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

Wang Hui, Michael Bell, Graeme Carroll

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.
### Table 1 Patients with rheumatoid arthritis

<table>
<thead>
<tr>
<th></th>
<th>RA (n = 20)</th>
<th>OA (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61 (13)</td>
<td>62 (15)</td>
</tr>
<tr>
<td>Sex</td>
<td>19 female, 1 male</td>
<td>11 female, 3 male</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>20 (10)</td>
<td>12 (7)</td>
</tr>
<tr>
<td>Rheumatoid factor &gt; 30 IU/ml</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Current treatment</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>DMARDs</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Aspiratory volume</td>
<td>21 (16) ml</td>
<td>31 (20) ml</td>
</tr>
<tr>
<td>Synovial fluid WCC (n=18)</td>
<td>10.7 (8.4) \times 10^{6}/ml (range 3.7-11.0 \times 10^{6}/ml)</td>
<td>5.9 (4.2) \times 10^{6}/ml (range 0.2-3.2 \times 10^{6}/ml)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>59.2 (24.3)%</td>
<td>32.8 (24.4)%</td>
</tr>
<tr>
<td>Monocytes</td>
<td>8.0 (4.4)%</td>
<td>8.0 (4.4)%</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>32.8 (24.4)%</td>
<td>32.8 (24.4)%</td>
</tr>
<tr>
<td>Haemoglobin (n=17)</td>
<td>120.2 (12.1) g/l (range 103.0-136.0 g/l)</td>
<td>120.2 (12.1) g/l (range 103.0-136.0 g/l)</td>
</tr>
<tr>
<td>Platelets (n=18)</td>
<td>389.6 (142.1) \times 10^{3}/ml</td>
<td>281.6 (112.1) \times 10^{3}/ml</td>
</tr>
<tr>
<td>Albumin (n=18)</td>
<td>37.6 (3.6) g/l (range 30.0-45.0 g/l)</td>
<td>37.6 (3.6) g/l (range 30.0-45.0 g/l)</td>
</tr>
</tbody>
</table>

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

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

### 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),1 TNFα,2 LIF,3 transforming growth factor β (TGF β),4 and insulin-like growth factor 1 (IGF1).5 IL1, TNFα, and LIF all act on chondrocytes to stimulate resorption of proteoglycans and inhibit proteoglycan synthesis in vivo and in vitro. Conversely TGFβ and IGF1 antagonise the actions of IL1 and TNFα by stimulating cartilage proteoglycan synthesis and down regulating the number of cell surface receptors for catabolic cytokines.
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Recently we have shown that OSM stimulates cartilage proteoglycan resorption and inhibits cartilage proteoglycan synthesis in pig cartilage explants. 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. Cytokines in this group elicit pleiotropic responses in multiple cell types. 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, TNFα, and LIF. Among these only LIF has been found to correlate to any appreciable degree with leucocyte numbers in the synovial fluid. Weak correlations have been described for TNFα. 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). It is worth noting that LIF, a related cytokine, stimulates the production of IL8 in synovial fibroblasts and chondrocytes.

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. The observed OSM concentrations were less than this threshold, however we have found that the effects of OSM and IL1α 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. 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.

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. 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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doi: 10.1136/ard.56.3.184

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