

Expression of arachidonate platelet-type 12-lipoxygenase in human rheumatoid arthritis type B synoviocytes

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Abstract In the present study, we have demonstrated platelet-type 12-lipoxygenase (12-LOX) expression in human rheumatoid arthritis (RA) type B synoviocytes by reverse-transcription polymerase chain reaction (RT-PCR). The presence of 12-LOX mRNA in these cells was revealed by classical RT-PCR analysis using platelet-type 12-LOX cDNA primers and the PCR fragment (246 bp) was purified, amplified and sequenced. By sequence analysis, this fragment was determined to be 100% identical to that from platelet-type 12-LOX cDNA. Immunofluorescence data demonstrate that interleukin-1 β (IL-1 β) increases cellular 12-LOX protein. Other results associate specific inflammatory cytokines with the activity of 12-LOX in human RA type B synoviocytes. IL-1 β increased 12S-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) production (4-fold) and we also observed an increase in 12-HETE production (2.5-fold) after incubation of human RA type B synoviocytes with TNF α . In contrast to the action of IL-1 β on 12-HETE synthesis, IL-4 and IL-6 did not enhance 12-HETE production. This is the first demonstration of platelet-type 12-LOX cDNA derived from the mRNA of cultured human RA type B synoviocytes.

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Key words: Rheumatoid arthritis; Human synoviocyte; 12-Lipoxygenase; Arachidonic acid; Cytokine

1. Introduction

Rheumatoid arthritis (RA) is an inflammatory joint disease characterized by progressive destruction of joint structures and tissues. This process is mediated by massive inflammation of the synovium, leading to pannus formation [1]. The cell populations at the site of cartilage erosion in RA have also been well characterized [2–4]. Thus, macrophages and fibroblasts, which dominate in the synovial lining, are also prominent in the eroding pannus at the synovium–cartilage junction. Based on these histologic and related experimental observations [5], it has been proposed that, firstly, fibroblast-like type B cells proliferate over the surface of the cartilage, followed by the accumulation of large numbers of macrophage-like type A cells which participate with other cells in the invasion of cartilage. Type B cells contain a prominent rough endoplasmic reticulum, few cell processes and vacuoles, less dense nuclear chromatin, but more developed nucleoli. Type B cells have the morphologic appearance of fibroblasts as well as the structural machinery to synthesize and secrete an impressive array of products, including proteoglycans, cytokines, arachidonic acid metabolites, and metalloproteinases [6]. Inflamed synovium contributed locally to joint destruction by the secretion of proteolytic enzymes, and also at a

distance by the synthesis and secretion of proinflammatory cytokines. Accumulating evidence suggests that some of these cytokines play a major role in the pathophysiology of arthritic disease, among which interleukin-1 (IL-1) and tumor necrosis factor- α (TNF α) appear to be likely candidates [1,7,8]. In rheumatoid synovium, IL-1 and TNF α are synthesized mainly by infiltrating macrophages/monocytes and synovial lining cells. These cytokines are very potent inducers of the synthesis of proteolytic enzymes, such as metalloproteinases and the plasminogen activator, which are involved in joint tissue degeneration [9–11]. Interleukin-6 (IL-6) is another proinflammatory cytokine synthesized in large quantities by both rheumatoid arthritic and osteoarthritic synovium [12]. This cytokine plays a role in the proliferation of B lymphocytes and likely potentiates the action of IL-1 which induces metalloproteinase synthesis by RA synovial fibroblasts [13,14].

Lipoxygenases are lipid-peroxidating enzymes that are implicated in the pathogenesis of a variety of inflammatory disorders such as arthritis, psoriasis, and asthma [15]. 5-Lipoxygenase (5-LOX) catalyzes the oxygenation of free arachidonic acid to its hydroperoxy derivative, 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid, the first step in the biosynthesis of the potent inflammatory mediators leukotrienes [15,16]. The 12- and 15-lipoxygenases (12-LOX and 15-LOX) are involved in the biosynthesis of other bioactive metabolites from free arachidonic acid, such as lipoxins [15]. Arachidonate 12-LOX is an oxygenase which incorporates one molecule of oxygen into arachidonic acid to produce 12S-hydroperoxy-5,8,10,14-eicosatetraenoic acid (12S-HPETE). The primary hydroperoxy product is reduced to 12S-hydroxy acid (12S-HETE). On the basis of enzymological, immunological, and molecular biological studies, two main types of 12-LOX ('platelet-type' and 'leukocyte-type') have been characterized [17], and a novel isoform of 12-LOX has been described [18,19]. The biological role of the 12-LOX is less clear. 12-LOX predominates in human platelets, and one potential role of its product, 12-HETE, is the modulation of adhesion receptors [20]. However, unlike cyclooxygenase (COX) and 5-LOX, the physiological functions of 12-LOX are still under of investigation.

