Interleukin-4 inhibits prostaglandin E_2 production by freshly prepared adherent rheumatoid synovial cells via inhibition of biosynthesis and gene expression of cyclo-oxygenase II but not of cyclo-oxygenase I

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Abstract

Objective—To characterise the effect of interleukin-4 (IL-4) on the biosynthesis of cyclo-oxygenases I (COX I) and II (COX II), the rate limiting enzymes of the synthesis of prostaglandin E_2 (PGE$_2$), in freshly prepared rheumatoid synovial cells.

Methods—Adherent synovial cells were obtained from rheumatoid synovium by collagenase digestion. The concentrations of PGE$_2$ in culture supernatants were determined by enzyme linked immunosorbent assay. The protein and mRNA concentrations of COX I and COX II were determined by Western blotting and reverse transcription polymerase chain reaction, respectively.

Results—Freshly prepared synovial cells produced large amounts of PGE$_2$. They also showed increased gene expression of COX I and COX II, and synthesised these proteins. IL-4 had suppressive effects on the production of PGE$_2$ by untreated or lipopolysaccharide (LPS) stimulated synovial cells. In addition, IL-4 inhibited the biosynthesis of COX II at the mRNA level. In contrast, it did not modify the protein concentration of COX I. In tests of cell specificity, IL-4 did not reduce the mRNA concentration of COX II in interleukin-1α (IL-1α) stimulated cultured synovial fibroblasts at passages 3–6, but it reduced considerably the mRNA concentrations of COX II in an LPS or IL-1α stimulated U937 monocyte/macrophage cell line.

Conclusions—These results suggest that IL-4 might inhibit overproduction of PGE$_2$ in rheumatoid synovia via selective inhibition of the biosynthesis of COX II, and that this inhibition might be specific to macrophage-like synovial cells.

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Rheumatoid arthritis (RA) is an inflammatory joint disease in which perpetuation of the chronic synovitis leads to bone and cartilage degradation.[1] Inflammatory cytokines or soluble factors are essential in pathogenesis of RA, and rheumatoid synovia are known to be rich sources of these mediators.[1][2] Among them, prostaglandin E_2 (PGE$_2$), actively produced by rheumatoid synovia, is widely considered to have an important role in the inflammatory process in rheumatoid joints.[3][4] Consequently, non-steroidal anti-inflammatory drugs (NSAIDs), the inhibitors of the production of prostaglandin metabolites, are extensively used in the treatment of RA.

Cyclo-oxygenase (COX), which converts arachidonic acid to prostaglandin endoperoxides, is the rate limiting enzyme in prostanoid synthesis.[5] At least two forms of COX have been identified, and their genes have been cloned:[6] type I COX (COX I) is expressed constitutively in most tissues and is involved in cellular 'housekeeping' activities such as coordinating actions of circulating hormones and regulating vascular homeostasis.[7] In contrast, type II COX (COX II) is highly inducible by serum, growth factors, and lipopolysaccharide (LPS) in certain cell types involved in the inflammatory process in RA, including macrophages,[8] fibroblasts,[9][10] and endothelial cells.[11] In addition, it has been demonstrated that COX II is expressed in rheumatoid synovia and synovial fibroblasts, and that interleukin-1β (IL-1β) increases the mRNA concentrations of COX II in these cells.[12][13] These observations suggest that COX II may play an important part in overproduction of PGE$_2$ in rheumatoid synovia.

Interleukin-4 (IL-4), originally described as a B cell growth factor, is a 20 kDa product of activated T cells.[14] We and other investigators previously reported that IL-4 inhibited the production of inflammatory cytokines such as IL-1β, IL-6, and IL-8 by rheumatoid synovia or freshly prepared rheumatoid synovial cells.[15-17] In addition to these anti-inflammatory actions, inhibitory effects of IL-4 on PGE$_2$ production by monocytes/macrophages,[18-20] fibroblasts,[10] and mesangial cells[21] have also been demonstrated. However, the mechanism(s) of the inhibitory action of IL-4 on PGE$_2$ production remains to be clarified.

We have investigated the effect of IL-4 on the production of PGE$_2$, and on the biosynthesis

and gene expression of COX I and COX II, in freshly prepared rheumatoid synovial cells.

Patients and methods

PATIENT POPULATION
Rheumatoid synovial tissues were obtained from the knee or wrist joints of patients who were undergoing surgical synovectomy or total joint replacement. The population consisted of five patients with RA (three women and two men, age range 52–63 years) who fulfilled the criteria of the American College of Rheumatology.[22] All five patients were treated with NSAIDs; two of the five were treated with gold sodium thiomalate, and another two with bucillamine.

