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Michihisa Zenmyo · Koji Hiraoka · Setsuro Komiya
Minoru Morimatsu · Yasuyuki Sasaguri

Histamine-stimulated production of matrix metalloproteinase 1 by human rheumatoid synovial fibroblasts is mediated by histamine H_1-receptors

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Abstract The purpose of this study was to investigate the role of histamine in human rheumatoid synovial fibroblasts in the production of factors responsible for tissue remodelling and cartilage breakdown in rheumatoid arthritis. We examined the effects of histamine of tritiated thymidine incorporation, production of matrix metalloproteinase-1 (MMP-1), histamine H_1-receptor expression, phosphoinositide metabolism and intracellular calcium ion concentration ($[Ca^{2+}]_i$) in human rheumatoid synovial fibroblasts. Tritiated thymidine incorporation studies demonstrated that histamine markedly stimulated the proliferation of rheumatoid synovial fibroblasts. Immunofluorescence and Northern blot analyses revealed that proMMP-1 production was also stimulated by histamine. The levels of inositol phosphates and $[Ca^{2+}]_i$ in the cells were elevated in response to histamine, indicating that the cells expressed histamine H_1-receptors; and Northern blot analysis indicated that these H_1-receptors were up-regulated by histamine. In in situ hybridization, large amounts of histamine H_1-receptor mRNA were also detected in rheumatoid synovial tissue. These results suggest that the interaction between H_1-receptor expression in rheumatoid synovial fibroblasts and histamine secretion by mast cells and macrophages in the affected sites is an important event responsible for tissue remodelling and joint destruction in rheumatoid arthritis.

Key words Histamine H_1-receptor · Matrix metalloproteinase-1 · Phosphoinositol metabolism · Intracellular calcium · Rheumatoid synovial fibroblasts

M. Zenmyo (✉) · M. Morimatsu
Department of Pathology, Kurume University School of Medicine, Kurume 830, Japan

K. Hiraoka · S. Komiya
Department of Orthopedic Surgery, Kurume University, School of Medicine, Kurume 830 Japan

Y. Sasaguri
Second Department of Pathology, University of Occupational and Environmental Health, School of Medicine, Kitakyushu 807, Yahatanishi-ku, Japan

Introduction

The synovial tissue of patients with rheumatoid arthritis shows pronounced hyperplasia of sublining fibroblasts associated with various types of inflammatory cells. This results in invasive resorption of cartilage and bone in the joints, a process known as tissue remodelling [5, 20, 21]. Such an inflammatory process can be initiated by a variety of events, including immune activation in the rheumatoid joint, in which activation is followed by cell migration, extracellular matrix degradation and cell proliferation. The matrix-degrading enzymes are considered to play key roles in tissue remodelling under pathological conditions [19, 28, 35] and in rheumatoid arthritis, metalloenzymes provide essentially all the proteolytic activity necessary to hydrolyze the collagens of the synovium and cartilage matrix [2, 8, 9, 19]. The joint destruction is caused by certain factors responsible for the degradation of extracellular matrix macromolecules [9, 19, 32] including the matrix metalloproteinases (MMPs), which are secreted by rheumatoid synovial fibroblasts [26, 33]. In previous studies [23, 24, 26], we demonstrated that interleukin 1, growth factors and lipid peroxide stimulate rheumatoid synovial fibroblasts to produce MMPs and that MMPs are responsible for tissue remodelling and development in arteries [36] as well as for destruction of stromal tissue by tumour cells [39].

Increases in the number of mast cells and histamine concentrations in rheumatoid synovium [10] and joint fluid [15] have been reported and consequently mast cells have attracted attention as potentially important participants in rheumatoid arthritis [6, 11, 18]. Histamine is a widely occurring chemical mediator that serves as a neurotransmitter and a modulator of gastrointestinal and smooth muscle cell functions [13] mediated by histamine receptors. At least three subtypes (H_1, H_2 and H_3) have been identified on the basis of their pharmacological properties [22]. Histamine H_1-receptors are known to be coupled to the phosphoinositide hydrolysis pathway and their activation results in an increase in the intracellular calcium concentration, $[Ca^{2+}]_i$ [29, 38]. H_2-receptors are

coupled to adenylate cyclase, and thus their activation increases the concentration of intracellular adenosine 3',5'-cyclic phosphate (cAMP) [17]. In previous reports [38, 40], we have also described the expression of histamine H_1-receptors in arterial smooth muscle cells in relation to the cell proliferation that results in the formation of atherosclerotic foci.

As histamine is also an initiator of inflammatory reactions, these findings led us to hypothesize that histamine plays a role in the pathophysiology of rheumatoid arthritis. However, the role of histamine in the regulation of synovial fibroblast growth and metabolism has yet to be clarified. In order to explore our hypothesis, we studied the effects of histamine on the production of MMP-1, expression of histamine receptors and DNA synthesis by cultured rheumatoid synovial fibroblasts. We also examined the accumulation of phosphatidylinositol phosphates (IPs), mobilization of $[Ca^{2+}]_i$ and levels of cAMP, which are second messengers of H_1- or H_2-receptors, in these cells in order to analyse the mechanism by which histamine acts on human rheumatoid synovial fibroblasts.

