of joint disease.

The concentration of a protein in synovial fluid depends upon its concentration in plasma, its molecular weight (M_r) and shape, synovial permeability, and whether local production or consumption occurs.¹ For many plasma proteins—for example, transferrin, caeruloplasmin, and α_2 macroglobulin, an inverse linear relation exists between the logarithms of the ratio of synovial fluid:serum concentration (SF:S) and M_r ,²⁻⁷ and between SF:S and molecular diameter.¹ This indicates that their site of production is extra-articular, and that they are not consumed inside the joint. Inflammation of the joint is associated with a decrease in the slope of the curve and an increase in the intercept.^{1,2} Comparison of the SF:S ratios of other proteins with this curve can provide evidence of local production or consumption within the joint.^{2-4,5,7}

C reactive protein (CRP) is the classical acute phase protein.⁸ Measurement of serum CRP concentrations is of use in the assessment of activity of rheumatoid arthritis (RA).^{9,10} It has been shown to be present in joints affected by RA,¹¹ and to be deposited in experimentally produced lesions, both in joints and other tissues.¹² We have previously reported lower than expected concentrations of CRP in synovial fluid from patients with RA,¹³ a finding which has recently been confirmed.⁵

In inflammatory joint disease synovial fluid IgG concentrations are raised.² In active RA

local IgG production occurs in the synovium: this can be inferred from the increased SF:S ratios,³ and confirmed by the demonstration of production of IgG by lymphocytes isolated from the synovium.¹⁴ The poor correlation between concentrations of total IgG in synovial fluid and serum¹⁵ may be due to local production, and because of differences between subjects in the slopes of the SF:S v M_r line.

This study was designed to investigate the concentrations of CRP and IgG and the relation between them within joints affected by several conditions using SF:S ratios.

Patients and methods

Seventy two inpatients and outpatients of St Bartholomew's Hospital and Whipps Cross Hospital, London, were studied: 29 with classical or definite RA (American Rheumatism Association criteria), 17 with osteoarthritis, 11 with crystal synovitis (gout or pyrophosphate arthropathy), eight with seronegative spondyloarthritis, and seven with undifferentiated monoarthritis. Many were taking non-steroidal anti-inflammatory drugs and 12 were receiving a slow acting antirheumatic agent, but none had had intra-articular steroids during the previous three months. Synovial fluid and serum samples were stored at -20°C until assay. Caeruloplasmin, α_2 macroglobulin, and CRP were assayed by electroimmunoassay,¹⁶ and transferrin and IgG by single radial immunodiffusion,¹⁷ using commercially available antisera to the proteins (Dako, High Wycombe, Bucks). Samples were diluted as appropriate, and paired serum and synovial samples were assayed in the same run. The coefficients of variation were less than 7% in the working ranges of the assays. The SF:S ratios were plotted against $\log(M_r)$. The median value was used to construct an overall curve for each group. The expected values for each patient's CRP and IgG SF:S ratios were calculated from the values obtained for the other three proteins, using least squares regression of $\log(\text{SF:S})$ value against $\log(M_r)$. From these, the ratio of observed to calculated (O:C) was obtained. These values were used to derive a CRP/IgG index for each patient.

The observed and calculated values in each group were compared with the binomial test. Between group comparisons of the O:C ratios were made by the Mann-Whitney test.

Results

The overall curve of SF:S v M_r for transferrin, caeruloplasmin, and α_2 macroglobulin was

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lower in osteoarthritis than in the other groups (fig 1), with significantly lower values for caeruloplasmin ($p<0.0001$ by Mann-Whitney test) and α_2 macroglobulin ($p=0.0018$). There was also a change in the slope of the lines, with RA and seronegative spondyloarthritis being associated with a smaller slope (fig 1), suggesting a more general effect on permeability than in the other conditions.

Observed:calculated ratios were calculated for CRP and IgG in 68 and 55 patients

Figure 1 Median synovial fluid:serum (SF:S) values v M_r (logarithmic scales) for transferrin (T), caeruloplasmin (C), and α_2 macroglobulin (α) in joint disease, showing raised levels in conditions other than osteoarthritis (OA) and reduced slopes in rheumatoid arthritis (RA) and seronegative spondyloarthritis (SA). CS=crystal synovitis; UA=undifferentiated arthritis.

