Reduction of tumor necrosis factor α and interleukin-1β levels in human synovial tissue by interleukin-4 and glucocorticoid

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Summary. The effects of recombinant human interleukin-4 (IL-4) and the glucocorticoid, dexamethasone, on tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) levels in cultures of rheumatoid and osteoarthritic synovial tissue were studied. Low concentrations of IL-4 and dexamethasone suppressed the levels of both cytokines in the supernatants of both types of tissue after stimulation with lipopolysaccharide (LPS); the IL-1β and TNFα levels were measured by ELISA. It is suggested that it is the monocyte/macrophage in the synovial tissues that is responsive to the inhibitors. It is proposed that glucocorticoids may act on synovial tissue in this manner in vivo and IL-4 may do so if administered intraarticularly.

Key words: Arthritis – Synovial tissue – TNFα – IL-1 – IL-4 – Glucocorticoid

Introduction

Chronic inflammation within the synovial tissue appears to be responsible for many of the manifestations of rheumatoid arthritis (RA), including persistent synovitis, local destruction of bone and cartilage and many systemic effects [1]. It is likely that local production of cytokines by the synovium accounts for many of these effects [1]. Two such cytokines, interleukin-1 (IL-1) and tumor necrosis factor α (TNFα), have been implicated in the pathogenesis of RA. They have many actions in vitro which, if operative in vivo, could account for the clinical picture in RA. For example, they both stimulate the production of neutral proteinases and vasoactive agents (e.g. prostaglandins) by human synoviocytes [2–4] and chondrocytes [5, 6], as well as stimulate human cartilage resorption [5, 7]. IL-1 can cause cartilage loss when injected intraarticularly into the joints of animals [8]. Both cytokines have been detected in arthritic synovial tissues and in their cultures [9, 10], and also in synovial fluids [1]. However, there is controversy in the literature concerning the relative levels of IL-1 and TNFα in different disease categories, some of the difficulties probably arising from the presence of inhibitors that interfere with the bioassays.

Given the possible deleterious effects of IL-1 and TNFα in joints, it would seem desirable to be able to control their production and/or action. Monocyte/macrophage-type cells are their likely sources in both inflamed and non-inflamed joints [11]. In previous studies we have shown that glucocorticoids and the lymphokine, interleukin-4 (IL-4), are quite active in suppressing the production of these and other cytokines in lipopolysaccharide-stimulated human monocyte/macrophages [12, 13]; it has been proposed that such suppression may form part of the efficacious action of this class of drug in inflammatory diseases, such as RA [12, 13]. IL-4 has not been found in rheumatoid arthritis synovial fluid nor is it produced by the RA synovial membrane [14]. In the present study we demonstrate that both IL-4 and a glucocorticoid are able to suppress the IL-1 and TNFα production by synovial tissue from patients with RA or osteoarthritis (OA), indicating that cells in such tissues are sensitive to the action of both of these agents.

Patients and methods

Patients and tissue preparation. Synovial tissue, removed at the time of joint arthroplasty was dissected from knee or hip joints within 3 h of operation. In all, 16 patients were studied, 8 with RA, fulfilling the American Rheumatism Association (ARA) criteria [15] (2 men, 6 women) and 8 with OA (4 men, 4 women). The tissue was dissected into 1–2 mm³ fragments under sterile conditions, washed three times in phosphate-buffered saline (PBS) to remove traces of blood and placed in 24-well plates (Nunc, Denmark). Each well contained 20–70 mg tissue suspended in 1.0 ml α-minimum essential medium (α-MEM) (supplemented with glutamine, 2-mercaptoethanol, MOPS, neomycin and NaHCO₃, pH adjusted to 7.10) plus 1% acid treated...
ELISA for TNFα. Levels of TNFα were measured using a sandwich ELISA. Microtiter plates (Nunc Immunoplates) were coated with anti-human TNFα monoclonal antibody (101-4) [16] overnight at room temperature. The wells were blocked with 2.5% bovine serum albumin (BSA) for 1 h at 37 °C, and samples or standard were then added and incubated for a further 2 h at 37 °C. TNFα bound to the plates was detected by incubation with a polyclonal anti-human TNFα antibody, followed by a sheep anti-rabbit immunoglobulin conjugated with horseradish peroxidase. The peroxidase reaction was developed using O-phenylenediamine dihydrochloride as substrate. The limit of detection for this assay was 10–15 pg/ml.