CELL CULTURE
Adherent rheumatoid synovial cells—Isolated rheumatoid synovia were aseptically dissected free from surrounding tissues, minced, and enzymatically digested with 0·5–1 mg/ml clostridium collagenase (Wako Pure Chemical Industries, Ltd, Osaka, Japan) and 5–10 µg/ml deoxyribonuclease 1 (Sigma Chemical Co St Louis, MO) for two to three hours. After digestion, the resultant single cell suspension was washed, filtered through sterile gauze and nylon mesh, washed thoroughly once again, and finally resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (FCS) (ICN Biomedicals, Australia), penicillin 100 U/ml, gentamicin 60 µg/ml, N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) 12·5 mmol/l, and L-glutamine 2 mmol/l (DMEM/ 10% FCS). The cells were cultured overnight in a humidified 5% carbon dioxide incubator to allow them to adhere to a plastic surface. The plate was washed to remove non-adherent cells, and the remaining adherent cells were used as adherent synovial cells.

Rheumatoid synovial fibroblasts—The freshly prepared adherent synovial cells obtained as above were cultured with DMEM/10% FCS at 37°C. When cellular confluency was attained (five to seven days), the cells were harvested by trypsinisation, washed, and one third of them replaced in culture dishes. Thereafter, one third of the cells were passaged when 95% confluency was reached. The synovial cells at three or more passages were morphologically fibroblast-like, and all negative for CD14 antigen and HLA-DR antigen on their surface (data not shown); they were therefore used as rheumatoid synovial fibroblasts in this study.

U937 cell line—U937 cells, human histiocytic lymphoma,[23] kindly provided by Japanese Cancer Research Resources Bank, were cultured in RPMI 1640 supplement with 10% FCS, HEPES, and L-glutamine. U937 cells were differentiated into monocyte/macrophage cells by 48 hours of incubation with 100 nmol/l of phorbol myristate acetate (PMA) (Sigma Chemical Co). After differentiation, the cells became adherent to the dish and expressed CD14 antigen on their surface (data not shown).

PGE2 MEASUREMENT
Adherent synovial cells, rheumatoid synovial fibroblasts, or U937 cells were adjusted to concentrations of 2–5 × 10^5/ml, and 1 ml of this suspension was added to each well of a 24 well macroplate and cultured in the presence or absence of various concentrations of human recombinant IL-4 (specific activity, 10^6 U/mg protein) (obtained from Ono Pharmaceutical Co, Osaka, Japan), for 24 hours. For other experiments, LPS (Sigma Chemical Co), human recombinant IL-1α (kindly provided by Dainippon Pharmaceutical Co, Osaka, Japan), dexamethasone, or indomethacin (Wako Pure Chemical Industries, Ltd) was added to the adherent cell culture. After incubation for various periods, the supernatants were stored at −20°C until required for measurement of PGE_2.

PGE_2 in the culture supernatant was measured using a commercially available enzyme linked immunosorbent assay kit according to the manufacturer's instructions (ELISA Technology, Lexington, KY).

WESTERN BLOT ANALYSIS
After incubation with various stimuli, freshly prepared adherent synovial cells were lysed in a buffer (50 mmol/l TRIS hydrochloric acid, pH 8·0, 1% Nonidet® P-40, 0·5% deoxycholic acid, 150 mmol/l sodium chloride, and 1 mmol/l phenyl methylsulphonyl fluoride). Proteins were separated by electrophoresis on a 7·5% sodium dodecyl sulphate-polyacrylamide gel, and transferred onto an Immobilon-P membrane (Millipore, Bedford, MA). After blocking, the membrane was incubated with antihuman COX I or antihuman COX II rabbit polyclonal antibodies (Oxford Biomedical Research, Oxford, MI), then with antirabbit donkey antibodies conjugated with horseradish peroxidase. Specific bands were visualised by an enhanced chemiluminescence detection system (ECL, Amersham International, Buckinghamshire, UK), and exposed to Fuji new RX films (Fuji Photo Film, Kanagawa, Japan).

EXTRACTION OF RNA, AND REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)
Freshly prepared adherent synovial cells, rheumatoid synovial fibroblasts, or PMA differentiated U937 cells were cultured with IL-1α or LPS in the presence or absence of IL-4, dexamethasone, or indomethacin for eight hours, and their total cellular RNAs were extracted by acid guanidine phenol/chloroform extraction[24] using Isogen (Wako Pure Chemical Industries, Ltd).

Two micrograms of total RNA was reverse transcribed to cDNA after annealing with 100 pmol of oligo deoxythymine $(dT)_{12-18}$ primer (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) in the presence of 100 U of Moloney murine leukaemia virus (M-MLV) reverse transcriptase (Gibco BRL Life Technologies, Inc, Gaithersburg, MD), 10 U of ribonuclease inhibitor (RNasin®, Promega Corporation, Madison, WI), 1 mmol/l dithiothreitol, 25

pmol of random primer (Takara Shuzo Co Ltd, Kyoto, Japan), and 10 pmol of each deoxynucleotide (Takara Shuzo Co) in a total volume of 10 μl for one hour at 37°C. Two microlitres of the resultant cDNA preparation was used directly for each amplification reaction.

