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DIALYSIS – TRANSPLANTATION

Involvement of interleukin-8 in dialysis-related arthritis

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Involvement of interleukin-8 in dialysis-related arthritis. To elucidate the role of interleukin (IL)-8, a chemotactic factor for neutrophils, in dialysis-related arthritis (DRA) of patients on long-term hemodialysis, the concentration of IL-8 was measured in the synovial fluids of DRA patients with acute arthralgia and joint swelling, and was compared with those in patients with rheumatoid arthritis (RA) and patients with osteoarthritis (OA). We noted a marked elevation of IL-8 in the joint fluids of patients with DRA and RA as compared with OA. Furthermore, to determine the role of IL-8 in synovitis, we examined the *in vivo* effect of intra-articular injection of human recombinant IL-8 on leukocyte infiltration into the joint space of rabbits. A single injection of IL-8 to the joints of rabbits induced rapid infiltration of neutrophils into the joint space and synovial tissues, which reached a maximum in four hours. The oral administration of indometacin farnesil (a prodrug that is converted to indomethacin after intestinal absorption) before the injection of IL-8 alleviated the infiltration of neutrophils. When human synovial cells were incubated with tumor necrosis factor (TNF)-α, the expression of IL-8 mRNA and IL-8 production in the cultured synovial cells were increased. The TNF-α-stimulated expression of IL-8 mRNA and IL-8 production in the cultured synovial cells were markedly inhibited by dexamethasone. In conclusion, IL-8 levels were markedly elevated in the joint fluids of patients with DRA. Interleukin-8 released from synovial cells may be an important factor to induce acute inflammation in DRA. Dexamethasone and indomethacin may be effective for DRA by inhibiting the production and chemotactic actions of IL-8, respectively.

The acute phase of inflammation is characterized by the accumulation of leukocytes, particularly neutrophils, at the site of tissue injury or infection. Neutrophils migrate according to the concentration gradient of chemotactic factor(s) [1]. A polypeptide leukocyte chemotactic cytokine, interleukin (IL)-8, consists of 72-amino acids with a molecular mass of 8 kDa in its mature form [2]. Interleukin-8 exhibits *in vitro* chemotactic activity against neutrophils [3], T lymphocytes [4] and basophils [5], affects the adhesion of neutrophils to the endothelium [6] and induces the *trans*-endothelial migration of neutrophils [7]. Interleukin-8 stimulates the degranulation of neutrophils, leading to the release of lysosomal enzymes and to the production of superoxide anion [2]; it is released from a variety of cells, including monocytes/macrophages, fibroblasts and endothelial cells in the presence of lipopolysaccharide (LPS), IL-1 and tumor necrosis factor (TNF)-α at the sites of infection or tissue injury [2, 7–9]. Intradermal injection of IL-8 causes infiltration of neutrophils as well as lymphocytes [4] and its intra-articular administration induces destruction of synovium in a neutrophil-dependent manner [10]. Furthermore, the administration of neutralizing antibody against IL-8 inhibits neutrophil infiltration and tissue damage in several types of acute infiltration [11, 12]. We demonstrated that hemodialysis (HD) using regenerated cellulose dialyzers markedly increased plasma IL-8 levels and IL-8 mRNA of peripheral blood mononuclear cells in uremic patients [13], and that plasma IL-8 can be used as a marker of biocompatibility of dialysis membranes [14]. These results suggest that overproduction of IL-8 is essentially involved in acute inflammatory lesions and occurs during HD in uremic patients.

Inflammation of the synovial joints is a common feature of rheumatoid arthritis (RA). During the course of disease, the local production of a number of cytokines by proliferative synovial cells as well as by infiltrating cells appears to account for various pathological and clinical manifestations in RA [15]. These cytokine factors can cause joint destruction either alone or in combination with other factors, and elevated levels of these mediators were documented in the synovial fluids of patients with RA [15, 16]. Neutrophils also increase in number in some chronic inflammatory lesions such as inflamed joints with RA. Particularly during acute inflammatory flares in RA, neutrophils have been shown to increase in synovial fluid [17]. Increased amounts of IL-8 were detected in synovial fluids of patients with active RA [10].