Materials and methods

Cell culture

Synovia were obtained from patients with active rheumatoid arthritis, as described previously [26]. Briefly, the tissue specimens were cut into small pieces and incubated in Dulbecco's modified Eagle's medium (DMEM; Nissui Pharmaceutical Company, Tokyo, Japan) supplemented with 10% (v/v) fetal calf serum (FCS; Filton, Brooklyn, Australia), 100 U/ml penicillin and 10 μg/ml streptomycin. Synovial fibroblasts grew from these explants within a week, and these cells were trypsinized and subcultured in the medium described above. Cells cultured up to the seventh passage were used in the experiments. Synovial fibroblasts obtained from patients with surgical orthopedic trauma and osteoarthritis were used as a control for the in in situ hybridization experiments.

Incorporation of tritiated thymidine

The incorporation of tritiated thymidine was determined by a previously described method [24]. Briefly, the cells were plated in 24-well culture plates with DMEM supplemented with 10% (v/v) FCS. When they had reached semiconfluence they were washed three times with phosphate-buffered saline (PBS; Nissui) and incubated in serum-free medium for 24 h to arrest cell growth. Then the medium was replaced with serum-free DMEM containing 1 μCi/ml of tritiated thymidine (Amersham Japan, Tokyo, Japan), and various concentrations of histamine (Wako Pure Chemicals, Osaka, Japan), which had been dissolved in distilled water and pH-adjusted to 7.0, were added. The cells were subsequently incubated for a further 24 h and harvested with 0.5 ml 0.5 M sodium hydroxide. After neutralization with an equal volume of 0.5 M hydrochloric acid (HCl), the cells were washed three times with 2 ml 0.6 M sodium chloride (NaCl)/0.06 M trisodium citrate on mixed cellulose acetate-cellulose nitrate filters (Millipore, Bedford, Mass., USA), and washed three times with 10% (v/v) trichloroacetic acid (TCA; Wako); then the radioactivity remaining on the filters was measured.

Immunofluorescence microscopy

Cells grown on a coverslip were cultured in DMEM for 24 h, incubated for a further 12 h in serum-free medium in the presence of 1 μM histamine, incubated in serum-free medium containing 1 μg/ml of monensin overnight, and fixed with 95% (v/v) cold acetone. Indirect immunofluorescence staining with sheep antibody against human proMMP-1 (a gift from Prof. Hideaki Nagase, Department of Biochemistry and Molecular Biology, Kansas Medical Center, Kansas, USA) was performed, as described previously [37]. After incubation with the antibody for 1 h, the cells were washed three times with PBS, incubated with fluorescein isothiocyanate-conjugated rabbit anti-[sheep IgG] IgG for 1 h, washed a further three times with PBS, and observed under an Olympus fluorescence microscope.

Northern blotting analysis

Partial cloning of the human proMMP-1 gene was performed as follows: a GeneAmp RNA PCR Kit (Perkin Elmer Cetus, Norwalk, Conn., USA), which employed the reverse transcriptase-polymerase chain reaction (RT-PCR) method, was used to isolate the proMMP-1 gene from a human aortic endothelial cell line [37] and a human smooth muscle cell line [30]. The total RNA was extracted from these cells by the acid guanidium isocyanate/phenol/chloroform method, and poly(A)+mRNA was isolated by oligo(dT)-cellulose chromatography. Single-strand cDNA was synthesized from it by use of morony murine leukaemia virus-RT, random hexamer and 100 ng RNA as a template. After the addition of 2.5 U Ampli *Taq* DNA polymerase, dNTP mixture and the synthetic oligonucleotide primers, PCR was performed, directly for 60 cycles (94° C, 1 min; 55° C, 1 min; 74° C, 1 min). The synthetic oligonucleotides (5': ATGCACAGCTTTCCTCCA; 3': TCAATTTTTCCTGCAGTT) for proMMP-1 gene that we made expaned the 1410 base pair (bp) DNA sequence corresponding to base residues 72–1481 of the human proMMP-1 gene [1]. The primers (5': ATGAGCCTCCCCAAT TCC; 3': GATGAGCTCCCGGTGCTG) for the human histamine H_1-receptor gene yielded the DNA sequences of a 680 bp product corresponding to residues 1 (translation initiation site)–680 of the human H_1-receptor gene [16]. These RT-PCR products were cloned in pBluescript SK(+) by use of a pCR-Script SK(+) cloning kit (Strategene, La Jolla, Calif., USA). We confirmed the sequences of the positive clones by automated laser fluorescent DNA squencing (Pharmacia, Uppsala, Sweden). After addition of various concentrations of histamine, the cells were incubated for 24 h and the total RNA was extracted.