The polymerase chain reaction (PCR) was performed in a 50 μl reaction mixture containing 20 pmol of each primer (see below), 20 pmol of each deoxynucleotide and 1·25 U Taq DNA polymerase (Takara Shuzo Co Ltd). Amplification of each specific PCR product was carried out separately, in a different tube. Primers used were: COX I sense primer, 5'-GAGGGGCAGGAAAGCAGCAT-3' (nucleotide 1804'-1823'); COX I antisense primer, 5'-CATCAGCCAGGGAACACAAC-3' (nucleotide 2210'-2229'); COX II sense primer, 5'-GAAACCCACTCCAAACACAG-3' (nucleotide 298-317); COX II antisense primer, 5'-CGTAGATGCTCAGGGACTTG-3' (nucleotide 887'-906'); hypoxanthine phosphoribosyl transferase (HPRT) sense primer, 5'-CTCCGCCTCCTCCTCTGCTC-3' (nucleotide 12-31'); HPRT antisense primer, 5'-TTGCGACCTTGACCATCTTT-3' (nucleotide 550'-569'); glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sense primer, 5'-CAAAAGGGTCATCATCTCTG-3' (nucleotide 408'-427'); GAPDH antisense primer, 5'-CCTGCTTCACCACCTTCTTG-3' (nucleotide 834'-853'). These primer sets yield PCR products of 426 bp, 609 bp, 558 bp, and 446 bp for COX I, COX II, HPRT, and GAPDH, respectively. Reactions were incubated in a Perkin-Elmer/Cetus DNA Thermal cycler for 25-30 cycles (denaturation, one minute, 94°C; annealing, two minutes, 60°C; extension, one minute, 72°C). Aliquots of PCR products were run on a 1·5% agarose gel in TAE buffer (40 mmol/l TRIS acetate, 2mmol/l sodium EDTA), and visualised by ethidium bromide staining.

SPONTANEOUS PRODUCTION OF PGE2 BY FRESHLY PREPARED ADHERENT SYNOVIAL CELLS

To evaluate the capacity of adherent synovial cells to produce PGE_2 (the major arachidonate metabolite produced by rheumatoid synovial cells), samples of these cells were cultured in DMEM/10%FCS without stimuli, the culture supernatant was replaced with fresh medium every 24 hours, and the replaced supernatant was measured for PGE_2.

EFFECT OF IL-4 ON PRODUCTION OF PGE2 BY ADHERENT SYNOVIAL CELLS

To examine if IL-4 inhibited the spontaneous production of PGE_2 by freshly prepared adherent synovial cells, the cells were cultured with increasing concentrations (0, 0·05, 0·5, 5, or 50 U/ml) of IL-4 for 24 hours, and the PGE_2 concentrations in the supernatants determined. Seven days after isolation, cells were cultured with or without 1 μg/ml of LPS in the presence or absence of IL-4 0, 1, 10, or 100 U/ml, dexamethasone 5 μmol/ml, or indomethacin 5 μmol/ml (as positive controls)

for 24 hours, and the concentrations of PGE_2 in the culture supernatants determined.

Different adherent synovial cells obtained from five patients with RA were cultured with or without 100 U/ml of IL-4 (patients Nos 1-4) or 50 U/ml of IL-4 (patient No 5) for 24 hours. The supernatants were collected and the concentrations of PGE_2 determined.

EFFECT OF IL-4 ON BIOSYNTHESIS OF COX I AND COX II IN ADHERENT SYNOVIAL CELLS

To clarify the mechanism by which IL-4 inhibited the production of PGE_2, we examined the effect of IL-4 on the biosynthesis of COX I and COX II proteins. Freshly prepared adherent synovial cells were incubated with or without IL-1α 10 ng/ml or LPS 1 μg/ml in the presence or absence of IL-4 100 U/ml or dexamethasone 5 μmol/l (as positive control[8] [10] [12]) for 24 hours. After incubation, the cells were lysed and the lysates analysed by western blotting using rabbit antihuman COX I and COX II. Adherent synovial cells were also cultured with LPS 1 μg/ml in the presence or absence of IL-4 1, 10, 100, or 1000 U/ml for 24 hours and the protein level of COX II in the cell lysate determined by western blotting.

EFFECT OF IL-4 ON THE GENE EXPRESSION OF COX II IN ADHERENT SYNOVIAL CELLS

To clarify further whether IL-4 might inhibit the synthesis of COX II at the mRNA level, we examined the effect of IL-4 on the gene expression of COX II by adherent synovial cells. Freshly prepared adherent synovial cells were cultured with or without IL-4 100 U/ml, dexamethasone 5 μmol/l, or indomethacin 5 μmol/l for 24 hours. After incubation, their total cellular RNAs were extracted, and the mRNA contents of COX I, COX II, and HPRT were determined by RT-PCR analysis. HPRT was the housekeeping gene used to assess the amount of RNA samples.