Frequent complications of dialysis-related arthritis (DRA) are recurrent or persistent arthralgia, restricted joint mobility and recurrent articular swelling with joint effusion and synovitis [18, 19]. These symptoms most frequently occur in the joints of shoulders and knees, followed by hips, wrists, elbows, acromioclavicular joints and feet. In joint destruction with DRA, β_2-microglobulin (β_2m) modified with advanced glycation end products (AGE) in amyloid deposits might induce monocyte chemotaxis and secretion of IL-1, IL-6 and TNF-α by infiltrating macrophages [20]. The local production of cytokines by proliferative synovial cells as well as by infiltrating cells may account for many pathological and clinical manifestations in DRA. The incidence of joint effusion reaches nearly 50% (5 of 11 cases) in patients receiving dialysis for more than 10 years [21]. Recent study using sonography also confirmed the high incidence (14 of 33 cases) of joint effusion in long-term HD patients [22]. Involvement is frequently bilateral and accompanied by mild joint discomfort. Occasionally, symptoms of acute pain are severe and flank arthritis is noted upon physical examination. Interleukin-8

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may be involved in the occurrence of the acute inflammatory lesions in DRA as observed in RA.

To elucidate the role of IL-8 in the acute inflammatory flares of DRA, IL-8 levels in the joint fluids of the patients with arthralgia and joint swelling were measured and the *in vivo* effect of IL-8 on the infiltration of leukocytes into joint space and synovial tissue was determined. Furthermore, the effects of indomethacin and dexamethasone on the IL-8-induced infiltration of leukocytes and TNF-α-stimulated expression of IL-8 mRNA and IL-8 production in cultured synovial cells, respectively, were evaluated.

METHODS

Patients

Joint fluids were aspirated from knee, hip or shoulder joints of six patients (5 males and 1 female) with DRA, when they complained of acute arthralgia and swelling in the joints. The patients were selected from a hemodialysis (HD) population of 411 patients. They ranged in age from 44 to 78 (mean 62.5) years, and all received HD therapy using cuprammonium rayon membranes for 4 hours three times a week. The duration of HD therapy was 12.2 $\pm$ 7.9 (mean $\pm$ SD) years. All the patients received a surgical operation for carpal tunnel syndrome associated with dialysis-related amyloidosis. Predonisolone (5 mg) was injected into their affected joints followed by alleviation of their symptoms. None of the patients with DRA had suffered from RA or osteoarthritis (OA).

Joint fluids were also aspirated from the knee joints of nine patients with RA (3 males and 6 females), and eight patients with OA (4 males and 4 females).

Assay of IL-8, IL-1α, IL-1β and TNF-α in joint fluids

Concentration of IL-8 in joint fluid and culture medium was measured by a two-step sandwich enzyme-linked immunosorbent assay (human IL-8 ELISA kit; Toray-Fuji Bionics, Tokyo, Japan). Concentrations of IL-1α, IL-1β and TNF-α in joint fluids were measured by two-step sandwich enzyme-linked immunosorbent assays (human IL-1α, IL-1β and TNF-α ELISA kits; Otsuka Pharmacol Co., Tokyo, Japan). Concentration of β_2m in joint fluids was measured by radioimmunoassay using a β_2-microglobulin kit Eiken (Eiken-Kagaku, Tokyo, Japan).

Intra-articular injection of IL-8

Twenty adult male New Zealand white rabbits (body weight 2.5 $\sim$ 3.5 kg) were used (IL-8 group, 8 rabbits; IL-8 + indometacin farnesil group, 8 rabbits; saline group, 4 rabbits). Carrier-free human recombinant IL-8 (0.5 μg; Bachem California, Torrance, CA, USA) was diluted in 0.5 ml of sterile endotoxin-free saline and injected into the joint space of the knee joint through the suprapatellar ligament [IL-8 group, $N = 8$ knee joints (4 rabbits) for 4 hour experiment, $N = 8$ knee joints (4 rabbits) for 8 hour experiment]. Further, to determine the effect of indometacin farnesil (Eisai, Tokyo, Japan) on the leukocyte accumulation in the joint, indometacin farnesil was orally given at a dose of 90 mg/kg body weight just before the injection of IL-8 [IL-8 + indometacin farnesil group, $N = 8$ knee joints (4 rabbits) for 4 hour experiment; $N = 8$ knee joints (4 rabbits) for 8 hour experiment]. Indometacin farnesil is a prodrug, and is converted after intestinal absorption to indomethacin by carboxyesterase in the liver, the kidneys and inflammatory lesions. An equal amount of saline (0.5 ml) was also injected into the contralateral knee joint as a control [saline group, $N = 4$ knee joints (2 rabbits) for a 4-hour experiment; $N = 4$ knee joints (2 rabbits) for an 8-hour experiment]. The animals were killed four or eight hours after injection of IL-8 or saline by the administration of Nembutal. One milliliter of sterile Hanks balanced salt solution (HBSS) was injected into the joint cavity. After manipulating the joint gently to mix the contents of the synovial cavity, the joint fluids from the saline and IL-8-treated joints were carefully collected and centrifuged for three minutes at 3000 g in a microcentrifuge. The infiltrated cells were resuspended in HBSS. The total cell number of leukocytes, after being stained with Turk solution, were determined using a hemocytometer. This was followed by Wright-Giemsa solution staining for differential leukocyte count. These cells were also examined for α-naphthyl butylate esterase staining and the rosette formation with 2-aminoethylisothiouronium bromide (AET)-treated sheep erythrocytes (SRBC).

