Interleukin-1 receptor antagonist production in cultured synovial cells from patients with rheumatoid arthritis and osteoarthritis

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Abstract

***Objective*—To measure the amounts of interleukin-1 receptor antagonist (IL-1ra) protein produced by cultured synovial cells obtained from patients with rheumatoid arthritis (RA) and osteoarthritis (OA).**

***Methods*—Synovial cells obtained from patients with either RA or OA were cultured and the supernatants were measured for IL-1ra by enzyme linked immunosorbent assay.**

***Results*—The synovial cells obtained from patients with RA produced significantly smaller amounts of IL-1ra than did those obtained from patients with OA, in a late passage (third to fifth) without stimulation and a first passage both with and without stimulation ($p < 0.025$, respectively). In addition, when the patients with RA were divided into two groups according to the maximum number of lining cell layers, the amounts of IL-1ra produced by the proliferative type were smaller than those produced by the less proliferative type ($p < 0.025$).**

***Conclusions*—The above findings suggest that IL-1ra production in RA synovial cells is suppressed, and that reduced IL-1ra protein production is one of the causes which leads to the proliferation of lining cells and persistent joint inflammation.**

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Rheumatoid arthritis (RA) is a systemic autoimmune disease characterised primarily by chronic inflammatory synovitis which often leads to joint destruction. Cytokines have been implicated as important mediators of both inflammation and joint destruction in RA.[1] Interleukin-1 (IL-1) is thought to mediate tissue damage in RA by inducing prostaglandin E_2 (PGE_2) and neutral protease production in synovial fibroblasts and articular chondrocytes.[2]

IL-1 receptor antagonist (IL-1ra) has been demonstrated to block the effects of IL-1 in various in vitro systems and animal disease models, and also to have no immunological cross reactivity with either IL-1α or IL-1β.[3–6] It is thus possible that sustained active synovitis is attributable not only to an excess production of agonist, but also to an inadequate synthesis of antagonist protein.

To investigate the possible role of IL-1ra in RA, the amounts of IL-1ra protein produced by cultured synovial cells from patients with RA and osteoarthritis (OA) were measured by an enzyme linked immunosorbent assay (ELISA).

Patients and methods

Synovial tissue specimens were obtained from 18 patients (10 with RA and eight with OA) at the time of knee joint replacement. Each patient satisfied the diagnostic criteria of the American College of Rheumatology.[7 8] All RA patients were treated with prednisolone, disease modifying antirheumatic drugs (DMARDs) and non-steroidal anti-inflammatory drugs (NSAIDs). All OA patients took NSAIDs without taking prednisolone.

HISTOLOGICAL ANALYSIS OF SYNOVIAL MEMBRANES

The synovial membranes of 10 patients with RA were fixed in neutral phosphate buffered 10% formalin and sections were stained with haematoxylin and eosin. The sections were examined without knowledge of either the patient's identity or the grade of joint damage. The degree of synovial lining cell proliferation was measured as the maximum number of synovial lining cell layers, and the degree of lymphocyte infiltration was graded from 1 to 4 depending on the number of infiltrated cells.[9 10]

SYNOVIAL CELL CULTURE

The synovial cells were cultured as previously described.[11] Cells taken at the first and the late (third to fifth) subcultures were examined by immunohistochemistry.[12] The first passage of rheumatoid synovial tissue cells comprises approximately 40–50% macrophages and 50–60% fibroblast like cells. The first passage of OA synovial tissue has a slightly greater percentage of macrophages (60–70%). The cells from both RA and OA synovium obtained at late passage consisted mostly of synovial fibroblasts like cells (>95%).