DIFFERENTIAL EFFECTS OF IL-4 ON PGE2 PRODUCTION BY CULTURED RHEUMATOID SYNOVIAL FIBROBLASTS AND A PMA DIFFERENTIATED U937 MACROPHAGE CELL LINE AND THEIR GENE EXPRESSIONS OF COX II

To determine the cell specificity of the suppressive effect of IL-4, we examined the effect of IL-4 on the production of PGE_2 and the gene expression of COX II by macrophage-like cells or fibroblast-like cells, which comprise the two major cell populations of freshly prepared adherent synovial cells.[25]

IL-1α stimulated cultured rheumatoid synovial fibroblasts—Rheumatoid synovial fibroblasts at three to six passages were cultured at a concentration of 2 × 10⁵/well in 24 well macroplates with or without IL-1α 10 ng/ml in the presence of IL-4 100 U/ml, dexamethasone 5 μmol/l, or indomethacin 5 μmol/l for 24 hours and the concentrations of PGE_2 in the culture supernatants determined. Additional cells were cultured with or without IL-1α 10 ng/ml in the presence of IL-4 10 or 100 U/ml (experiment

1) or 100 U/ml (experiment 2), or dexamethasone 5 µmol/l for eight hours. After incubation, their total cellular RNAs were extracted, and the mRNA contents of COX II and HPRT were determined by RT-PCR analysis.

IL-1α or LPS stimulated PMA differentiated U937 macrophage cell line—For differentiation to macrophages, U937 cells were cultured with PMA 100 nmol/l for 48 hours. The cells were cultured at a concentration of 2×10^5/well in 24 well macroplates with or without IL-1α 10 ng/ml or LPS 1 µg/ml in the presence of IL-4 100 U/ml or dexamethasone 5 µmol/l for 24 hours, after which the concentrations of PGE_2 in the culture supernatants were determined. PMA differentiated U937 cells were also cultured with or without IL-1α 10 ng/ml or LPS 1 µg/ml in the presence of IL-4 100 U/ml or dexamethasone 5 µmol/l for eight hours. After incubation, their total cellular RNAs were extracted, and the mRNA contents of COX II and GAPDH were determined by RT-PCR analysis. GAPDH was the housekeeping gene used to assess the amount of RNA samples.

Results

SPONTANEOUS PRODUCTION OF PGE2 BY FRESHLY PREPARED ADHERENT SYNOVIAL CELLS

Freshly prepared adherent synovial cells produced large amounts of PGE_2 for one to two days, after which the production decreased rapidly (fig 1), suggesting that these adherent synovial cells were spontaneously activated to produce PGE_2, but that the culture conditions did not continuously stimulate them to produce it. The same was observed in three different cultures of adherent synovial cells. Consequently, we carried out subsequent experiments using freshly prepared adherent synovial cells obtained within two days of the preparation of rheumatoid synovia.

SUPPRESSIVE EFFECT OF IL-4 ON PGE2 PRODUCTION BY FRESHLY PREPARED ADHERENT SYNOVIAL CELLS

IL-4 inhibited the production of PGE_2 by freshly prepared adherent synovial cells in a dose dependent manner that became effective at concentrations as small as 0·05 U/ml (fig 2A). This effect was consistently observed in patients with RA: a mean (SD) inhibition of 56 (3)% was observed in the five patients studied (fig 3).

LPS markedly enhanced the production of PGE_2 by these cells, and IL-4 inhibited this augmented production in a dose dependent manner (fig 2B). However, the effect of IL-4 seemed to be less potent than that of either dexamethasone or indomethacin.

EFFECT OF IL-4 ON THE BIOSYNTHESIS OF COX I AND COX II IN FRESHLY PREPARED ADHERENT SYNOVIAL CELLS

Bands of both COX proteins were detected in freshly prepared adherent synovial cells. The COX I protein consisted of a major band around 70 kDa, whereas the COX II protein was composed of three bands around 70 kDa
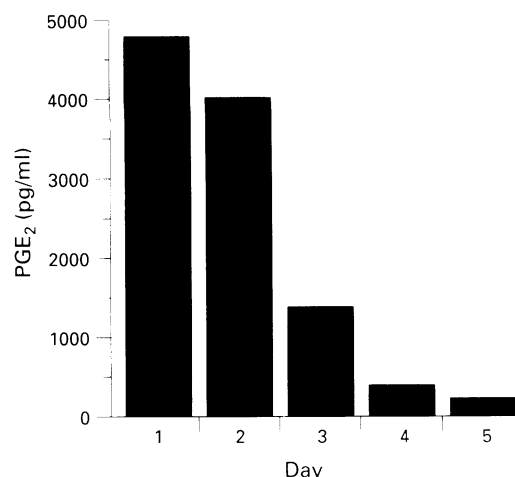

Figure 1 Spontaneous production of prostaglandin E_2 (PGE_2) by freshly prepared adherent rheumatoid synovial cells. The culture supernatants were collected on the days indicated. Values are the mean of triplicate determinations.