Light microscopic analysis

At specified intervals following a single injection of recombinant IL-8 or saline, synovial tissue was dissected for histopathological examination at different sites of injection. Tissue samples were fixed in neutral buffered formalin and embedded in paraffin wax. Thin sections (3 $\sim$ 4 μm) were stained with hematoxylin and eosin and examined by light microscopy.

Isolation of synovial cells and its cultures

Synovial tissues were obtained from knee joints by arthroscopic biopsy performed on patients with traumatic injury. Synovial cells were isolated from the explant by collagenase digestion and cultured in a plastic culture flask (Falcon Plastic Inc., Los Angeles, CA, USA) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 50 U/ml penicillin G, 50 μg/ml streptomycin (GIBCO BRL/Life Technologies, Inc., Grand Island, NY, USA), 1 mg/ml amphotericin B (ICN Biomedicals Inc., Costa Mesa, CA, USA) and 10% fetal bovine serum (Cell Culture Lab., Cleveland, OH, USA). The cells between the 4th and 10th passages were used for the experiments.

Treatment of the synovial cells

When synovial cells were grown to confluency, the medium was replaced with DMEM containing 1 mg/ml of bovine serum albumin (BSA; Itohan Foods Inc., Hyogo, Japan) (BSA-DMEM) and incubated for 25 to 28 hours. The experiment was initiated by replacing the medium with BSA-DMEM with or without $10^{-6} \sim -8$ M dexamethasone. After incubation for one hour, TNF-α (0.5 ng/ml) was added and the cells were incubated another six hours for Northern blot analysis or 24 hours for ELISA.

Human recombinant TNF-α (2.5×10^4 U/μg) was obtained from Asahi Chemical Industry Co. Ltd. (Tokyo, Japan) and diluted with DMEM (GIBCO BRL/Life Technologies, Inc., Grand Island, NY, USA) immediately before use.

Northern blot analysis

cDNA for IL-8 and human glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) were prepared by polymerase chain reaction (PCR) using the product of reverse transcription of mRNA from TNF-α stimulated synovial cells. Oligonucleotide primers to

amplify the respective cDNA were as follows: IL-8, 5′-ATTCTCAGCCCTCTTCAAAA-3′ and 5′-TCTGCAGCTCTGTGTGAAGG-3′; GAPDH, 5′-TTCATTGACCTCAACTACAT-3′ and 5′-GAGGGGCCATCCACAGTCTT-3′. The expected sizes of the cDNA were 240 and 465 base pairs for IL-8 and GAPDH, respectively. After PCR amplification each fragment was cloned into pCR1000 plasmid (TA cloning system; Invitrogen, San Diego, CA, USA) according to the supplier's protocol. The cloned cDNA was used as probes for Northern blot analysis.

Total RNA was extracted from synovial cells by the method of Chomcynski and Sacchi [23]. Total RNA (4 ~ 10 μg) was denatured in 10 mM phosphate buffer (pH 7.0), 50% dimethyl sulfoxide and 1 M glyoxal, and was separated by electrophoresis on 0.8% agarose gels. The RNA was transferred onto a nylon filter (Gene Screen Plus; New England Nuclear, Boston, MA, USA). The membrane was prehybridized in buffer containing 50% formamide, 900 mM NaCl, 5 mM EDTA, 50 mM sodium phosphate (pH 7.4), 1% sodium dodecyl sulfate (SDS), 5 × Denhardt's solution [0.1% Ficoll (molecular weight 400,000), 0.1% polyvinylpyrrolidone (molecular weight 360,000), 0.1% BSA], and denatured herring sperm DNA (0.1 mg/ml) for at least four hours at 42°C. Heat denatured cDNA probes were labeled with [^{32}P]dCTP (111 TBq/mmol; New England Nuclear) using the Random Primed DNA Labeling Kit (Boehringer Mannheim, Mannheim, Germany). Labeled cDNA probes were added to the solution and the hybridization was continued for 20 hours at 42°C. After hybridization, the membranes were washed in 2 × SSC (300 mM NaCl, 30 mM sodium citrate) at 20°C (2 × 5 min), 2 × SSC and 1% SDS at 65°C (2 × 30 min), and 0.1 × SSC (15 mM NaCl, 1.5 mM sodium citrate) at 20°C (2 × 10 min) and exposed to Kodak X-AR film (Eastman Kodak Co., Rochester, NY, USA) for autoradiography. The amount of RNA was determined by scanning densitometry (Image Analyzer, Immunomedica Co. Ltd., Shizuoka, Japan) of the band and expressed in arbitrary units.