In our work, we sought to determine if human RA type B synoviocytes were really able to express 12-LOX mRNA leading to the synthesis of 12-HETE. Because cytokines have been implicated in the cellular response seen in arthritis [21], we examined the effect of different cytokines produced by cells present in RA on 12-LOX activity.

2. Materials and methods

2.1. Preparation of human synovial cells

RA synoviocytes were isolated from fresh synovial biopsies ob-

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tained from RA patients undergoing hip synovectomy. Synovia were minced and digested with 1.5 mg/ml collagenase-dispase, 1 mg/ml hyaluronidase and 0.15 mg/ml DNase I for 3–4 h at 37°C. After centrifugation, cells were resuspended in Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL) supplemented with 10% fetal calf serum, 4.5 g/l D-glucose, 25 mM HEPES, 100 U/ml penicillin and 100 µg/ml streptomycin in a humidified atmosphere containing 5% (v/v) CO₂ at 37°C. After 48 h, non-adherent cells were removed. Adherent cells were cultured in complete medium and, at confluence, cells were trypsinized and passed. Cells were used between passages 4 and 8 when they morphologically resembled 'fibroblast-like' synoviocytes [22]. Cells were cultured 45–60 days prior to experimentation. This delay allowed the elimination of all possible interactions resulting from an eventual pre-operative treatment (NSAID analgesics). No patients were receiving corticotherapy or chemotherapy.

2.2. Culture of human RA type B synoviocytes

Between passages 4 and 8, cells were trypsinized. Cell count and viability were determined and 10⁶ cells were plated in 25 cm² tissue culture flask (Falcon, Oxnard, CA). Viability, measured by trypan blue dye exclusion [23] at the start and the end of culture, was always greater than 95%. Cells (10⁶) from RA patients were used for indirect immunofluorescence study. The following monoclonal antibodies were used: 5B5 (anti-prolyl hydroxylase) for fibroblasts at 1:50 dilution (Dako, Burlingame, CA), JC/70A (anti-CD31) for endothelial cells at 1:50 (Dako), RMO52 (anti-CD14) for macrophages at 1:50 (Immunotech, Marseille, France). The negative control was a mouse antibody of the same isotype (Immunotech). Incubations were performed at room temperature for 30 min. Binding of monoclonal antibodies was visualized using fluorescein (DTAF)-conjugated goat anti-mouse antibody (Immunotech) at 1:50 dilution.

2.3. RT-PCR analysis of 12-LOX synoviocyte culture extracts and verification of PCR products

Total cellular RNA from synoviocytes was isolated using a RNA extraction kit (RNazol[®]) based on a modification of the technique described by Chomczynski and Sacchi [24], reverse transcribed into cDNA and amplified. RNA (2 µg) was added to 0.5 µg oligodT, heated to 70°C for 10 min and quick-chilled on ice. The following components were added to the reaction mixture: 1× synthesis buffer, 1 mM dNTP, 0.2 mM DTT and 200 U of RT for a final reaction volume of 20 µl. After incubation for 50 min at 42°C, the reaction was terminated by heating at 70°C for 15 min and quick-chilled on ice. Residual RNA was destroyed by the addition of 2 U RNase H for 20 min at 37°C. An 21-base sense primer of human platelet 12-LOX cDNA and an antisense primer consisting of 21 bases were designed to specifically amplify 246 bp of 12-LOX cDNA (Table 1) [25]. Reverse-transcribed cDNA (10 µl) was incubated with 10 pmol of sense and antisense primers, 1 mM dNTP, 0.1 U Taq polymerase in 1× PCR buffer at a final volume of 50 µl. The mixture was overlaid with mineral oil and amplified in a thermal cycler (Crocodyle II, Appligene): 94°C for 1 min during 1 cycle, denaturation at 94°C for 45 s, primer annealing at 60°C for 45 s and extension at 72°C for 1 min during 35 cycles with appropriate controls. PCR products were extracted and visualized by electrophoresis on a 1.2% agarose gel containing ethidium bromide in Tris-acetate EDTA buffer. Human platelet 12-LOX product was verified by digestion with (*Ban*I) (Boehringer-Mannheim) under the following conditions. 12-LOX amplification product (10 µl) was incubated with *Ban*I for 1 h at 37°C. Resulting fragments were visualized by electrophoresis on a 1.5% agarose gel and size was calculated by linear regression with two molecular weight ladders (Boehringer-Mannheim and Appligene).