(fig 4A). These findings are consistent with those of previous studies of COX.[12] The heterogeneity in COX II protein is likely to reflect different degrees of glycosylation.
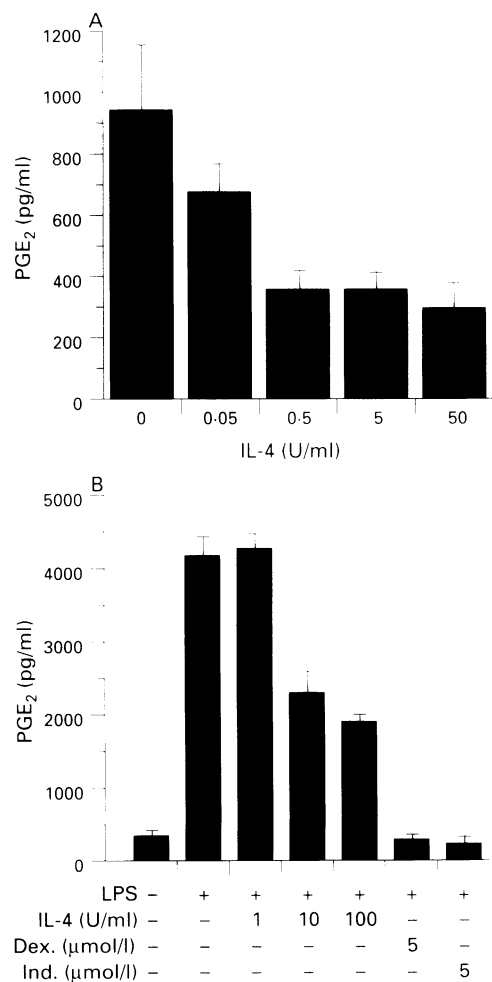

Figure 2 Suppressive effect of interleukin-4 (IL-4) on the production of prostaglandin E_2 (PGE_2) by adherent rheumatoid synovial cells. A: Cells cultured with or without increasing concentrations of IL-4 for 24 hours. Values are the mean (SD) of triplicate determinations. B: Cells cultured, seven days after isolation, with or without lipopolysaccharide (LPS) 1 µg/ml in the presence or absence of IL-4 1, 10, or 100 U/ml, dexamethasone (Dex.) 5 µmol/l, or indomethacin (Ind.) 5 µmol/l for 24 hours. Data are representative of three separate experiments.
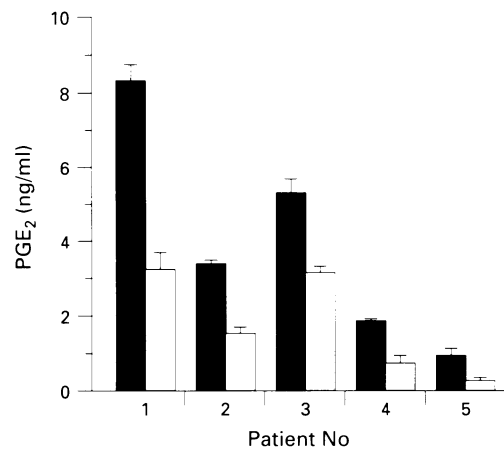
Figure 3   Suppressive effect of interleukin-4 (IL-4) (☐) 100 U/ml (patients 1–4) or 50 U/ml (patient 5) compared with medium alone (■) on the spontaneous production of prostaglandin E₂ (PGE₂) by adherent rheumatoid synovial cells obtained from five patients with rheumatoid arthritis. Results are the means (SD) of triplicate determinations for each patient.

Addition of IL-4 reduced the protein content of COX II (fig 4A). This inhibition of COX II biosynthesis by IL-4 was dose dependent and was effective at concentrations as small as 10 U/ml (fig 4B). IL-4 also reduced the remarkably increased protein content of COX II observed when the adherent synovial cells were incubated in the presence of IL-1α or LPS (fig 4A). In contrast, IL-4, LPS, or

IL-1α did not alter the protein level of COX I at all. Dexamethasone was more potent than IL-4 in reducing the protein levels of COX II.

SUPPRESSIVE EFFECT OF IL-4 ON THE GENE EXPRESSION OF COX II IN FRESHLY PREPARED ADHERENT SYNOVIAL CELLS

The mRNA of COX II was spontaneously expressed in these adherent synovial cells. This gene expression of COX II was considerably reduced by both IL-4 and (as previously reported[12]) dexamethasone (fig 5). In contrast, IL-4 and dexamethasone did not alter the mRNA content of COX I, and indomethacin had no effect on the concentrations of either COX I or COX II mRNA (fig 5).

DIFFERENTIAL EFFECTS OF IL-4 ON PGE2 PRODUCTION BY CULTURED RHEUMATOID SYNOVIAL FIBROBLASTS AND A PMA DIFFERENTIATED U937 MACROPHAGE CELL LINE AND THEIR GENE EXPRESSIONS OF COX II

Unexpectedly, IL-4 did not inhibit PGE₂ production by IL-1α stimulated rheumatoid synovial fibroblasts (fig 6A), but dexamethasone and indomethacin each had a potent inhibitory effect on its production. IL-4 also failed to alter the content of COX II mRNA in these cells (fig 6B).