STATISTICS

The results are expressed as mean ± SE with a significance of $P < 0.05$. For comparison of multiple groups ANOVA was performed and Fisher's PSLD was used.

RESULTS

Levels of IL-8 and the other cytokines in the synovial fluids from affected joints of patients with DRA

As shown in Figure 1, the IL-8 levels were markedly increased in the joint fluids of patients with DRA (7920 ± 4330 pg/ml, $P < 0.05$) as well as RA (12700 ± 2700 pg/ml, $P < 0.01$), compared with OA (284 ± 211 pg/ml). The concentrations of IL-1α in the joint fluids of all the patients with DRA, RA and OA were below the detection limit (less than 7.8 pg/ml) and the concentrations of IL-1β in the joint fluids of all the patients with DRA, RA and OA, except one case with DRA (73.4 pg/ml) and one with RA (22.8 pg/ml), were below the detection limit (less than 15.6 pg/ml). Tumor necrosis factor-α in the joint fluid was detected in three patients with DRA (mean 22.9 pg/ml ranging from 17.6 to 27.7 pg/ml) and two patients with RA (95.3 and 35.9 pg/ml), but could not be detected in the other patients with DRA and RA nor all the patients with OA (less than 7 pg/ml). The levels of IL-8 were significantly higher in the synovial fluids of the three DRA patients (15100 ± 6500 pg/ml, $P < 0.05$) with detectable levels of TNF-α in their synovial fluids than the other DRA patients (727 ± 87 pg/ml) without detectable levels of TNF-α. The levels of β_2m in the synovial fluids were significantly increased in the DRA patients (17.1 ± 4.8 μg/ml, $P < 0.05$) as compared with those in OA (3.1 ± 1.0 μg/ml) and RA (2.7 ± 0.5 μg/ml). However, the β_2m levels in the synovial fluids of DRA patients did not show any significant correlation with their levels of IL-8 or TNF-α.

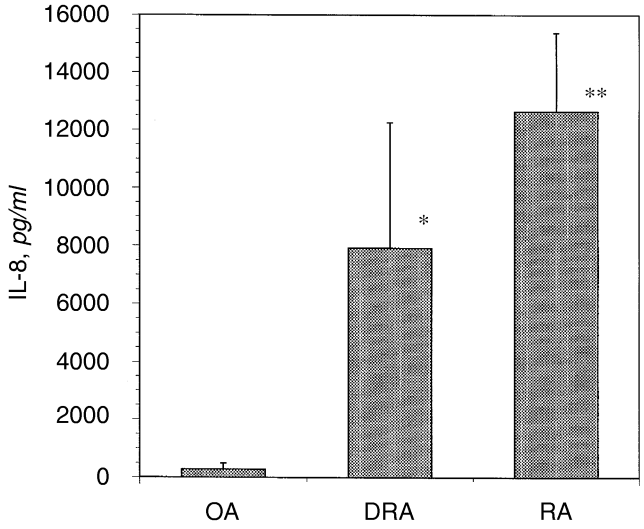

Fig. 1. Concentrations of interleukin (IL)-8 in joint fluids of patients with dialysis-related arthritis (DRA), rheumatoid arthritis (RA) and osteoarthritis (OA). IL-8 levels in DRA and RA were markedly increased compared with OA. Data are expressed as mean ± SE ($N = 8$ for OA, $N = 6$ for DRA and $N = 9$ for RA). $^*P < 0.05$ and $^{**}P < 0.01$ vs. OA.