2.4. Cloning and sequencing

The PCR fragment was purified from the PCR reaction using *High Pure* PCR Product Purification Kit (Boehringer-Mannheim), and ligated into pGEM[®]-T Easy Vector (3 kb) that had been previously cut with *Eco*RV with a 3'-terminal thymidine added to both ends (Promega). The high copy number pGEM[®]-T Easy Vector contain T7 and SP6 RNA Polymerase promoters flanking a multiple cloning site (MCS) within the α-peptide coding region of the enzyme β-galactosidase. Recombinant plasmid was transformed into competent cells (*E. coli* JM 109), isolated and purified with Wizard[®] Plus Minipreps DNA Purification System (Promega). Recombinant plasmid was amplified with Quiagen/Filter, Plasmid Maxi Kit (Quiagen GmbH) and

plasmid insert was sequenced by PCR amplification in a final volume of 20 µl using 100 ng of PCR product, 5 pmol of pUC/M13 Forward Primer and 9.5 µl of DyeTerminators premix according to Applied Biosystems protocol. Sequencing was performed at Genome Express (Zone Astec, Grenoble, France).

2.5. Immunocytochemistry

Subconfluent human RA type B synoviocytes were dislodged by trypsin/EDTA treatment, 10⁵ cells were seeded onto poly-L-lysine coated glass coverslips in 6-well plates, and cultured in DMEM (3 ml/well) supplemented with 10% fetal calf serum for 24 h. Following adherence and spreading, IL-1β (50 ng/ml) or vehicle (DMEM with 10% fetal calf serum) was added to the cells and culture continued for an additional 20 h. For subsequent immunofluorescent labeling of 12-LOX protein, cells were fixed and permeabilized with methanol (5 min, −20°C) and acetone (10 s, −20°C), and non-specific Fc-binding sites were blocked by incubation of all coverslips with 3% (w/v) bovine serum albumin (BSA) in phosphate-buffered saline (PBS) pH 7.2 for 30 min before incubation for 4 h with rabbit polyclonal antiserum to purified human platelet type 12-LOX (1:100 in PBS/3% (w/v) BSA; Oxford Biomedical Research) or with rabbit pre-immune serum (1:100 in PBS/3% (w/v) BSA) as control. After washing 3×5 min with PBS, cells were labeled for 1 h with FITC-conjugated goat anti-rabbit IgG (1:100 in PBS/3% (w/v) BSA/0.02% (w/v) Evans blue) (Sigma), washed again 3 times with PBS, and mounted with immunount (Shandon). Immunofluorescence pictures were taken with a Nikon microscope OPTIPHOT-2 (Nikon Corporation).

2.6. Arachidonic acid metabolism

Exogenous [1-¹⁴C]arachidonic acid metabolism of synoviocytes was analyzed by reverse-phase high performance liquid chromatography (RP-HPLC). After trypsinization, 10⁶ cells were pelleted and washed twice with PBS to eliminate culture medium. Then, cells were resuspended in 0.05 M phosphate buffer (pH 8.2) and pre-incubated for 5 min at 37°C with 2 mM CaCl₂ and 0.5 mM MgCl₂. Exogenous [1-¹⁴C]arachidonic acid (1 µCi, specific activity: 55 mCi/mmol, Isotopchim) and 0.5 µg calcium ionophore A23187 (Sigma) were added in an ethanolic solution that never exceeded 0.4% and had no effect on arachidonic acid metabolism. After 15 min at 37°C, the reaction was stopped by the addition of 0.5 ml methanol containing 50 ng prostaglandin B₂ (Sigma) as internal standard. Precipitated material was removed by centrifugation at 600×g for 10 min and the pH adjusted to 3–4 with phosphoric acid. Samples were stored at −80°C until RP-HPLC analysis. To confirm the nature of 12-LOX product, synoviocytes were trypsinized, pelleted and washed twice with PBS, resuspended in 0.05 M phosphate buffer (pH 8.2) and pre-incubated without (control) and with 1 µM of indomethacin (IND) (Sigma) or 1 µM of nordihydroguaiaretic acid (NDGA) (Sigma) for 30 min at 37°C prior to the addition of [1-¹⁴C]arachidonic acid. In other experiments, the effects of various cytokines was shown. IL-1β, interleukin-4 (IL-4), IL-6 and TNFα were purchased from R&D Systems. Human RA type B synoviocytes were cultured with and without cytokines at 1, 10, and 50 ng/ml for 20 h prior to the addition of [1-¹⁴C]arachidonic acid.