When PGE₂ production and the gene expression of COX II by the PMA differentiated U937 macrophage cell line were also examined, the amounts of PGE₂ produced were small, but were increased by IL-1α and LPS, while addition of IL-4 reduced the enhanced amount of PGE₂ to basal levels (fig 7A). In RT-PCR analysis of COX II mRNA in these cells, basal concentrations of COX II mRNA were not detectable, but the protein was markedly upregulated after stimulation with IL-1α or
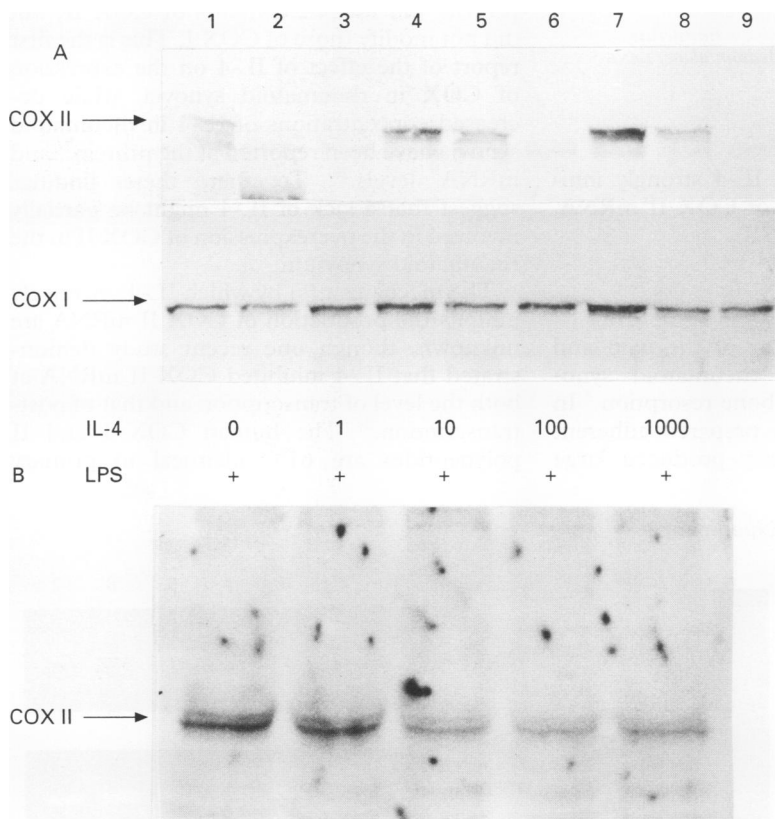

Figure 4   Suppressive effect of IL-4 (IL-4) on the biosynthesis of cyclo-oxygenase (COX) in freshly prepared adherent rheumatoid synovial cells. A: Western blot using rabbit antihuman COX I or COX II. Cells cultured with or without IL-1α 10 ng/ml or lipopolysaccharide (LPS) 1 μg/ml in the presence or absence of IL-4 100 U/ml or dexamethasone 5 μmol/l for 24 hours. LANE 1 = Medium; LANE 2 = IL-4; LANE 3 = dexamethasone; LANE 4 = IL-1α; LANE 5 = IL-1α + IL-4; LANE 6 = IL-1α + dexamethasone; LANE 7 = LPS; LANE 8 = LPS + IL-4; LANE 9 = LPS + dexamethasone. B: Western blot. Cells cultured with LPS 1 μg/ml in the presence or absence of IL-4 0, 1, 10, 100, or 1000 U/ml for 24 hours.
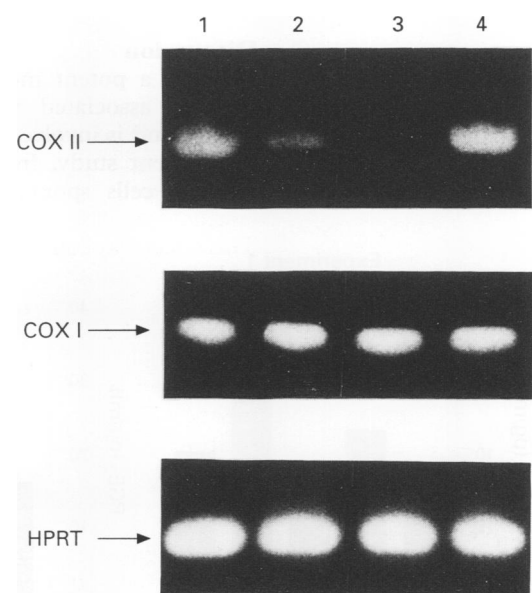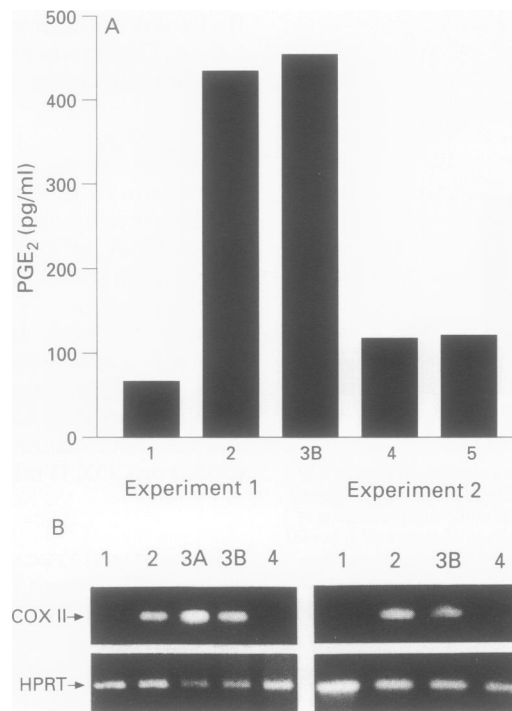