The cells were seeded into 60 mm Petri dishes (1×10^6/dish) and cultured in 5 ml of medium. The cells were incubated in serum-free medium for 24 h prior to the experiment to arrest cell growth. After removal of the old medium, fresh medium containing various concentrations of histamine was added, the cells were incubated for a further 24 h, and the total RNA was extracted from these cultures. Ten micrograms of RNA was separated by electrophoresis on 1.4% (w/v) agarose gel containing 6.2% (w/v) formaldehyde and 1×3-(N-morpholino)propanesulfonic acid (MOPS), transferred to Gene Screen Filters (New England Nuclear-Du Pont, Mass., USA) and prehybridized. Next, the cDNA fragments for human proMMP-1 were labelled with $\alpha[^{32}P]$dCTP by the random priming method and hybridized at 42° C overnight with the filters at 65° C in a solution containing 50% (v/v) formamide, 5×Denhardt's solution, 5×saline sodium phosphate EDTA (SSPE), 1% (w/v) sodium dodecyl sulphate (SDS) and 100 μg/ml denatured salmon sperm DNA. The filters were washed three times with 2×SSPE at room temperature for 15 min, with 2×SSPE and 2% (w/v) SDS at 65° C for 45 min, and finally with 0.1×SSPE at room temperature for 15 min. Autoradiography was performed with Kodak X-OMATTMAR films (Eastman Kodak Company, Rochester, New York, USA) at −70° C for 2 days. The autoradiographs were analysed quantitatively with a densitometer, as described previously [40].

Measurement of tritiated IPs formation

The amounts of tritiated IPs formed were determined as described previously [38]. Rheumatoid synovial fibroblasts in six-well cul-

ture plates were labelled with tritiated inositol (1 μCi/well) in inositol-free medium for 48 h, then washed three times with Krebs-N-2-hydroxyethylpiperazine-N′-2-ethanesulfonioc acid (HEPES) bicarbonate (KHB) buffer, which comprised 125 mM NaCl, 4.7 mM potassium chloride, 2.2 mM calcium chloride, 1.2 mM magnesium chloride ($MgCl_2$), 1.2 mM potassium KH_2PO_4, 15 mM sodium bicarbonate, 11 mM glucose, and 15 mM HEPES (pH 7.4), and preincubated in 1.8 ml KHB buffer containing 10 mM lithium chloride (LiCl) for 5 min at 37° C. Histamine (100 μM) in KHB buffer containing 10 mM LiCl was added to the solution, and the reaction was carried out for 0, 0.5, 1, 5 and 10 min at 37° C and then terminated by quick aspiration of the buffer. Next, 1 ml of 5% (w/v) TCA was added to each well and tritiated inositol monophosphate (IP_1), tritiated inositol biphosphates (IP_2), and tritiated inositol triphosphates (IP_3) were separated by Bio-Rad AG1-X8 chromatography, as described by Berridge et al. [4]. The radioactivity in each eluate was determined by liquid scintillation counting.

Measurement of $[Ca^{2+}]_i$

Synovial fibroblasts were incubated in 10 ml of Hanks' medium containing 10 mM HEPES (pH 7.4) and 0.25% (w/v) bovine serum albumin, and then incubated for 40 min at room temperature with 1 mM fura-2/AM dissolved in dimethyl sulphate [final concentration, 0.1% (v/v)]. After the cells had been loaded with the indicator, the cells were washed twice and resuspended in KHB buffer at a concentration of 1×10^5 cells/ml. Fluorescence excitation was made with a Hamamatsu 75Xe lamp, and excitation wavelengths of 340 and 380 nm were selected by computer-controlled movement of filters in the light path. Paired recordings were made every 5 s, and fluorescence images were captured with a Hamamatsu SIT camera (C2400-08th) and stored in a digital image processor (Argues-100). The $[Ca^{2+}]_i$ was calculated from the ratio of the fluorescence intensity obtained with excitation at 340 and 380 nm on a pixel basis, as described previously [38].

Measurement of cAMP

Rheumatoid synovial fibroblasts (3×10^5/well) in 24-well plates were washed twice with 1 ml of KHB buffer (pH 7.4) at 37° C and preincubated for 10 min in 800 μl of the buffer, and then 100 μl of KHB buffer containing 10 μM histamine and 100 μl of 10 mM 3-isobutyl-1-methylxanthine were added to each well. After incubation for various times (0, 1, 5, 10, 15 and 20 min), the reaction was terminated by aspirating the medium quickly; and next 1 ml of 5% (w/v) cold TCA was added to the cells. The resulting solution was centrifuged at 3,000 rpm for 10 min, the extracted supernatant was washed twice with 6 ml water-saturated diethyl ether, and the amount of cAMP in the extract was measured by use of an Amersham cAMP [^{125}I] assay system. After incubation with rabbit anti-succinyl cAMP serum followed by centrifugation at 3,000 rpm for 10 min, the supernatants were discarded; and the radioactivity in the precipitated samples was counted with a gamma counter. The results obtained with standards were used to construct a standard curve.