Acidified samples and synthetic standards were injected on a 5 µm Radial-Pack[®] C18 cartridge (Waters-Millipore, Saint-Quentin en Yvelines, France) protected by a Waters C18[®] Guard Pack precolumn. Lipoxigenase metabolites were eluted at a flow rate of 2 ml·min^{−1} using a tertiary methanol-acetonitrile-H₂O gradient as previously described [26]. Radioactivity was detected at the exit of the column by a Flow One/Beta A500-Packard system. Metabolites of [1-¹⁴C]arachidonic acid were identified by comparing their retention times with those of synthetic standards, by coelution experiments with pure 12-HETE, by shift retention time studies after methylation of carboxylic functions and suppression of UV absorbance after catalytic hydrogenation [27]. Products were quantified by measuring the areas under the respective peaks. Each peak count was adjusted relative to the total radioactivity of the corresponding sample. For each incubation, the amount of every metabolite was compared with the control assay.

2.7. Statistical analysis

Statistical analysis of differences was carried out by analysis of variance (ANOVA). A *P*-value of less than 0.05 was considered to indicate significance.

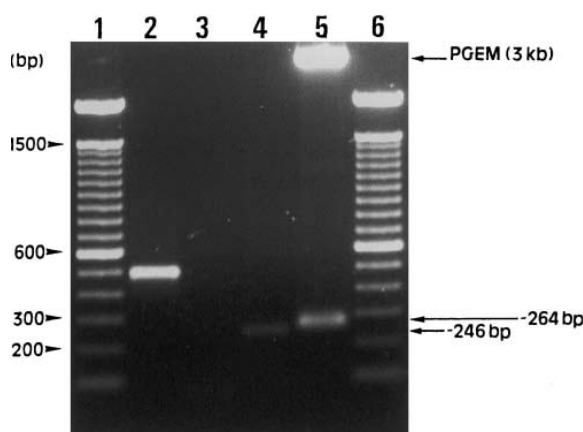


Fig. 1. RT-PCR analysis of human RA type B synoviocytes platelet 12-lipoxygenase mRNA. After reverse transcription, PCR was performed for 35 cycles with appropriate controls. PCR products were extracted and visualized by electrophoresis on a 1.2% agarose gel containing ethidium bromide in Tris-acetate EDTA buffer: the 246 bp PCR fragment (lane 4) amplified from synoviocyte mRNA was purified, cloned into the vector and amplified using the Quiagen Kit as previously described in Section 2. The fragment was determined to be 264 bp after appropriate restriction (*EcoRI*) in length (lane 5). Lanes 1 and 6: Molecular weight ladders. Lane 2: Positive control: cDNA from chloramphenicol acetyl transferase mRNA with chloramphenicol acetyl transferase primers (band at 523 bp). Lane 3: Negative control: same cDNA as positive control with 12-LOX primers (no band). Arrow indicates the pGEM[®]-T Easy Vector (3 kb) after restriction enzyme digestion (*EcoRI*).

3. Results

3.1. Morphological and Immunofluorescence results

Cells were used between passages 4 and 8. Direct observation with phase-contrast microscopy demonstrated human RA type B synoviocytes similar to fibroblast cells. Morphological observations were correlated to the immunofluorescence study: 99% of cells were stained with 5B5 antibody. One percent of cells were stained with anti-CD14 antibody. There was neither anti-CD31 nor control antibody staining (data not shown) [28].

3.2. RT-PCR analysis of 12-LOX synoviocyte culture extracts and verification of PCR products

Total RNA isolated from human RA type B synoviocytes was subjected to reverse-transcription and PCR amplification with the primers specific for human platelet 12-LOX cDNA (Table 1). Subsequent agarose gel electrophoresis revealed PCR product and the amplified cDNA fragment (246 bp) was observed in the reaction of human RA type B synoviocytes with the primers for human platelet 12-LOX cDNA (Fig. 1, lane 4). The possible contamination of genomic DNA in RT-PCR reaction was excluded by the fact that no amplification of the large intron (3 kb) in the genomic DNA

was amplified by our primers. The identity of PCR product was verified by restriction enzyme digestion (data not shown). Incubation of 12-LOX amplification product with *BanI* gave two fragments (83 and 163 bp) corresponding to expected fragments.

