Detection of oncostatin M in synovial fluid from patients with rheumatoid arthritis

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Abstract

Objective—To measure oncostatin M (OSM) in synovial fluid from patients with rheumatoid arthritis (RA) and osteoarthritis (OA).

Methods—20 samples of synovial fluid from patients with RA and 10 samples from patients with OA were examined using an OSM specific sandwich ELISA.

Results—OSM was detected at concentrations ranging from 2.36 to 901.82 pg/ml in 18 (90%) of 20 samples of synovial fluid from RA patients. There was no detectable OSM in synovial fluid from OA patients. In the RA patients, the OSM concentration in synovial fluid correlated significantly with the synovial fluid white blood cell count ($r=0.67$, $p<0.01$), but not with other laboratory parameters of disease activity.

Conclusion—These findings suggest that OSM may contribute to joint inflammation in RA.

(*Ann Rheum Dis* 1997;56:184-187)

Rheumatoid arthritis (RA) is a systemic inflammatory disease characterised by chronic joint inflammation, severe cartilage and bone breakdown, joint deformity, and loss of joint function. The pathology of cartilage and bone destruction is a complex process in which the inflammatory cells and the cells resident in the joint contribute. Cytokines produced by these cells are thought to play a central part in this process. Several cytokines have been shown to promote cartilage destruction *in vivo* and *in vitro*. These include interleukin 1 α and β (IL1 α and β), tumour necrosis factor α (TNF α) and leukaemia inhibitory factor (LIF).¹⁻³ The concentrations of these cytokines correlate with markers of disease activity in the synovial fluid and serum of patients with RA.⁴⁻⁷

Oncostatin M (OSM) is a 28 kDa glycoprotein that is related to LIF through shared elements in their receptor complexes.⁸ OSM was originally characterised on the basis of its ability to inhibit the growth of the human melanoma cell line A375. It was first purified from the conditioned medium of the human histiocytic lymphoma cell line U937 treated with phorbol 12 myristate 13 acetate but subsequently has been shown to be produced by mitogen activated T lymphocytes and endotoxin stimulated macrophages. OSM is a pleiotropic cytokine that exerts numerous effects in many different cell types. Observed effects include stimulation of the acute phase response in liver cells and modulation of the

growth and differentiation of several normal and tumour cell lines. It also stimulates tissue inhibitor of metalloproteases (TIMP-1) and plasminogen activator in synovial fibroblasts. Recently we have demonstrated that OSM stimulates cartilage proteoglycan resorption and inhibits proteoglycan synthesis in cultured pig cartilage explants in a manner similar to IL1, TNF α , and LIF.⁹ To better understand the biology of OSM in RA we measured OSM concentrations in the synovial fluid of patients with RA and osteoarthritis (OA).

Methods

PATIENTS

Twenty patients who met the ACR criteria for RA and 10 patients who satisfied the ACR criteria for OA of the knee joint were studied. Samples of synovial fluid were collected consecutively from patients who required diagnostic or therapeutic aspiration. Twenty samples were collected from the patients with RA and 10 from the patients with OA. Except for two samples retrieved from elbow joints in RA patients, all samples of synovial fluid were obtained from the knee joint. Table 1 shows the characteristics of the patients with RA. The age of the patients with OA was 76 (5.8) (mean (SD)) years. No reliable data concerning disease duration for these patients were available. In all 10 of the patients with OA the synovial fluid white cell count was less than $1.0 \times 10^6/\text{ml}$.

PROCESSING OF SYNOVIAL FLUID

Samples of synovial fluid were collected into sterile vials using a no-touch aseptic technique and sterile needles and syringes. For cell counts an aliquot of synovial fluid was placed into a dry vial containing 5 mol EDTA. The cell counts were performed manually within four hours using a haemocytometer. For OSM assays specimens were collected into dry sterile plastic vacutainer tubes containing no anticoagulant. Samples were held at room temperature for up to two hours then centrifuged at $1000 \times g$ for 10 minutes at 4°C. Aliquots were then stored in sterile plastic vials at -70°C until assayed.

OSM ELISA ASSAY

The OSM ELISA assay was performed according to the methods recommended by the manufacturer (R & D Systems, Minneapolis, MN, USA).