Measurement of prostaglandin E_2 (PGE_2)

Rheumatoid synovial fibroblasts were cultured in the presence of 100 μM histamine in 2 ml of PBS for 0, 5 and 10 min at 37° C, and the reaction was terminated by quick aspiration of the buffer. The aspirated solutions were stored at –70° C until analysed. Measurement of PGE_2 was performed with a PGE_2 [^{125}I] radioimmunoassay kit (Du Pont de Nemours and Company, Boston, Mass., USA). In this assay, an analogue of PGE_2 was used as a tracer and rabbit anti-PGE_2 as the antiserum (specific antibody). PGE_2 in the sample was extracted with a BOND-ELUT C18 extraction column (Analytichem International, Harbor City, Calif., USA); the resulting extracted sample was incubated with the tracer and antiserum overnight at 4° C, cold precipitating reagent was added, and the mixture was incubated at 4° C for 30 min. After centrifugation at 3,000 rpm for 30 min, the supernatants were discarded and the radioactivity of each precipitate was counted in a gamma counter. The results obtained with known amounts of PGE_2 were used to construct a standard curve.

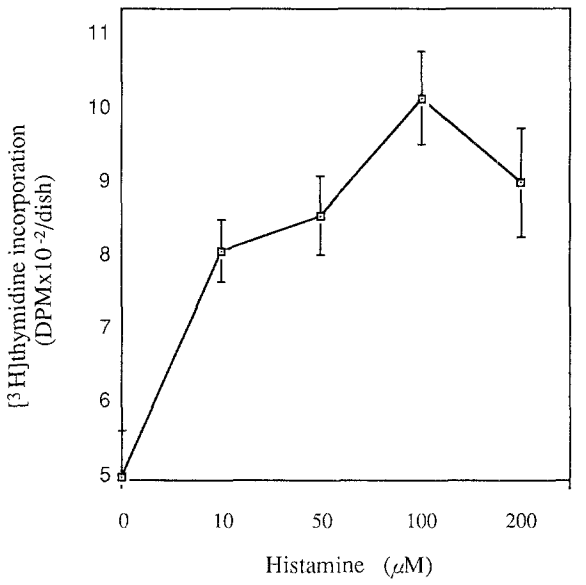

Fig. 1 Effect of histamine on DNA synthesis by rheumatoid synovial fibroblasts. The amount of DNA synthesized was determined by measuring the incorporation of tritiated thymidine, as described in Materials and methods. Histamine (10–100 μM) stimulated cell proliferation in a concentration-dependent manner. DPM=Disintegration per minute. Mean±standard deviation (SD), n=4

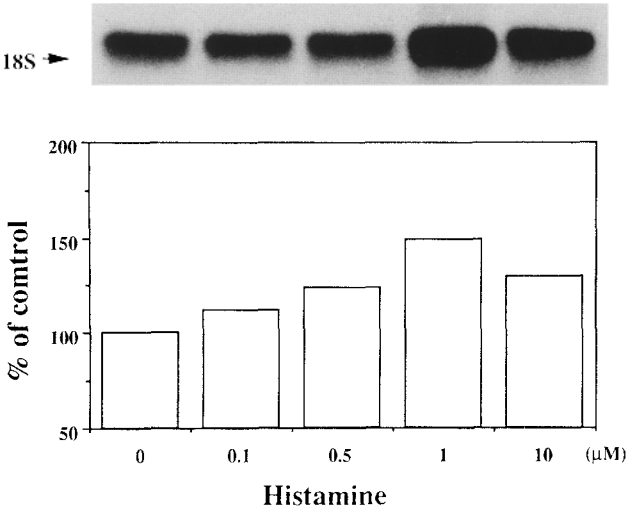

Fig. 2 Effect of histamine on promatrix metalloproteinase (MMP)-1 gene expression in rheumatoid synovial fibroblasts. The intensity of each band was measured densitometrically, and the amount of proMMP-1 mRNA was calculated as a percentage of the control value. The *bars* represent the amounts of proMMP-1 gene transcribed in rheumatoid synovial fibroblasts

In situ hybridization

Human tissues were fixed in 4% paraformaldehyde in PBS at 4° C overnight, dehydrated, and embedded in low-melting-point paraf-