3.3. Expression of platelet 12-LOX in human RA type B synoviocytes

The 246 bp PCR fragment amplified from synoviocytes mRNA was purified using *High Pure* PCR Product Purification Kit, cloned into the vector (pGEM[®]-T Easy), amplified using the Quiagen Kit and sequenced as previously described in Section 2. The amplified fragment was determined to be 264 bp after appropriate restriction (*EcoRI*) in length (Fig. 1, lane 5) and the cDNA fragment (246 bp) was showed 100% identity to the corresponding human platelet 12-LOX cDNA.

3.4. Immunocytochemistry detection

Human RA type B synoviocytes were incubated with the anti-12-LOX antiserum, labeled with FITC-conjugated goat anti-rabbit IgG and counterstained with Evans blue. Control incubation with pre-immune serum in place of the anti-12-LOX antiserum gave no positive fluorescence signals (Fig. 2A). 12-LOX protein was detectable, localized in a diffuse manner (Fig. 2B,C) and treatment of cells for 20 h with IL-1 β (50 ng/ml) resulted in an overall increase in cellular 12-LOX protein (Fig. 2D,E).

3.5. Arachidonic acid metabolism

When 10^6 human RA type B synoviocytes were incubated with exogenous [1^{14} C]arachidonic acid as described in Section 2, the metabolic profile shown in Fig. 3 demonstrated that 12-LOX activity was present in these cells. To confirm 12-LOX activity, an extract of synoviocytes was pre-incubated with a potent COX inhibitor (IND) or a LOX inhibitor (NDGA) used at the same concentration of 1 μ M. IND at 1 μ M had no significant effect ($P=0.3520$) on 12-HETE production (Table 2). At 1 μ M, selectively NDGA inhibited the biosynthesis of 12-HETE (63%, $P=0.0009$; Table 2).

On the other hand, 10^6 synoviocytes were cultured for 20 h with various proinflammatory cytokines and screened for 12-HETE synthesis. These cytokines were selected because of their relevance to RA and the doses were selected for their ability to induce relevant biological effects in other cell systems. After incubation of synoviocytes with IL-1 β for 20 h, we observed a large increase in 12-HETE production, especially (4-fold, $P<0.0001$) with 50 ng/ml of IL-1 β (Table 2). In the same way, incubation of synoviocytes with TNF α (50 ng/ml for 20 h) led to a 2.5-fold increase ($P<0.0001$) in 12-HETE production (Table 2). On the contrary, IL-4 and IL-6 at 50 ng/ml had no significant effect ($P=0.89$ and $P=0.91$, respectively) on 12-HETE production but, surprisingly, these cytokines at lower concentrations (1 and 10 ng/ml) seemed to decrease 12-HETE production (Table 2).

Table 1
Oligonucleotides and PCR product size for human platelet 12-LOX

cDNA species	5'-Primer	Corresponding nucleotides	3'-Primer	Corresponding nucleotides	Size of PCR product (bp)	Reference
Human platelet 12-Lipoxygenase	TCAGATTTC AAC TGCACGAG	1048–1068	CCGACGGAGCAA CTGTACATG	1273–1293	246	[25]