ELISA for IL-1β. Levels of IL-1β were measured as described previously [12]. Microtiter plates were coated with anti-IL-1β monoclonal antibody (H67) overnight at 4 °C and non-specific binding was blocked with 2.5% BSA. After washing, sample or standard was incubated on the wells with biotinylated anti-IL-1β monoclonal antibody (H6) overnight at 4 °C and non-specific binding was blocked with 2.5% BSA. After washing, sample or standard was incubated with biotinylated anti-IL-1β monoclonal antibody (H67) for 2 h at room temperature, before being transferred to the coated plates. An avidin-biotinylated horseradish peroxidase complex was then added and incubated for a further 30 min. The peroxidase reaction was developed using 3,3',5,5'-tetramethylbenzidine as substrate. The sensitivity of the assay was 15 pg/ml and the absorbance was linearly related to IL-1β concentration up to 500 pg/ml.

Measurement of protein synthesis. Protein synthesis in synovial explants was monitored by the incorporation of 3H-leucine into trichloroacetic acid insoluble protein. After dissection, the tissue was preincubated for 2 h in leucine-poor RPMI (Flow) supplemented with 1% acid treated FCS. At 0, 22 or 70 h, the samples were labelled for 2 h in medium containing 20 μCi/ml 3H-leucine (at 24 h the samples that were to continue to 72 h had fresh media and reagents added, as in the cytokine study protocol). The medium was removed and the tissue was washed at 4 °C with 1 ml aliquots of 0.6 M trichloroacetic acid supplemented with 20 mM leucine until the washings contained only background radioactivity. Samples were then weighed and digested at 65 °C overnight with papain (30 mg/ml 0.1 M sodium acetate, 5 mM EDTA, 5 mM cysteine-HCl buffer). Aliquots of the digested samples were assayed for radioactivity.

Reagents. These were obtained as gifts from the following people: recombinant human IL-4 (greater than 400 U/μg) [12] (Dr. F. Lee, DNAX, Palo Alto, Calif., USA), recombinant human TNFα (rhTNFα; Dr. G. R. Adolf, Ernst-Boehringer Institut, Vienna, Austria); recombinant human IL-1β (rhIL-1β; Dr. A. Shaw, Glaxo, Geneva, Switzerland), rabbit polyclonal antibody to rhTNFα (Dr. K. Ashman, Dept of Veterinary Science, University of Melbourne, Australia), two monoclonal antibodies (H6 and H67) to different epitopes of IL-1β (Dr. A. Allison, Syntex, Palo Alto, Calif., USA). Peroxidase-conjugated swine anti-rabbit IgG (1.3 mg/ml) was obtained from Dakopatts and 3H-leucine (53 Ci/mmol) from NEN (Boston, Mass., USA).

Statistical methods. Statistical significance was determined using the Friedman and Wilcoxon signed rank test for non-parametric statistics. Results were considered significant if P < 0.05.

Results

TNFα and IL-1β levels in RA synovial tissue

When TNFα levels were measured in 24 h cultures of untreated synovial tissue from eight RA patients, undetectable or very low (4 pg/mg tissue or less) levels were found. In order to study whether IL-4 and glucocorticoid could suppress TNFα levels, the cultures were, therefore, treated with LPS (100 ng/ml) in the presence or absence of these reagents. As seen in Fig. 1a, both IL-4 (4 U/ml) or dexamethasone (Dex.) (10−7 M) for 24 h. TNFα (a) and IL-1β (b) levels in supernatants were measured by ELISA. Mean values of either duplicate or triplicate cultures are presented depending on availability of tissue. The P values shown are derived from the Wilcoxon signed rank test.