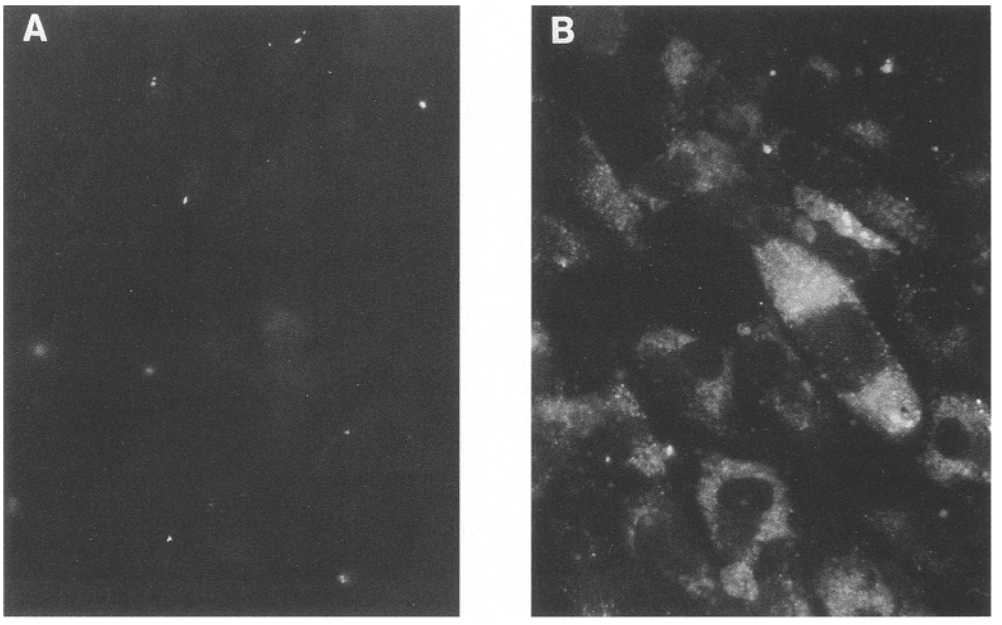

Fig. 3A, B Immunocytochemical staining of proMMP-1 produced by rheumatoid synovial fibroblasts. Immunofluorescence staining revealed that histamine stimulated production of proMMP-1 by rheumatoid synovial fibroblasts. **A** Untreated control cells, **B** cells treated with 1 μM histamine

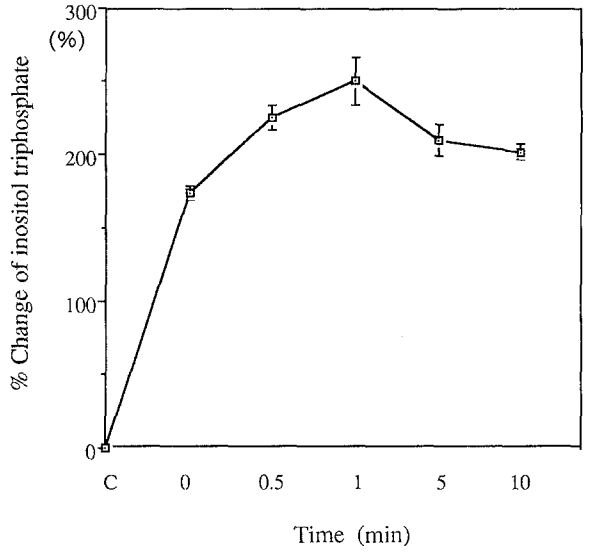

Fig. 4 Time course of tritiated inositol triphosphate accumulation by lithium chloride-treated rheumatoid synovial fibroblasts. Mean±SD, *n*=3

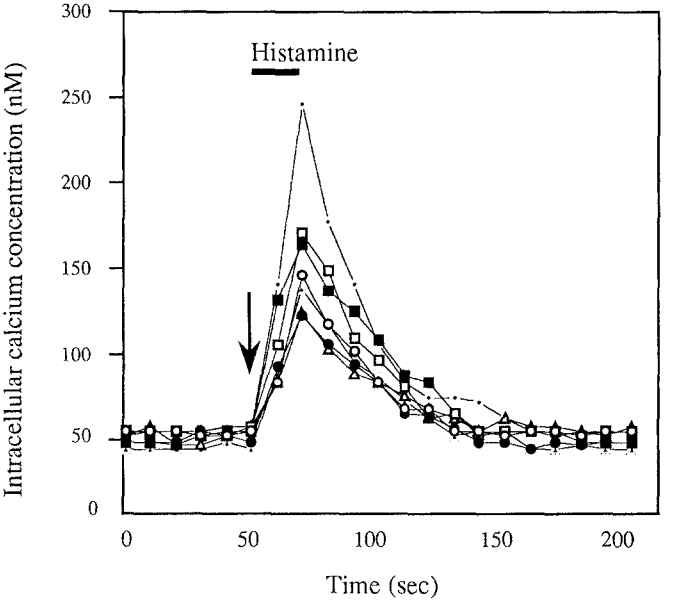

Fig. 5 Time course of the intracellular calcium ion concentration $[Ca^{2+}]_i$) response of rheumatoid synovial fibroblasts to histamine. Fura-2 loaded cells were exposed to histamine (100 μM) for the time designated by the *bar*. The lines show the time course of seven independent cells, indicating synchronous elevation of $[Ca^{2+}]_i$ in response to histamine