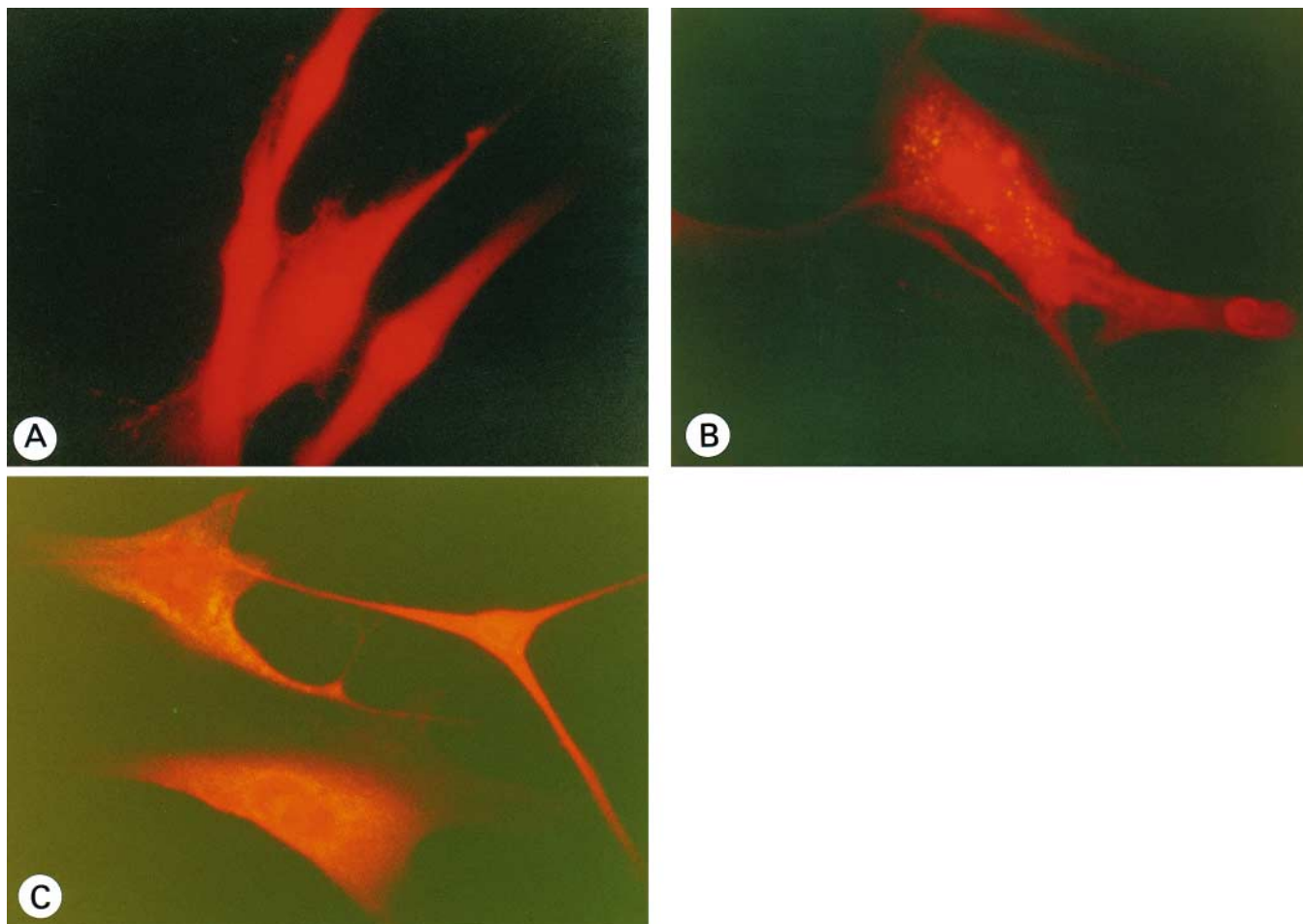


Fig. 2. Immunofluorescence detection of 12-LOX protein in human RA type B synoviocytes. Cells were cultured for 24 h in medium containing 10% fetal calf serum before addition of fresh medium without (A,B,C) or with IL-1 β at 50 ng/ml (D,E). A: control: after 20 h, cells were fixed and incubated with rabbit pre-immune serum and immunofluorescent (FITC-labeled, stained with Evans blue) secondary antibody. B,C,D,E: after 20 h, cells were fixed and stained for 12-LOX protein using 12-LOX-specific primary and immunofluorescent (FITC-labeled, stained with Evans blue) secondary antibody. Immunofluorescence pictures were taken with a Nikon microscope OPTIPHOT-2 (magnification $\times 400$).

4. Discussion

5-LOX metabolizes arachidonic acid into leukotrienes, a series of potent inflammatory mediators which clearly play an important role in inflammatory responses [15]. The properties of 12- and 15-LOX are not clear. Although a great number of biological functions have been attributed to the metabolites of these enzymes, no clear roles have been demonstrated in physiological or pathological states [29,30]. 12-LOX exists in at least two isoforms which differ in substrate specificity, catalytic and immunological properties [31]. They are referred to as 'leukocyte-type' or 'platelet-type' in reference to results obtained with 12-LOX from porcine or bovine leukocytes or human or bovine platelets, respectively [31].

In our laboratory, Bonnet et al. [28] have shown the expression of 5-LOX and FLAP mRNAs in human cultured synovial cells. Moreover, these authors observed 12- and 15-HETE production by synovial cells and suggested the presence of two other types of LOX activity, 12- and 15-LOX. On the other hand, in situ RT-PCR combined with immunocytochemistry demonstrated that mature oligodendrocytes and astrocytes express 12-LOX mRNA [32].