Figure 2 Observed:calculated (O:C) ratios of C reactive protein (CRP) in joint disease. For abbreviations see fig 1 caption.

respectively. In RA CRP values (fig 2) were lower ($p<0.001$ by Mann-Whitney test), and IgG concentrations (fig 3) higher ($p=0.036$) than expected, while the ratio of CRP (O:C):IgG (O:C) (fig 4) was lower than expected ($p<0.0001$). There was no correlation between SF:S or O:C values of CRP and IgG. Although there was a tendency to higher than expected CRP (fig 2) and IgG (fig 3) concentrations in seronegative spondyloarthritis, and to raised concentrations of CRP in undifferentiated arthritis (fig 2), these did not reach statistical significance. The distributions of these values were reflected in their binomial probabilities, with

Figure 3 Observed:calculated (O:C) ratios of IgG in joint disease. For abbreviations see fig 1 caption.

Figure 4 Ratio of CRP (O:C): IgG (O:C) in joint disease. For abbreviations see fig 1 caption.

highly significant deviations from unity for CRP ($p=0.008$), IgG ($p=0.006$), and their ratio ($p=0.0016$) in RA, and significant deviation for CRP ($p=0.048$) in osteoarthritis.

Discussion

Synovial inflammation causes an increase in permeability of the synovium to plasma proteins, with a consequent change in the slope and intercept of the regression line of SF:S ratio against $\log (M_r)$ of the protein (fig 1). This makes it necessary to use the overall permeability equation for several proteins in order to interpret the results for a single protein of interest.

Synovial fluid CRP concentrations are increased in RA.^{6 18} In seropositive disease they remain lower than in serum.¹⁹ Horne and colleagues reported that mean CRP SF:S values were little different from those expected in RA.⁴ Our initial observations¹³ of lower than expected SF:S CRP values are confirmed in this study, however, and by another recent study.⁵

Native CRP undergoes calcium dependent binding to many molecules, including phosphorylcholine,²⁰ nuclear chromatin,²¹ and plasma very low density lipoprotein,²² with activation of the classical complement system.²³ Aggregated CRP binds to many other molecules and can stimulate platelet²⁴ and polymorph function.²⁵ It localises in damaged tissue,¹² though deposition may be limited to areas of tissue necrosis,²⁶ and its presence can be shown in synoviocytes and histiocytes of joints affected by RA, though not in patients with osteoarthritis or controls.¹¹

Although the functions of CRP are unknown, its conservation throughout evolution²⁷ argues a useful function,²³ and its binding properties have led to the proposal that CRP may act as a scavenger for molecules released by tissue damage.²³ This hypothesis is supported by the finding that DNA must be bound by CRP in order to undergo complement dependent solubilisation.^{28 29} Thus CRP might, under normal circumstances, be responsible for the removal of damaged molecules and the prevention of autoimmune induction.²⁹

The finding of lower than expected concentrations of CRP in synovial fluid in RA may indicate that it is deposited in areas of tissue damage, and that it fulfils a useful function in collaboration with complement, which is also low in synovial fluid in joints affected by RA,³ by limiting immune mediated damage.

Raised IgG SF:S ratios have been shown in RA,^{2 4} with weak correlation between synovial fluid IgG concentrations and disease activity.¹⁰ The raised levels are due to synovial synthesis of immunoglobulins by plasma cells,¹⁴ and significantly increased levels of rheumatoid factors,^{3 7} immune complexes,⁷ and antibodies to denatured collagen^{15 30} can be shown. Synovial synthesis of IgG and variations in synovial permeability might explain the poor correlation between total and specific IgG concentrations in synovial fluid and serum.¹⁵

We have shown that synovial fluid concentrations of IgG in RA are raised. It is tempting to link this finding with the low CRP concen-

trations and suggest that CRP exerts a protective effect in active inflammation, but we found no correlation between the two indices, even after allowing for variations in synovial permeability.

The relatively raised concentrations of CRP found in other joint diseases suggest local production, preferential transport into the joint, or binding to soluble molecules. There is no evidence for either of the first two mechanisms, but the third would have the effect of removing CRP from the exchangeable pool, and thus allowing its concentration in the synovial fluid to rise, without denaturing the immunological determinants. Attempts to show the presence of complexed CRP in serum have failed,³¹ perhaps because the binders used had far higher affinity for CRP than other molecules to which CRP might be bound. The nature of such binders is a matter for speculation, but they may include molecules released from the joint surface, which bind CRP, rather than being localised to the tissue, as seems to occur in RA. The raised concentration of CRP in synovial fluid may thus signal the absence of a local inflammatory reaction, though it does not preclude an active role in scavenging for the products of damaged tissue.

Raised IgG concentrations were found in seronegative arthritis, indicating active local IgG production, in contrast with osteoarthritis, undifferentiated arthritis, and crystal synovitis. Seronegative arthritis differed from RA in producing higher than expected synovial fluid CRP concentrations. The measurement of SF:S values of CRP and IgG may be a valuable adjunct in the diagnosis of inflammatory joint disease.

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