Effect of indometacin farnesil on IL-8-induced infiltration of leukocytes into the synovial cavity and synovial tissue

Figure 2 shows the effect of IL-8 injection on leukocyte accumulation in the joint space and its time course. Saline caused a small number of leukocytes to accumulate four hours after injection. An intra-articular injection of IL-8 (0.5 μg) rapidly induced infiltration of leukocytes into the joint space with maximal accumulation four hours after injection. The oral administration of indometacin farnesil just before the injection of IL-8 reduced leukocyte infiltration into the joint space. Accumulation of neutrophils and mononuclear cells were present in the joint wash fluids of the IL-8-induced synovitis. Neutrophils consisted of 89% of the infiltrated leukocytes. By light microscopic examination, most of these mononuclear cells were small, round and positive for α-naphthyl butylate esterase staining. About 80% of the infiltrated mononuclear cells were positive for rosette formation with AET-treated SRBC, indicating that these small mononuclear cells were T lymphocytes, which were 9% of the infiltrated leukocytes.

As shown in Figure 3A, a large number of leukocytes had infiltrated into the superficial layers of the synovium four hours after the injection of IL-8 comparing with the synovium four hours after injection of saline (Fig. 3C). The oral administration of indometacin farnesil just before the injection of IL-8 reduced leukocyte infiltration into the synovial tissue (Fig. 3B).

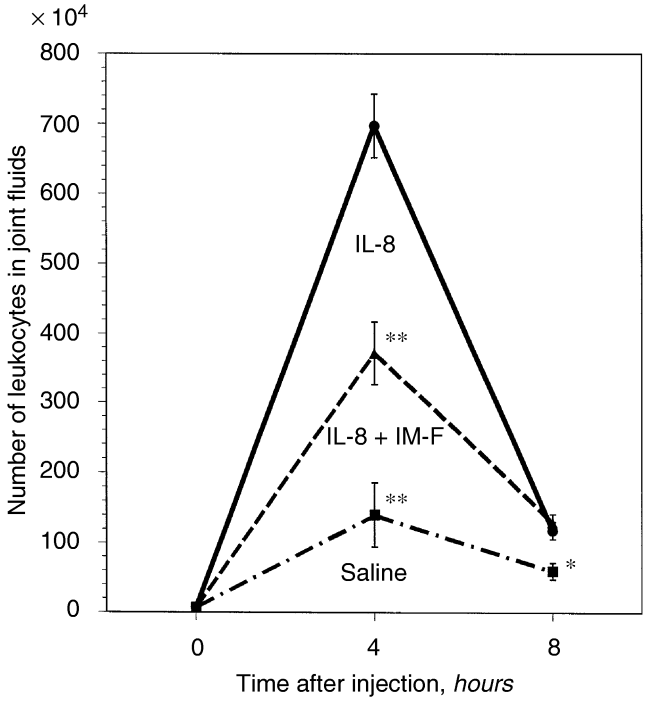

Fig. 2. Time course of leukocyte infiltration in joint fluids induced by intra-articular injection of IL-8. Symbols are: (—) IL-8; (– –) IL-8 + IM-F; (– · –) saline controls. Leukocyte infiltration occurred with maximal accumulation 4 hours after injection of IL-8 (0.5 μg). The oral administration of indometacin farnesil (IM-F) reduced leukocyte infiltration into the joint space in the IL-8-induced synovitis. Data are expressed as mean ± SE ($N = 8$ for IL-8, $N = 8$ for IL-8 + IM-F and $N = 4$ for saline). $^*P < 0.05$ and $^{**}P < 0.01$ vs. IL-8.

Effect of dexamethasone on TNF-α-stimulated expression of IL-8 mRNA

When synovial cells were incubated with TNF-α for six hours, the level of IL-8 mRNA was markedly increased (Fig. 4). To examine whether dexamethasone suppresses TNF-α-stimulated expression of IL-8 mRNA, the synovial cells were incubated with $10^{-6} \sim {}^{-8}$ M dexamethasone for one hour before the addition of TNF-α. The incubation with dexamethasone and TNF-α was continued for six hours. As shown in Figure 4, the dexamethasone reduced TNF-α-stimulated expression of IL-8 mRNA in a concentration dependent manner, reaching a plateau at 10^{-7} M. The dose of dexamethasone (10^{-7} M) reduced IL-8 mRNA to about 40% of the level stimulated by TNF-α.

Effect of dexamethasone on TNF-α-stimulated production of IL-8

To examine whether dexamethasone suppresses TNF-α-stimulated production of IL-8, the synovial cells were incubated with 10^{-7} M dexamethasone for one hour before the addition of TNF-α. The incubation of the cells with dexamethasone and TNF-α was continued for 24 hours. As shown in Figure 5, the addition of TNF-α resulted in a marked increase in IL-8 (7720 ± 740 pg/ml, $N = 4$) compared with controls (14.9 ± 0.8 pg/ml, $N = 4$). Dexamethasone reduced the TNF-α-stimulated IL-8 synthesis to about 50% (4000 ± 180 pg/ml, $N = 4$).