Aliquots of the cells (0·5 ml, 1×10^5 cells/ml) were plated in a 24 well plate and cultured for five to seven days until the cells reached confluency, at which time the number of cells in each well averaged $1{\cdot}8–2{\cdot}0 \times 10^5$ cells/well. The medium was replaced with 0·5 ml of fresh medium and the cells cultured on at 37°C in a 5% carbon dioxide incubator for 72 hours in the presence of lipopolysaccharide (LPS, Sigma Chemical Co, St Louis, Missouri, 10 μg/ml) or

phosphate buffered saline (PBS) (0·01 mol/l phosphate, 0·138 mol/l NaCl, pH 7·4) (unstimulated controls). The plates were then centrifuged at 400 *g* for 10 minutes and the supernatants collected and frozen at −80°C until required for assay for IL-1ra.

MEASUREMENT OF CYTOKINES

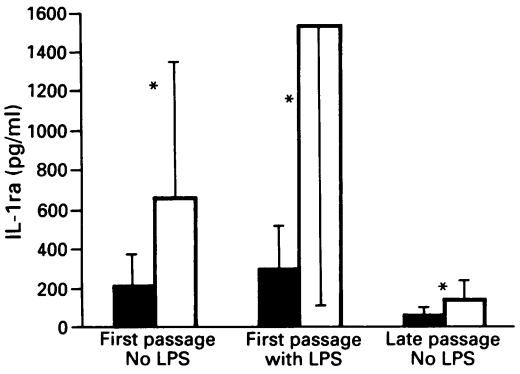
IL-1ra, IL-1α and IL-1β were measured by ELISA kits (Amersham International plc, Amersham, England, for IL-1ra; Ohtsuka Pharmaceutical Co, Tokyo, Japan, for IL-1α and IL-1β). These assays recognise both natural and recombinant IL-1ra, IL-1α, or IL-1β. No significant cross reactivity or interference was observed. The lower limits of detection of these kits are IL-1ra 6·5 pg/ml, IL-1α 0·5 pg/ml, and IL-1β 0·5 pg/ml.

STATISTICAL ANALYSIS

Data are expressed as mean (SD). Statistical analyses were by Student's *t* test.

Results

The figure shows the amounts of IL-1ra produced by synovial cells (2×10^5 cells/well). Unstimulated synovial cells of the first passage obtained from patients with RA produced less IL-1ra (214·0 (157·0) pg/ml, n = 10) than unstimulated synovial cells of the first passage obtained from patients with OA (659·0 (686·4) pg/ml, n = 8) ($p < 0·025$). Similarly, stimulated synovial cells of the first passage from patients with RA produced significantly smaller amounts of IL-1ra (301·6 (217·4) pg/ml, n = 10) than did those cells obtained from patients with OA (1526·5 (1415·4) pg/ml, n = 8) ($p < 0·025$). In late passage, unstimulated cells from patients with RA also produced significantly smaller amounts of IL-1ra (57·2 (39·9) pg/ml, n = 10) than did synovial cells from patients with OA (136·6 (100·7) pg/ml, n = 8) ($p < 0·025$). When the delta values of IL-1ra production attributable to LPS stimulation were calculated, those for synovial cells obtained from RA patients (46·2 (36·2) pg/ml, n = 10) were statistically smaller than those for OA patients (201·1 (188·8) pg/ml, n = 8) ($p < 0·0125$).



IL-1ra production (mean, SD) by synovial cells. Synovial cells of the first passage obtained from patients with either rheumatoid arthritis (■) (n = 10) or osteoarthritis (□) (n = 8) were cultured (2×10^5 cells per well) with or without lipopolysaccharide (LPS) 10 μg/ml; synovial cells of the late passage were also cultured, without LPS. IL-1ra was measured in the supernatants.
**p < 0·025 between groups.*

Histological findings in synovial membranes from patients with rheumatoid arthritis

	No of patients	*Lining cell layer*	*Production of IL-1ra (pg/ml)*
Proliferative group	7	6·4 (1·0)	126·7 (72·6)
Less proliferative group	3	2·7 (0·5)*	458·3 (156·8)*

Values are mean (SD). *p < 0·05 compared with proliferative group.