Figure 5   Effect of interleukin-4 (IL-4) on the gene expression of cyclo-oxygenase (COX) in freshly prepared rheumatoid adherent synovial cells. Cells cultured in medium alone (LANE 1), IL-4 100 U/ml (LANE 2), dexamethasone 5 μmol/l (LANE 3), or indomethacin 5 μmol/l (LANE 4) for 24 hours. HPRT = Hypoxanthine phosphoribosyl transferase (housekeeping gene).

Figure 6 **A:** *Production of prostaglandin E₂ (PGE₂) by interleukin-1α (IL-1α) 10 ng/ml stimulated rheumatoid synovial fibroblasts (RSFs) cultured with interleukin-4 (IL-4) 100 U/ml (column 3B), dexamethasone 5 μmol/l (column 4), or indomethacin 5 μmol/l (column 5). Column 1 = Medium alone; column 2 = IL-1α alone. Values are means of triplicate determinations.* **B:** *Gene expression of cyclo-oxygenase II (COX II) by IL-1α stimulated RSFs cultured in the presence of IL-4 10 U/ml (LANE 3A in experiment 1) or 100 U/ml (LANE 3B in experiments 1 and 2), or dexamethasone 5 μmol/l (LANE 4). HPRT = Hypoxanthine phosphoribosyl transferase (housekeeping gene). LANE 1 = Medium alone; LANE 2 = IL-1α alone.*

amounts of PGE_2. However, the capacity of these cells to produce PGE_2 was rapidly lost during the period of culture, suggesting that these cells maintained the nature of the inflammatory process that was seen in the patient. Consequently, use of freshly prepared adherent synovial cells was appropriate in this study, though these cells were heterogeneous.

We were able to demonstrate that the protein and mRNA contents of COX II were spontaneously expressed in freshly prepared adherent synovial cells, and that IL-1α and LPS increased their protein contents. In contrast, these stimuli did not modify the protein contents of the constitutive COX I. These results are consistent with those of previous studies showing that the mRNA level of COX II was upregulated by IL-1β in cultured synovial cells from patients with RA[12] or osteoarthritis,[26] and suggest that overproduction of PGE_2 in rheumatoid synovia might be mainly attributable to new synthesis of COX II enzyme in response to various stimuli including IL-1.

We and other investigators have previously demonstrated the anti-inflammatory effects of IL-4 on rheumatoid synovia or synovial cells by inhibiting the production of proinflammatory cytokines such as IL-1β, IL-6, and IL-8.[15-17] We have now shown that IL-4 inhibited the spontaneous production of PGE_2 by 56%, and that these suppressive effects were also observed in adherent synovial cells stimulated by LPS or IL-1α. In addition, IL-4 inhibited the protein and mRNA contents of COX II, but did not modify those of COX I. This is the first report of the effect of IL-4 on the expression of COX in rheumatoid synovia, while decreased concentrations of IL-4 in rheumatoid synovia have been reported at the protein[27] and mRNA levels.[28] Together, these findings suggest that a lack of IL-4 might be partially involved in the overexpression of COX II in the rheumatoid synovium.

The mechanism(s) by which IL-4 selectively inhibits the production of COX II mRNA are unknown, though one recent study demonstrated that IL-4 inhibited COX II mRNA at both the level of transcription and that of post-transcription.[29] The human COX I and II polypeptides are 61% identical in primary