DISCUSSION

In this study we have shown that IL-8 levels were increased in the synovial fluids of patients with DRA. We demonstrated that intra-articular injection of IL-8 increased leukocyte infiltration into joint space and the oral administration of indometacin farnesil reduced leukocyte infiltration in IL-8-induced acute synovitis. Further, TNF-α stimulated the expression of IL-8 mRNA and IL-8 production in cultured synovial cells, and the TNF-α-stimulated expression of IL-8 mRNA and IL-8 production were inhibited by dexamethasone. Although TNF-α was detectable in the synovial fluids in three of the six patients with DRA, the levels of IL-8 in the synovial fluids were significantly higher in the three DRA patients with detectable levels of TNF-α in their synovial fluids than the other DRA patients. Thus, elevation of IL-8 is not unique in the setting of inflammatory arthritis, and the elevation of IL-8 in the synovial fluids of patients with DRA may be partly due to TNF-α-induced IL-8 production by synovial cells. Interleukin-8 may be involved in acute inflammatory flares of DRA, since IL-8 induces neutrophil infiltration and neutrophil-mediated cartilage breakdown as a result of induction of neutrophil degranulation by the cytokine [24].

The intra-articular injection of IL-8 induced rapid infiltration of leukocytes into the synovium and joint cavity, with a maximal response occurring four hours after the injection. At that time neutrophils were present in the greatest number and they subsequently decreased. The intra-articular injection of IL-1 led to the same kinetics as in the present study [25], suggesting that the leukocyte infiltrating activity of IL-1 was mediated by IL-8 production in synovial cells as demonstrated *in vitro* [7, 8]. However, a definite statement about the involvement of IL-1 in DRA cannot be made from our clinical data. We demonstrated *in vivo* that indomethacin inhibited the IL-8-induced infiltration of leukocytes. Indomethacin was also reported to inhibit adhesivity and chemotaxis of neutrophils in response to zymosan-activated serum or N-formyl-methionyl-leucyl-phenylalanine (FMLP) [26] and the release of lysosomal enzymes and the production of superoxide anions by neutrophils [27].

The IL-8-induced accumulation of inflammatory cells is associated with joint redness, which is not accompanied by significant joint swelling. Rampart et al [28] noted that the administration of IL-8 and vasodilator substances such as PGE_2 into the skin synergistically induced both neutrophil accumulation and plasma extravasation. It is thus evident that IL-8 alone directly induces leukocyte infiltration, but does not form edema. Thus, indomethacin may also inhibit edema formation by suppressing PGE_2 synthesis.

Tumor necrosis factor-α stimulated IL-8 production in cultured synovial cells. Interleukin-8 production is mainly controlled at the activation step of the transcription factor, NF-κB [29, 30]. NF-κB may be activated in synovial cells and endothelial cells in joints of patients with RA (particularly those with active disease), and it regulates the expression of many genes involved in immune and inflammatory proteins such as cytokines, enzymes and adhesion molecules [31, 32]. The proinflammatory cytokines IL-1β and TNF-α activate NF-κB, and their transcriptions are also activated by NF-κB. Thus, products of the genes that are regulated by NF-κB cause the activation of NF-κB [32]. This type of positive regulatory loop may amplify and perpetuate local inflammatory responses. In fact, it has been reported that NF-κB is activated in the synovial tissues of patients with RA [33]. Since IL-8 is