Briefly, to precoat (anti-OSM MAb) microtitre plates was added 50 μl of anti-heterophilic antibody solution followed by 200 μl of either assay buffer, OSM standards

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Accepted for publication 13 December 1996

Table 1 Patients with rheumatoid arthritis

Age	61 (13) (range 25-81)
Sex	19 female, 1 male
Disease duration	20 (10) years (range 3-42)
Rheumatoid factor > 30 IU/ml	18
Current treatment	19
DMARDS	12
Corticosteroids	7
Aspirate volume	21 (16) ml (range 3.0-68.0 ml)
Synovial fluid WCC (n=18)	10.7 (8.4) $\times 10^6$ /ml (range 0.2-32.5 $\times 10^6$ /ml)
Neutrophils	59.2 (24.3)%
Monocytes	8.0 (4.4)%
Lymphocytes	32.8 (24.4)%
Haemoglobin (n=17)	120.2 (12.1) g/l (range 103.0-136.0 g/l)
Leucocytes (n=17)	7.9 (2.0) $\times 10^6$ /ml (range 3.7-11.0 $\times 10^6$ /ml)
Platelets (n=18)	389.6 (142.1) $\times 10^3$ /ml (range 186.0-702.0 $\times 10^3$ /ml)
Albumin (n=18)	37.6 (4.56) g/l (range 30.0-45.0 g/l)
ESR (n=17)	49.8 (35.5) mm/h (range 1.0-118.0 mm/h)

Data shown as mean (SD) unless otherwise stated. DMARDS = disease modifying antirheumatic drugs; WCC = white cell count; ESR = erythrocyte sedimentation rate.

or diluted test samples. The plate was then incubated for two hours at room temperature before all wells were washed three times with the wash buffer supplied with the kit. To all wells was then added 200 μ l of the anti-OSM polyclonal antibody conjugated to horseradish peroxidase and the plate was incubated for a further two hours at room temperature. The wells were then washed a further three times before 200 μ l of substrate solution was added to all wells. The plate was then incubated for 20 minutes at room temperature and the reaction finally stopped by the addition of 50 μ l of the stopping reagent to all wells. The absorbance was read at 450 nm and the results calculated by interpolation of the linear standard curve.

STATISTICAL TESTS

The Wilcoxon rank test sum was used to determine if the median OSM concentration in synovial fluid was significantly greater than zero.

Results

PERFORMANCE OF THE OSM ELISA ASSAY

Before measuring concentrations of OSM in synovial fluid the maximum sensitivity of the assay, the within assay coefficient of variation (CV), the between assay CV, and the stability of OSM in synovial fluid were determined. The maximum sensitivity of the assay was found to be 2.0 pg/ml for RA synovial fluid and 4.0 pg/ml for OA synovial fluid respectively. The within assay CV was 3.6% while the between assay CV was 10.8%. In specimens of synovial fluid to which OSM (500 pg) had been added back, the OSM was found to be quite stable with only a negligible loss of OSM (<1%) being observed for up to 12 hours after the addition of the OSM. This result shows that OSM is stable in synovial fluid and that rheumatoid synovial fluid does not contain any appreciable proteolytic activity against OSM.

OSM CONCENTRATIONS IN SYNOVIAL FLUID

Twenty samples of synovial fluid from patients with RA and 10 samples from patients with OA were examined in this study. OSM was detected at concentrations ranging from 2.36 to 901.82 pg/ml in 18 of 20 (90%) ($p < 0.001$) samples from patients with RA. There was no

Figure 1 Concentration of OSM in RA and OA synovial fluid. OSM concentrations were determined by ELISA as described in Methods. Each point represents a mean result obtained from two independent assay runs. The horizontal bar depicts the median value ($p < 0.001$).

detectable OSM in the synovial fluid obtained from patients with OA (fig 1).

RELATION OF SYNOVIAL FLUID OSM TO DISEASE ACTIVITY

To determine whether the concentrations of OSM in synovial fluid correlated with disease activity in RA, OSM concentrations were evaluated in relation to objective parameters of disease activity. The synovial fluid OSM concentration correlated significantly with synovial fluid total leucocyte numbers ($r = 0.667$, $p < 0.01$) and synovial fluid neutrophil numbers ($r = 0.723$, $p < 0.01$) (fig 2). No relation was observed between synovial fluid OSM concentrations and either patient age, disease duration, synovial fluid monocyte or lymphocyte numbers, peripheral white blood count, erythrocyte sedimentation rate, haemoglobin concentration, platelet numbers or serum albumin concentrations. Moreover, no significant difference was noted between those patients treated or not treated with either disease modifying antirheumatic drugs or maintenance corticosteroids. No clinical indices of global disease activity were available.

Discussion

Cartilage resorption is a recognised consequence of chronic active joint inflammation in RA and related diseases. The secreted products of the inflammatory cells that enter the joint cavity and those of resident synoviocytes and chondrocytes are thought to play an important part in cartilage proteoglycan and collagen catabolism. Cytokines known to affect cartilage metabolism include; IL1 (α and β),⁵ TNF α ,² LIF,³ transforming growth factor β (TGF β),¹¹ and insulin-like growth factor 1 (IGF1).¹⁰ IL1, TNF α , and LIF all act on chondrocytes to stimulate resorption of proteoglycans and inhibit proteoglycan synthesis in vivo and in vitro. Conversely TGF β and IGF1 antagonise the actions of IL1 and TNF α by stimulating cartilage proteoglycan synthesis and down regulating the number of cell surface receptors for catabolic cytokines.