In the present study, we demonstrated 12-LOX expression

in human RA type B synoviocytes by RT-PCR analysis followed by enzymatic digestion. The PCR fragment amplified from human RA type B synoviocytes mRNA was purified, amplified and sequenced. The cDNA fragment showed 100% identity to the corresponding human platelet 12-LOX cDNA. RT-PCR analysis in combination with cDNA sequencing the amplified fragment provide novel and distinct evidence for the presence of the platelet-type 12-LOX isoform in fibroblast-like synovial cells and define it as identical with human platelet 12-LOX cDNA.

We also demonstrated 12-LOX expression in human RA type B synoviocytes by RT-PCR in situ (data not shown) and the presence of 12-LOX protein was confirmed by immunofluorescence. Moreover, our data showed that treatment of human RA type B synoviocytes with IL-1 β markedly increased the cellular expression of 12-LOX protein as observed in RIN m5F insulinoma cells [33].

Other results associate specific inflammatory cytokines with the activity of 12-LOX in human RA type B synoviocytes. In RA, the destruction of cartilage and bone occurs by erosion mainly at the junction of cartilage, bone and synovium, a region known as the pannus [34]. Cytokines are important in cartilage damage, especially IL-1 and TNF α which induce

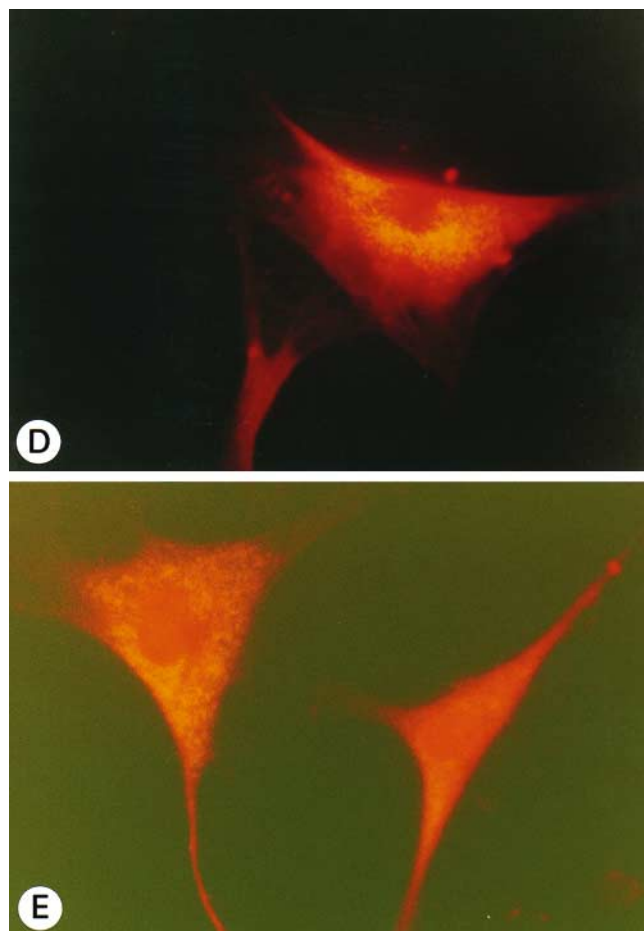


Fig. 2. (continued)

destructive matrix metalloproteinases involved in cartilage destruction [11,35]; IL-1 and TNF α also induce bone resorption in *in vitro* cultures [36,37]. There is increasing evidence that IL-6 may inhibit bone formation and induce bone resorption through its stimulatory effects in osteoclasts [38]. In common with transforming growth factor- β (TGF- β), IL-4 also displays some immunoregulatory effects such as inhibition of lipopolysaccharide-induced IL-1, TNF α , PGE $_2$, and 92-kDa gelatinase production in human monocytes [39]. However, in contrast to TGF- β , IL-4 has not been found in rheumatoid synovial tissue cultures [40] nor in T cells cloned from RA synovial biopsies [41], although it has been detected in reactive arthritis [42].

In order to eliminate the possible production of 11-HETE which is a COX by-product, COX and LOX inhibitors were used: IND (1 μ M) had no effect on 12-HETE synthesis whereas NDGA (1 μ M) inhibited synthesis of LOX metabolites.