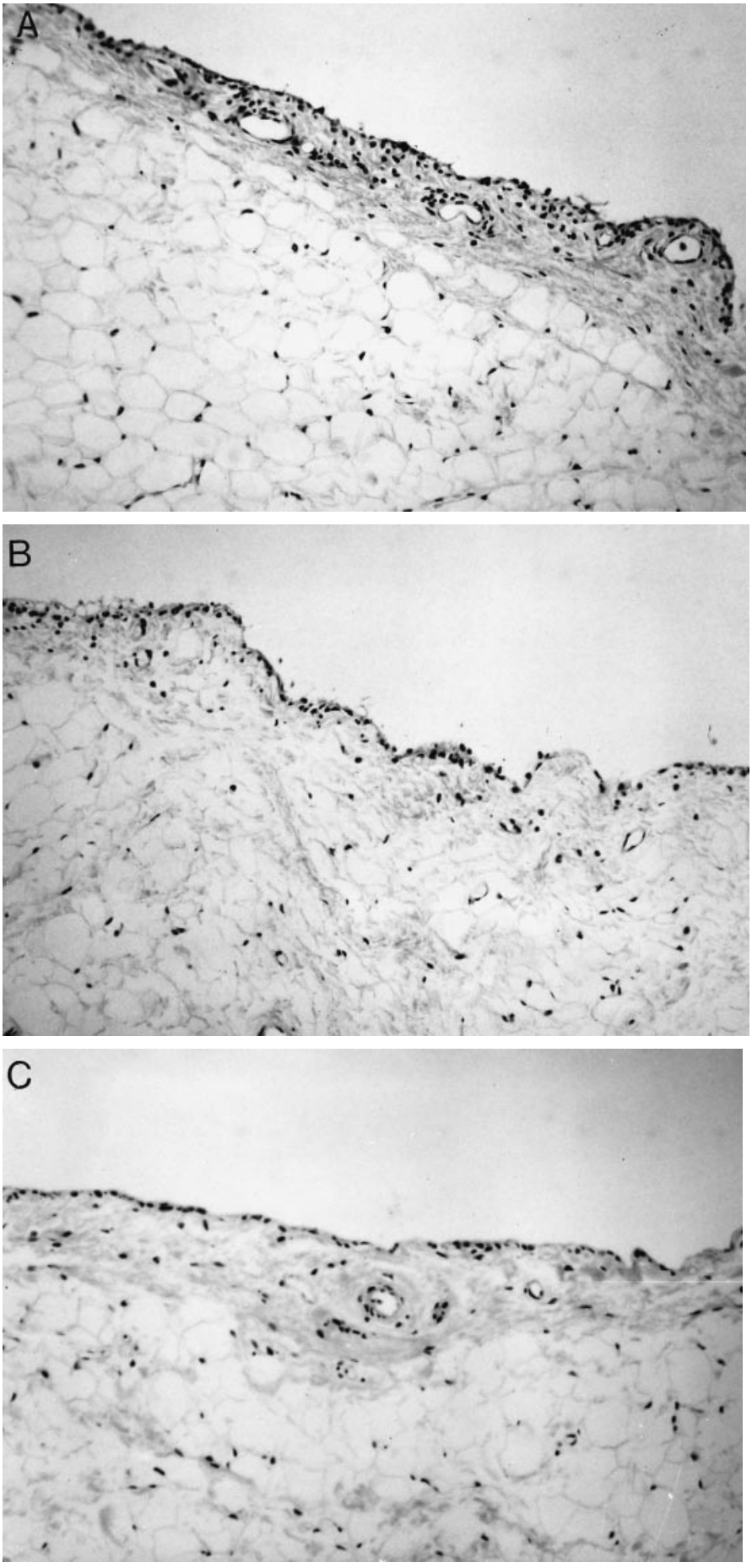

Fig. 3. Light microscopy of the synovial tissues obtained 4 hours after injection of interleukin (IL)-8 with or without oral administration of indometacin farnesil (hematoxylin and eosin stain, ×200). (*A*) Synovial tissue after intra-articular injection of IL-8, showing infiltration of a large number of neutrophils on the superficial layers of the synovium. (*B*) Synovial tissue after intra-articular injection of IL-8 with oral administration of indometacin farnesil, showing that neutrophil infiltration was reduced. (*C*) Synovial tissue after intra-articular injection of endotoxin-free saline, showing normal appearance.