fin wax. Sections 5 μm thick were mounted on silane-coated glass slides and dried at 37° C. Some sections were stained with haematoxylin and eosin to confirm the presence of pathological changes of active rheumatoid arthritis or osteoarthritis. After removal of the wax with alcohol, the sections were treated with 20 μg/ml proteinase K, post-fixed in 4% paraformaldehyde in PBS, treated with 0.25% acetic anhydride in 0.1 mol/l triethanolamine, and dehydrated again. A Digoxigenin (DIG) Oligonucleotide 3'-End Labelling Kit (Boehringer Mannheim Biochemica, Mannheim, Germany) was used for experiments. DIG-labelled single-stranded sense or antisense RNA probes were synthesized with T_7 or T_3 RNA polymerase after linearization with *Eco*R I or with *Sac* I following mung bean nuclease treatment. The probe was added to the hybridization solution (50% formamide, 10 mmol/l TRIS-HCl [pH 7.6], 1 mmol/l ethylenediamine tetraacetic acid, 600 mmol/l NaCl, 1×Denhardt's solution, 0.25% SDS, 10% dextran sulphate, 200 μg/ml yeast-tRNA) at a final concentration of 1 μg/ml. After hybridization at 50° C overnight, a washing procedure that included RNAse treatment (20 μg/ml) was performed. After incubation with buffer containing 100 mM maleic acid, 100 mM TRIS-HCl (pH 7.5) and 150 mM NaCl, the sections were incubated with buffer 1 containing alkaline phosphatase-conjugated anti-DIG antibody followed by washing with buffer containing 100 mM TRIS-HCl (pH 9.5), 100 mM NaCl and 50 mM $MgCl_2$. The sections were then visualized with nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate p-toluidine salt (Wako). Counterstaining was performed by immersing the slides in 0.5% methyl green in 0.1 mol/l sodium acetate solution (pH 4.0) for 5 min at room temperature.

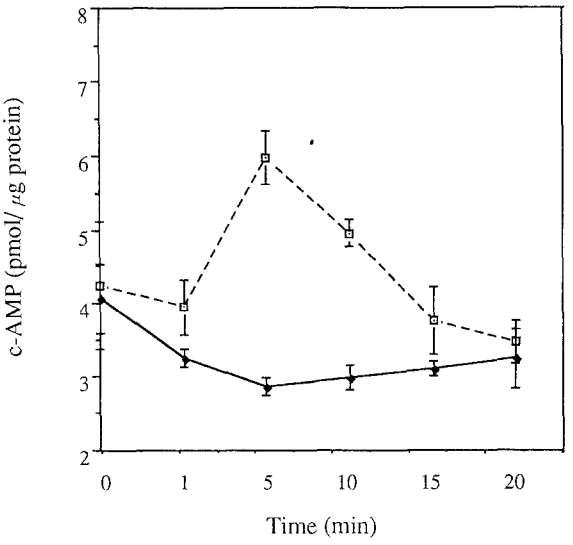

Fig. 6 Formation of cAMP by rheumatoid synovial fibroblasts. ⊡ Histamine (100 μM), ● histamine (100 μM)+indomthacin (1 μg/ml). Mean±SD, n=5

Results

Histamine, at concentrations of 10–100 μM, stimulated DNA synthesis by rheumatoid synovial fibroblasts in a concentration-dependent manner, and maximal stimulation (twofold) was observed with 100 μM (Fig. 1). The effect of histamine on proMMP-1 mRNA production by rheumatoid synovial fibroblasts was analysed by Northern blotting analysis of the total cytoplasmic RNA from the synovial fibroblasts stimulated by histamine. As shown in Fig. 2, the amount of mRNA for proMMP-1 transcribed by the cells increased in the presence of histamine in a concentration-dependent manner.

Immunofluorescence staining showed that histamine stimulated proMMP-1 production; proMMP-1 was detected in the cytoplasm of histamine-treated rheumatoid synovial fibroblasts but not in that of the untreated control specimens (Fig. 3). This result is consistent with that of the Northern blotting analysis.

The accumulation of IP_3, which is a second messenger of the H_1-receptor, was investigated. Histamine (100 μM) increased the accumulation of IP_3 by rheumatoid synovial fibroblasts compared with that by untreated cells, and maximal stimulation (2.5-fold) was seen 1 min after the addition of histamine (Fig. 4). The accumulation of IP_1 and IP_2 also increased in response to histamine in a time-dependent manner (data not shown). As shown in Fig. 5, consequently, activation of phospholipase C via IP_3 led to the synchronous elevation of $[Ca^{2+}]_i$ immediately after the addition of the histamine. The accumulation of cAMP, which acts as a second messenger of histamine H_2-receptors, was also investigated. Five minutes after the addition of 100 μM histamine to the culture, increased cAMP accumulation by rheumatoid synovial fibroblasts was seen. This reaction, however, was inhibited markedly by the addition of 1 μg/ml indomethacin, an inhibitor of prostaglandin production (Fig. 6). The addition of 100 μM histamine stimulated PGE_2 production by rheumatoid synovial fibroblasts in a time-dependent manner. The cells had produced 3.767±0.095 pg PGE_2/mg protein by 5 min after the addition of the histamine (Table 1).