LPS for eight hours, and IL-4 strongly inhibited the enhanced levels of COX II mRNA, as did dexamethasone (fig 7B).
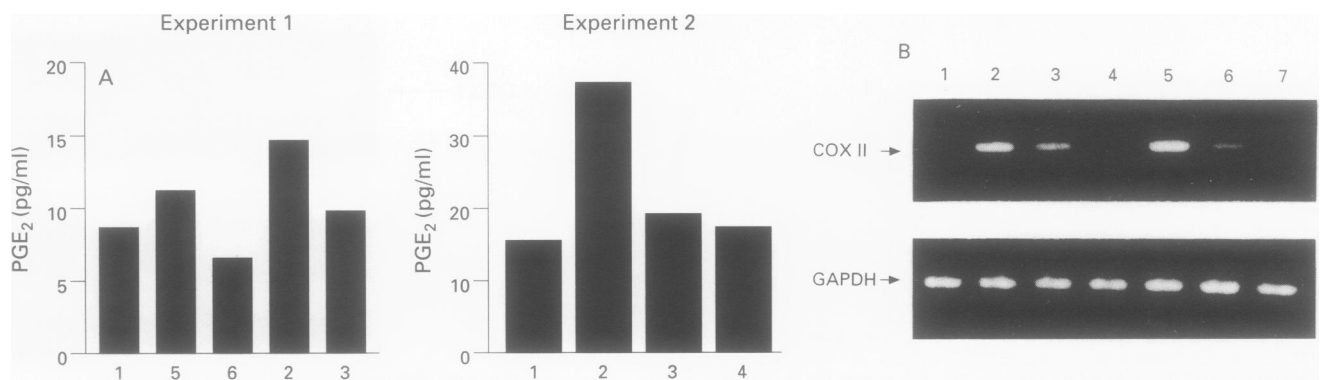
Discussion

PGE_2 is a potent mediator of the pain and oedema associated with rheumatoid synovitis,[3 4] and is involved in bone resorption.[3] In the present study, freshly prepared adherent synovial cells spontaneously produced large

Figure 7 *Effect of interleukin-4 (IL-4) on production of prostaglandin E₂ (PGE₂) and gene expression of cyclo-oxygenase II (COX II) by a phorbol myristate acetate differentiated U937 macrophage cell line stimulated with interleukin-1α (IL-1α) 10 ng/ml or lipopolysaccharide (LPS) 1 μg/ml.*
A: *Production of PGE₂. Stimulated cells cultured with IL-4 100 U/ml or dexamethasone 5 μmol/l. Values are mean of triplicate determinations.* **B:** *Reverse transcription polymerase chain reaction analysis of gene expression of COX II. Stimulated cells cultured with IL-4 100 U/ml or dexamethasone 5 μmol/l. GAPDH = glyceraldehyde-3-phosphate dehydrogenase (housekeeping gene).* **A, B**—*Columns or LANES: 1 = Medium alone; 2 = LPS alone; 3 = LPS + IL-4; 4 = LPS + dexamethasone; 5 = IL-1α alone; 6 = IL-1α + IL-4; 7 = IL-1α + dexamethasone.*

sequence,[30] [31] but it has recently been demonstrated that the transcriptional regulatory sequences of the COX I gene were considerably different from those of COX I,[31] therefore it is likely that IL-4 modulates transcriptional factors involving COX II, but not COX I. For example, the human COX II gene contains two nuclear transcription factor-κB (NF-κB) sites.[31] As it has been reported that IL-4 decreases the nuclear concentration of NF-κB in monocytes,[32] these effects may be associated with the selective inhibition of the mRNA level of COX II.

It has been reported that dexamethasone selectively inhibits the expression of COX II in rheumatoid synovium,[12] macrophages,[8] and endothelial cells.[11] The suppressive effect of IL-4 on PGE_2 production and COX II expression appeared to be less potent than that of dexamethasone. These findings prompted us to examine the cell specificity of the IL-4 effect. Our previous study demonstrated that the adherent rheumatoid synovial cells isolated by enzymatic digestion consisted mainly of macrophage-like cells and fibroblast-like cells, as determined by flow cytometric analysis.[25] Interestingly, IL-4 did not reduce the mRNA level of COX II in the cultured rheumatoid synovial fibroblasts stimulated with IL-1α, whereas it considerably reduced those in an IL-1α or LPS stimulated U937 monocyte/macrophage cell line. In contrast, dexamethasone completely inhibited the mRNA level of COX II in both types of cells. Endo et al[10] reported that IL-4 did not inhibit the activity and mRNA content of COX II in lung fibroblasts. Although we could not examine the effect of IL-4 on synovial macrophage-like cells, these findings and ours suggested that the main suppressive effect of IL-4 might be specific to macrophage-like cells in rheumatoid synovia. Although the reason for the different response of the two types of cell to IL-4 is unknown, it might be important to clarify the amounts of IL-4 receptors on their cell surfaces, and any difference in transcriptional factors involved in their expression of COX II mRNA.

COX I is constitutively expressed in most tissues, and its activation leads to the production of prostacyclin, which is antithrombotic in endothelium,[33] and cytoprotective in the gastric mucosa.[34] These clearly represent physiological functions for COX I. NSAIDs, inhibitors of COX, are widely used for the treatment of RA. As these drugs inhibit the activity of COX I more actively than that of COX II,[35] the gastrointestinal and renal side effects limit their use in high dose regimens. In this study, we have demonstrated that IL-4 selectively inhibited the biosynthesis of COX II in rheumatoid synovia, and that its inhibition was specific to macrophage-like cells. For the future development of COX II selective inhibitors, it would be useful first to define the mechanism by which IL-4 selectively inhibited the gene expression of COX II.

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