After demonstrating the expression of a platelet 12-LOX in human RA type B synoviocytes, we characterized effects of IL-1 β , IL-4, IL-6 and TNF α on this enzyme activity. Our results showed that IL-1 β increased 12-HETE production (4-fold) as observed in RIN m5F insulinoma cells [33] and in isolated pancreatic islets [43]. We also observed an increase in 12-HETE production (2.5-fold) after incubation of human RA type B synoviocytes with TNF α . This result is consistent with the fact TNF α is known to be the major regulator of

Table 2

12-HETE released from human RA type B synoviocytes with different stimuli

Stimulus ^b	Concentration ^a (12-HETE)
Control	36.6 $\pm$ 6.3
IND (1 μ M)	40.4 $\pm$ 7.6 ^{##}
NDGA (1 μ M)	13.5 $\pm$ 4.1 [#]
IL-1 β (1 ng/ml)	47.2 $\pm$ 7.0
IL-1 β (10 ng/ml)	90.6 $\pm$ 14.3
IL-1 β (50 ng/ml)	152.7 $\pm$ 15.8*
IL-4 (1 ng/ml)	13.9 $\pm$ 3.9
IL-4 (10 ng/ml)	20.9 $\pm$ 5.3
IL-4 (50 ng/ml)	35.7 $\pm$ 8.1 ^{##}
IL-6 (1 ng/ml)	13.1 $\pm$ 2.2
IL-6 (10 ng/ml)	12.4 $\pm$ 0.9
IL-6 (50 ng/ml)	35.9 $\pm$ 4.9 ^{##}
TNF α (1 ng/ml)	37.4 $\pm$ 3.6
TNF α (10 ng/ml)	43.2 $\pm$ 8.1
TNF α (50 ng/ml)	91.3 $\pm$ 5.2*

^aConcentration of 12-HETE is expressed as ng/10⁶ cells.

^bStimulus abbreviations: control, spontaneous release by unstimulated human RA type B synoviocytes; IL-1 β , interleukin-1 β ; IL-4, interleukin-4; IL-6, interleukin-6; TNF α , tumor necrosis factor α . Other abbreviations: IND, indomethacin; NDGA, nordihydroguaiaretic acid.

Values are expressed as mean $\pm$ SEM of three experiments (*P* relative to control group): **P* < 0.0001, #*P* = 0.0009, ##*P* was not significant.

IL-1 [44]. In contrast to the action of IL-1 β on 12-HETE synthesis, IL-4 and IL-6 (50 ng/ml) did not enhance 12-HETE production.

In our work, we demonstrated that human RA type B synoviocytes are able to express platelet-type 12-LOX mRNA leading to the synthesis of 12-HETE which is modu-

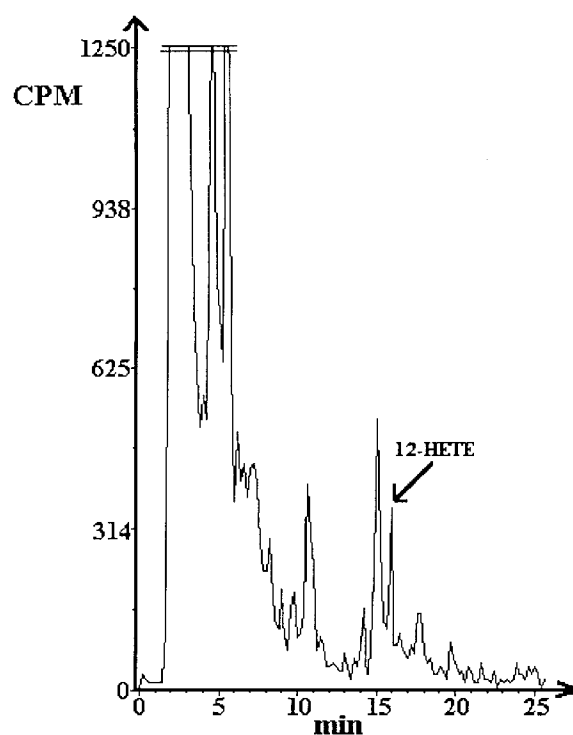


Fig. 3. Extended scale of radioactive profile of human RA type B synoviocytes (10⁶ cells) incubated with 1 μ Ci [1-¹⁴C]arachidonic acid in the presence of calcium ionophore A23187. Arrow indicates 12-HETE.

lated by various cytokines that play a major role in the pathophysiology of RA especially IL-1 and TNF α .

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