Table 1 Production of prostaglandin E_2 (PGE_2) in rhumatoid synovial fibroblasts stimulated by histamine

Time (min)	PGE_2 (pg/μg protein; mean ±standard deviation)
0	0.517±0.148
5	3.797±0.739
10	3.767±0.095

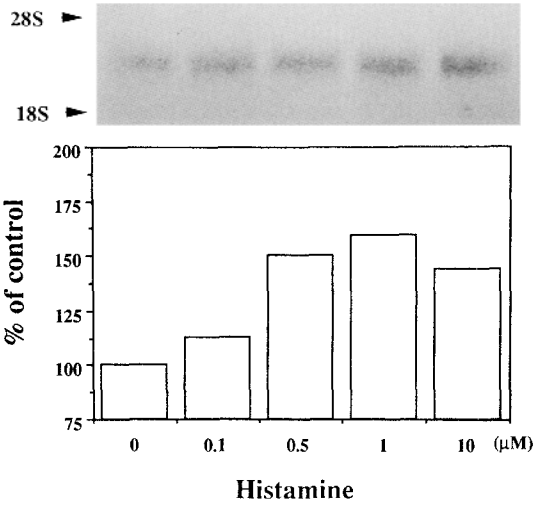

Fig. 7 Effect of histamine on H_1-receptor gene expression. The intensity of each band was measured densitometrically, and the amount of H_1-receptor mRNA was calculated as a percentage of the control value. The bars represent the amounts of the H_1-receptor gene transcribed in rheumatoid synovial fibroblasts

The expression of H_1-receptor mRNA was investigated by Northern blotting analysis and in situ hybridization. The amount of H_1-receptor mRNA transcribed increased in the presence of histamine. Maximal stimulation (1.5-fold) was observed in the presence of 1 μM histamine (Fig. 7). As shown in Fig. 8, in situ hybridization demonstrated that large amounts of histamine H_1-receptor mRNA were expressed in synovial epithelium and stromal fibroblasts in the rheumatoid synovial tissue (Fig. 8B) and in osteoarthritis, the expression of the H_1-receptor was localized mainly in the epithelium of on the surface of synovial tissue. However, the amount of the receptor expressed in the synovial tissue affected with osteoarthritis was less than that in the rheumatoid synovial tissue (Fig. 8E).

Discussion

Histamine is produced from histidine by histidine decarboxylase (HCD) through a single enzymatic reaction [3,

