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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 mm^3 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 $NaHCO_3$, pH adjusted to 7.10) plus 1% acid treated

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fetal calf serum (FCS) (Commonwealth Serum Laboratories, Australia). Where tissue availability allowed, triplicate cultures were incubated in the presence or absence of lipopolysaccharide (LPS; 100 ng/ml) and/or with various concentrations of dexamethasone or recombinant human IL-4 (rhIL-4). The plates were incubated at 37 °C in 5% CO_2 and the supernatants were harvested at 24 h and 72 h. The supernatants were spun at 13,000 *g* for 5 min to remove cell debris and stored at −20 °C. The wet weight of the tissue was determined. Plastic disposable equipment was used wherever possible and media and buffers were filtered through Zetapor membranes (AMF Cuno, Meriden, CT) to remove LPS [12].

ELISA for TNFα. Levels of TNFα were measured using a sandwich ELISA. Microtitre 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]. Microtitre plates were coated with 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 on the wells 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 ^{3}H-leucine into trichloracetic 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 ^{3}H-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 m*M* leucine until the washings contained only background radioactivity. Samples were then weighed and digested at 65 °C overnight with papain (50 mg/ml 0.1 *M* sodium acetate, 5 m*M* EDTA, 5 m*M* 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 ^{3}H-leucine (53 Ci/mmo/l) 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 $P < 0.05$.

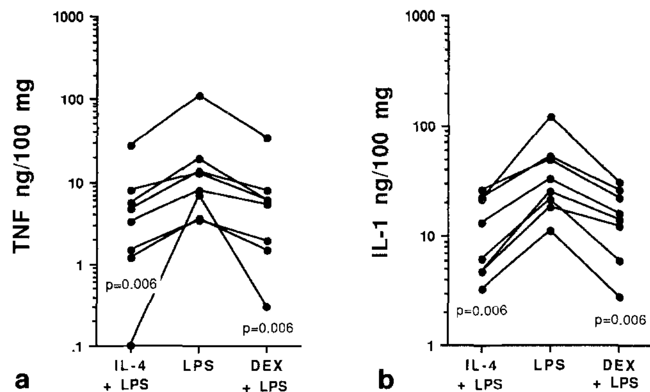

Fig. 1 a, b. Effect of IL-4 and glucocorticoid on LPS-mediated induction of TNFα and IL-1β in rheumatoid synovial tissue cultures. Synovial tissues from eight rheumatoid donors were cultured in LPS (100 ng/ml) with or without IL-4 (4 U/ml) or dexamethasone (Dex.) (10^{-7} *M*) for 24 h. TNFα (**a**) 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

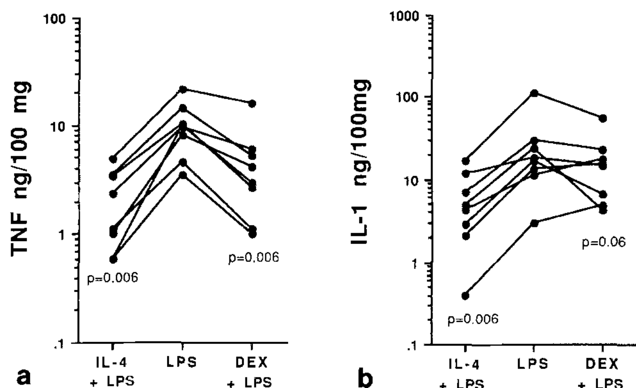

Fig. 2. Effect of IL-4 and glucocorticoid on LPS-mediated induction of TNFα and IL-β in osteoarthritic synovial tissue cultures. Synovial tissues from eight osteoarthritic donors were cultured in LPS (100 ng/ml) with or without IL-4 (4 U/ml) or dexamethasone (Dex) (10^{-7} *M*) for 24 h. Details as for Fig. 1

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, undetected 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 = 480 p*M*) and dexamethasone (10^{-7} *M*) were capable of lowering the LPS-induced TNFα levels in cultures of tissue from all of the patients over 24 h (mean 79% decrease by IL-4, range 66–94%; mean 58% decrease by dexamethasone, range 27–76%). The IL-4 and glucocorticoid were active when tested at concentrations as low as 0.2 U/ml and 10^{-8} *M*, respectively. The production of TNFα over the period from 24 to 72 h was 10-fold less than over the first 24 h (data not shown). Suppres-

sion studies were, therefore, performed over the first 24 h of culture. Neither IL-4 (4 U/ml) nor dexamethasone (10^{-7} *M*) inhibited total protein synthesis in the synovial tissue cultures over a 72-h period.