Figure 2 Correlation of OSM concentration with the total white cell count in RA synovial fluid. Synovial fluid OSM concentration and total white cell numbers were determined by ELISA and haemocytometer count respectively as described in Methods.

Recently we have shown that OSM stimulates cartilage proteoglycan resorption and inhibits cartilage proteoglycan synthesis in pig cartilage explants.⁹ OSM is a member of a cytokine superfamily that includes LIF, interleukin 6 (IL6), interleukin 11 (IL11) and ciliary neurotrophic factor. This family has been delineated on the basis of shared elements (gp130) in their receptor complexes.⁸ Cytokines in this group elicit pleiotropic responses in multiple cell types.⁸ Effects observed include stimulation of the acute phase response, the regulation of bone and cartilage metabolism, and regulation of cell proliferation and differentiation. Prompted by our *in vitro* findings concerning the action of OSM on cartilage proteoglycan metabolism and the involvement of cytokines in the pathology of RA, we decided to measure OSM concentrations in RA and OA synovial fluids to better understand the involvement of OSM in the pathology of RA.

OSM was not detectable in samples of synovial fluid from patients with OA. In contrast detectable concentrations of OSM were observed in most samples from patients with active RA. Whether such concentrations are biologically important is difficult to determine in the absence of data concerning the broader physiology and pathophysiological role of

OSM. There are several lines of evidence however that suggest the observed concentrations may have pathogenetic significance. Firstly, a correlation was observed between the OSM concentrations and both the total leucocyte and neutrophil subset counts in the samples from the rheumatoid patients. Other cytokines with activity against cartilage have also been detected in synovial fluid. These include IL1, TNF α , and LIF.⁴⁻⁷ Among these only LIF has been found to correlate to any appreciable degree with leucocyte numbers in the synovial fluid.⁴ Weak correlations have been described for TNF α .^{6,7} Whether OSM contributes to leucocyte recruitment in RA is not known. OSM does not have inherent chemokine activity but it may stimulate the production of chemokines however and in particular IL8, which has been detected in rheumatoid synovial fluid, has potent activity against neutrophils, and has been shown in some studies to correlate with synovial fluid neutrophil counts in RA ($r=0.66$).¹² It is worth noting that LIF, a related cytokine, stimulates the production of IL8 in synovial fibroblasts and chondrocytes.¹³

A second line of evidence suggesting biological significance arises from studies concerning the effects of human OSM on animal cartilage. In an earlier study we reported statistically significant inhibition of proteoglycan synthesis in pig cartilage explants at OSM concentrations of 1 ng/ml or higher.⁹ The observed OSM concentrations were less than this threshold, however we have found that the effects of OSM and IL1 α on proteoglycan synthesis are additive suggesting that OSM concentrations in the subnanogram per ml range may have the capacity to modulate proteoglycan synthesis *in vivo* (unpublished results). Furthermore, we have observed increased proteoglycan catabolism in pig cartilage explants exposed to rhOSM at 10 ng/ml but not 1 ng/ml.⁹ Here again the observation of additive effects between OSM and IL1 in respect to proteoglycan and collagen catabolism in pig and bovine cartilage explants suggests the observed concentrations of OSM in rheumatoid synovial fluid may be relevant to cartilage degradation.^{9,14} It is acknowledged that in the absence of data concerning the effects of these cytokines on human cartilage, the case for relevance cannot be more convincingly argued.

The origin of the OSM in rheumatoid synovial fluid is of interest. Activated lymphocytes and macrophages are able to produce OSM. Although the concentrations of OSM did not correlate with the numbers of lymphocytes and mononuclear cells in the synovial fluid, it is possible that the lymphocytes and/or monocyte/macrophage cells in the synovial membrane are a significant source of OSM. It is worth noting that LIF, a closely related cytokine is produced by synovial cells and chondrocytes in culture.¹⁵ Accordingly OSM may be elaborated by synovial cells and perhaps even by chondrocytes *in vivo*.

In summary OSM was detected at subnanogram per ml concentrations in most of the 20 synovial fluid specimens obtained from active

rheumatoid joints but in none of the 10 synovial fluid specimens from patients with OA. In RA a statistically significant and moderately strong correlation was observed between the synovial fluid concentrations of OSM and the total leucocyte count and neutrophil subset count. These findings together with observations on the effects of OSM on proteoglycan metabolism in cartilage explants suggest that OSM may promote inflammation and also perturb cartilage proteoglycan metabolism in RA.

This work was generously supported by the Royal Perth Hospital Medical Research Foundation and the Arthritis Foundation of Western Australia.

We thank Dr N Nicola for helpful advice and Mrs Ilse Nicolai for secretarial services.

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