The production of IL-1α by cultured synovial cells from patients with RA and OA examined by ELISA was almost undetectable. Greater amounts of IL-1β were produced by synovial cells obtained from patients with RA (1·58 (1·34) pg/ml, n = 5) than by those from patients with OA (0·76 (0·45) pg/ml, n = 5) and the ratios of IL-1β to IL-1ra concentrations for each cell culture were greater in RA ($4·42\ (3·25) \times 10^{-3}$, n = 5) than in OA ($2·89\ (2·07) \times 10^{-3}$, n = 5). However, these differences in both IL-1β production and IL-1β:IL-1ra ratio were not statistically significant.

No clinical parameter (erythrocyte sedimentation rate, C reactive protein, rheumatoid factors, platelet count, haemoglobin concentration, leucocyte count, joint score, duration of morning stiffness, strength of hand grip, and activity index) was found to correlate with the amount of IL-1ra produced by stimulated or unstimulated synovial cells of the first passage obtained from patients with RA (data not shown).

When patients with RA were compared in two groups according to the maximum number of lining cell layers (proliferative group = more than four layers; less proliferative group = fewer than three layers), the amounts of IL-1ra produced by synovial cells from the proliferative group (126·7 (72·6) pg/ml, n = 7) were found to be significantly smaller than those from the less proliferative group (458·3 (156·8) pg/ml, n = 3) ($p < 0·025$) (table). However, there was no correlation between the degree of lymphocyte infiltration and the amount of IL-1ra produced by the synovial cells obtained from RA.

Discussion

This is the first report of IL-1ra protein production by cultured synovial cells obtained from patients with RA and OA using a sensitive and specific sandwich ELISA. Malyak *et al*[13] reported that IL-1ra concentrations in RA synovial fluid were increased compared with OA and other non-inflammatory arthropathies, and they suggested that the increased concentrations of IL-1ra in RA synovial fluid might not be adequate to block the proinflammatory effects of IL-1 within the synovial tissue. Our own experimental study of IL-1ra production by RA peripheral blood mononuclear cells (submitted for publication) indicated that IL-1ra production by those cells was increased compared with normal peripheral blood mononuclear cells. However, Deleuran *et al*,[14] using

monoclonal antibodies and immunohistochemical techniques, demonstrated that there was a greater number of cells containing IL-1ra in the OA synovium than in the RA synovium. Their results also suggested that RA synovial cells, which proliferated in rheumatoid joints, produced smaller amounts of IL-1ra than did OA synovial cells. Our comparative study between RA and OA showed that the amounts of IL-1ra produced by synovial cells obtained from patients with RA were decreased compared with those obtained from patients with OA. These results support those of Deleuran *et al* and thus also imply a possible role of IL-1ra in RA.

All the patients with RA in this study were treated with prednisolone and DMARDs; none of the patients with OA was receiving these medications. Our study confirmed that glucocorticoids suppressed production of IL-1ra by cultured synovial cells. It is possible that the suppression of IL-1ra production by cultured synovial cells from patients with RA was the result of an in vivo effect of glucocorticoids. Alternatively, suppression of IL-1ra production may be implicated as part of the pathogenesis of RA, in which case past treatment with oral glucocorticoids and DMARDs would have little influence on the production of IL-1ra by cultured synovial cells.

A primary characteristic of RA synovial tissue is proliferating synovial cells.[1] IL-1 can stimulate fibroblast growth[15] and induce production of collagenase and PGE_2[1] in synovial cultures. Muirden *et al*[9] reported that joint damage recorded by surgeons correlated directly with the grade of lining cell proliferation. Our results also showed that the amounts of IL-1ra produced by the proliferative group of patients were significantly smaller than those produced by the less proliferative group. This reduced production of IL-1ra would exacerbate any underlying biological effects of IL-1 on the pathological processes occurring in rheumatoid joints.

In conclusion, we have demonstrated reduced IL-1ra production by cultured synovial cells obtained from patients with RA in comparison with cells from patients with OA. These findings suggest that reduction in the amount of IL-1ra produced may be one cause for both the proliferation of lining cells and persistent joint inflammation.

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