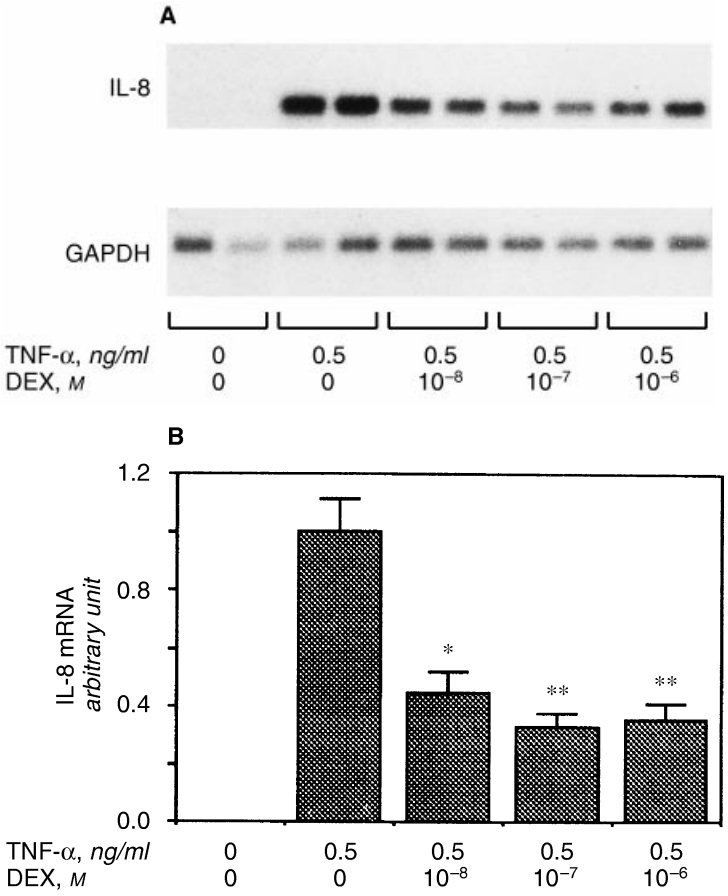

Fig. 4. Effect of dexamethasone (DEX) on tumor necrosis factor alpha (TNF-α)-stimulated expression of interleukin (IL)-8 mRNA. Human synovial cells were incubated with dexamethasone for 1 hour, followed by the addition of TNF-α (0.5 ng/ml) and further incubation for 6 hours. (*A*) Autoradiograph of a Northern blot. (*B*) Densitometric analysis of the autoradiographs from two separate experiments. The amount of IL-8 mRNA was corrected with that of GAPDH mRNA (ANOVA $P < 0.01$). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. TNF-α (0.5 ng/nl) and DEX (0 M).

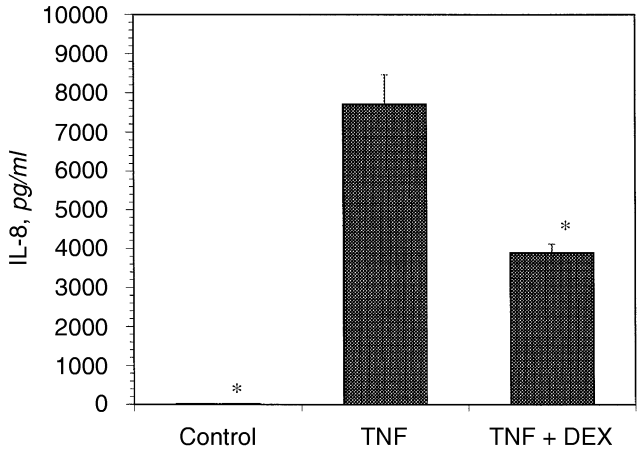

Fig. 5. Effect of dexamethasone (DEX) on tumor necrosis factor-alpha (TNF-α)-stimulated production of interleukin (IL)-8. Human synovial cells were incubated with 10^{-7} M dexamethasone for 1 hour, followed by addition of TNF-α (0.5 ng/ml) and further incubation for 24 hours. $^{*}P < 0.05$ vs. TNF, by ANOVA.

abundant in synovial fluids of patients with DRA, NF-κB may also be activated in the affected synovial tissue of DRA.

We demonstrated that dexamethasone suppressed TNF-α-induced IL-8 synthesis in cultured synovial cells. Activation of NF-κB, for example by cytokines, is blocked by glucocorticoids [32, 34]. Glucocorticoid-receptor complexes bind the p65 subunit of NF-κB and this prevents NF-κB activation of inflammatory genes. Synthesis of IκBα is stimulated by the binding of glucocorticoid-receptor complexes to a glucocorticoid response element in the promoter region of the IκBα gene.

In conclusion, IL-8 levels were markedly elevated in the joint fluids of patients with DRA. Interleukin-8 released from synovial cells may be an important factor to induce acute inflammation in DRA. Dexamethasone and indomethacin may be effective for the arthritis through inhibition of production and chemotactic action of IL-8, respectively.

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APPENDIX

Abbreviations used in this article are: AET, 2-aminoethylisothiouronium bromide; AGE, advanced glycation end products; β_2m, beta-2-microglobulin; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; DRA, dialysis:-related arthritis; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; HBSS, Hank's balanced salt solution; HD, hemodialysis; IL, interleukin; LPS, lipopolysaccharide; OA, osteoarthritis; PCR, polymerase chain reaction; RA, rheumatoid arthritis; SDS, sodium dodecyl sulfate; SRBC, sheep erythrocytes; TNF-α, tumor necrosis factor alpha.

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