When IL-1β levels were measured in the same untreated culture supernatants, cytokine levels were again either undetectable or very low (9 pg/mg tissue or less). Likewise, when the IL-1β levels were monitored in the same LPS-treated cultures examined above, it can be observed in Fig. 1 b that both IL-4 and dexamethasone lowered the LPS-induced IL-1β levels in all of the cultures (mean 69% decrease by IL-4, range 58–82%; mean 53% decrease by dexamethasone, range 32–75%). Similar dose responses for both inhibitors were observed in the case of IL-1β formation as for TNFα (data not shown).

TNFα and IL-1β levels in OA synovial tissue

We then determined whether OA synovial tissue would respond similarly to IL-4 and glucocorticoid. As for the RA synovial tissues, untreated OA synovial tissue (eight samples studied) released non-measurable or small amounts (5 pg/mg tissue or less) of TNFα. In cultures treated with LPS, TNFα levels were once more dramatically elevated by LPS in comparable amounts to RA cultures. The levels of both TNFα and IL-1β were greatest in the first 24 h of culture and 10-fold less in the period 24 to 72 h (data not shown). Production of TNFα was reduced by IL-4 and dexamethasone in the supernatants of all the eight tissues cultured (Fig. 2a; mean 66% decrease by IL-4, range 38–98%; mean 57% decrease by dexamethasone, range 32–95%). In OA cultures, LPS-induced IL-1β production was effectively inhibited by IL-4 (Fig. 2b; mean 73% decrease, range 35–87%) but dexamethasone (10^{-7} *M*) failed to suppress IL-1β production in three of the eight tissues tested. Synovial cultures from the remaining five patients demonstrated equivalent suppression to that seen for TNFα, giving an overall mean suppression of IL-1β production of 13% (range −58–82%). This difference may reflect a sampling bias as the total number of cases in our series was small.

Discussion

We showed that, at optimal concentrations, both IL-4 and the glucocorticoid, dexamethasone, could suppress the TNFα and IL-1β levels of LPS-stimulated RA and OA synovial tissue, IL-4 being the slightly more potent agent. The dose-response curves for each of these agents (data not shown) and their degree of inhibition of cytokine formation were similar to those shown previously for LPS-treated human monocytes [12, 13]. These observations, as well as the finding that LPS was stimulatory for IL-1β and TNFα, suggested that cells of the monocyte/macrophage class produce these cytokines in the synovial tissue cultures, a notion put forward in a recent review [11]. In the rheumatoid tissue, these cells could be from the synovial membrane (type A cell) or from blood, although dendritic cells may contribute [1]; for the OA tissue, it is likely that a resident macrophage (type A cell) is responsible, although a role for circulating monocytes cannot be excluded since OA lesions can have an inflammatory component. There are also immunohistochemical data to support a monocyte/macrophage origin of these cytokines in arthritic synovial tissue [9, 17].

We determined TNFα and IL-1β levels in the synovial tissue cultures by ELISA to avoid the possible problems of inhibitors and non-specificity of the bioassays. Basal levels of IL-1β and TNFα production were very low for both OA and RA tissue, in accordance with the observation of some [18] but not others [9, 19] for synovial mononuclear cells. Miyasaka et al. [10] have found IL-1 bioactivity in RA but not in OA synovial tissue cultures. The timing of joint replacement surgery is generally late in the natural history of both OA and RA, often when the disease has few clinical signs of inflammation. Discrepancies in the literature may result from differences in the timing of tissue collection, reflecting differences in tissue cellularity [20], as well as from the presence of LPS, which is sometimes a contaminant in tissue culture.

The suppressive actions of glucocorticoids on the cytokine production by monocytes/macrophages have been invoked as forming a part of their anti-inflammatory action, leading to the suggestion of a possible similar application for IL-4 [12, 13]. Our findings reported here indicated that glucocorticoids can act on synovial tissue in the same way and this action might presumably occur in vivo; whether IL-4 might have efficacy in suppressing some of the synovial tissue mediated manifestations of arthritic disease is unknown but perhaps is now worthy of investigation.

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