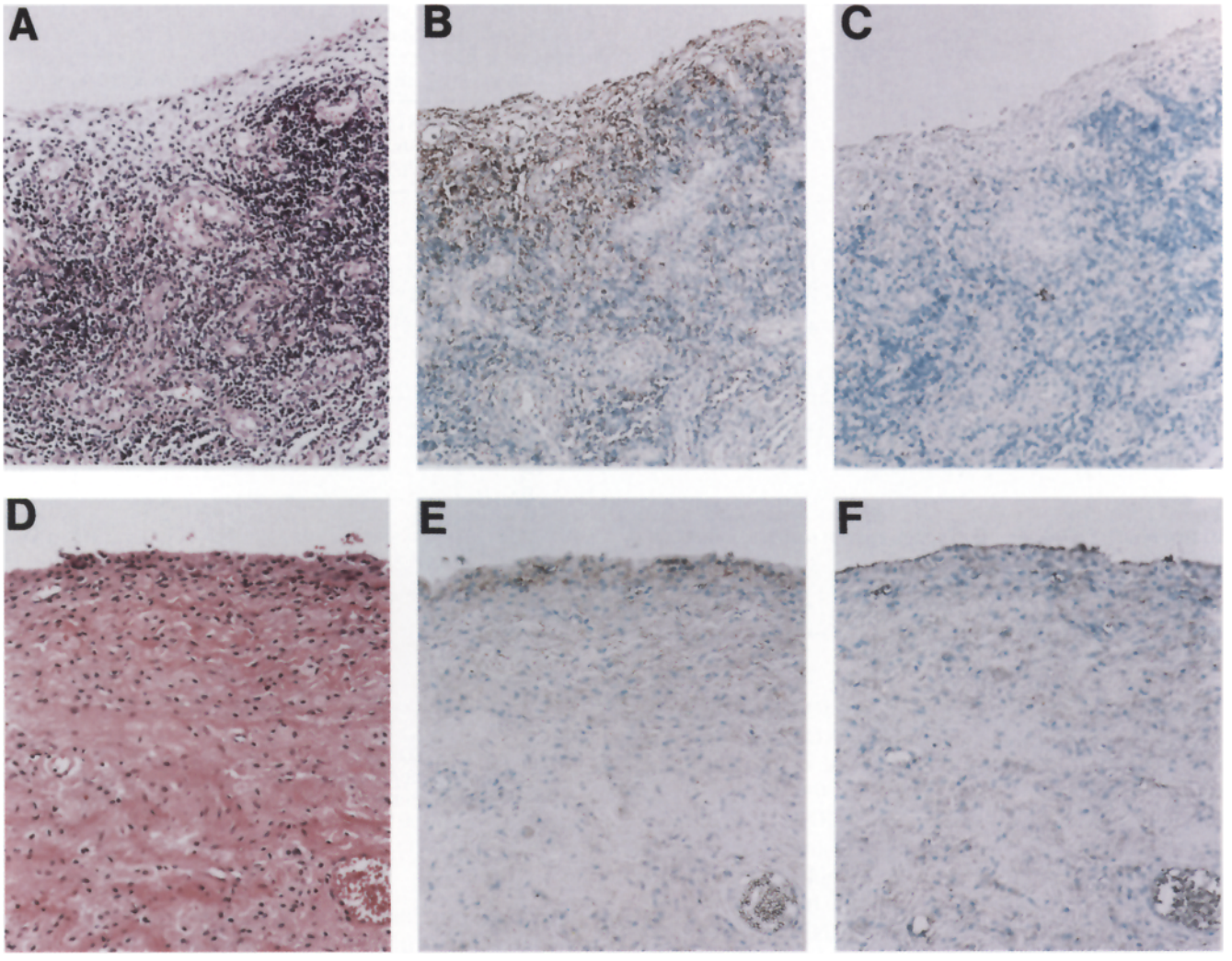

Fig. 8A–F In situ hybridization of synovial tissue with the human histamine H_1-receptor RNA probe. Light micrographs show rheumatoid arthritis with an intense infiltrate composed of lymphocytes (**A**), which are positive for methyl green but not with the H_1-receptor RNA probe (**B**). Synovial epithelium and fibroblasts are strongly stained with the H_1-receptor RNA probe (**B**). In osteoarthritis, the amount of expression of the H_1-receptor in them is less than that in rheumatoid arthritis (**D**). **A–C** Rheumatoid synovial tissue, **D–F** synovial tissue of osteoarthritis. **A–D** Haematoxylin and eosin staining; **B, E** antisense; **C, F** sense. (Original magnification, ×40 for all)

25]. HCD is distributed mainly in mast cells, basophils and macrophages. Therefore, it is likely that macrophages and mast cells, which are involved in the pathological process of rheumatoid arthritis, are possible producers of histamine [44]. In addition, various researchers have reported that histamine can stimulate the growth of carcinoma cells, smooth muscle cells and fibroblast cell lines [27, 34, 43]. In the present study, we found that histamine stimulated DNA synthesis and proMMP-1 production by rheumatoid synovial fibroblasts and confirmed, using Northern blotting analysis with a human proMMP-1 cDNA probe, that the expression of proMMP-1 mRNA was up-regulated by histamine. MMP-1 is capable of degrading types I, II and III of interstitial collagen [45], and collagens are major structural proteins in the intercellular spaces and play important roles in morphogenesis [14, 31]. Therefore, degradation of collagen is controlled precisely by collagenolytic enzymes, including proMMPs. Histamine has been also reported to stimulate human articular chondrocytes to produce collagenase [46]. Therefore, ability of rheumatoid synovial fibroblasts and chondrocytes to synthesize MMP-1 suggest that histamine secreted by both mast cells and macrophages participates in the regulation of the turnover of pathological extracellular matrix macromolecules as well as in the cell proliferation in joints affected by rheumatoid arthritis.

Of the three types of histamine receptors, the H_1-receptors are linked to PI breakdown [12] and calcium mobilization [7]; and the H_2-receptors are linked to cAMP formation [17]. We demonstrated that histamine stimulated hydrolysis of IPs and $[Ca^{2+}]_i$ elevation via phospholipase C activation. Both these processes increase the transcription of *jun* and *fos* protooncogenes, resulting in the enhancement of activator protein-1 (AP-1) complex

formation, and this complex can then stimulate the transcription of some other genes. In fact, the regulatory region for the proMMP-1 gene contains an AP-1-responsive element. Therefore, our results indicate that synovial fibroblasts express the H_1-receptors on their surface. We confirmed this expression using Northern blotting analysis with a human histamine H_1-receptor cDNA probe and by in situ hybridization: relatively large amounts of the H_1-receptors were expressed in synovial fibroblasts when compared with synovial tissue obtained from patients with osteoarthritis. We also demonstrated that histamine stimulated transcription of the H_1-receptor gene in these cells. We believe that focally produced histamine is the most important factor involved in pathological processes limited to a specific organ(s) or focal site(s), even though histamine is present even in serum. Therefore, we suggest that expression of large amounts of the H_1-receptor and secretion of histamine are pathologically important events in rheumatoid arthritis.

Our study showed that histamine slightly increased the amount of cAMP accumulated by rheumatoid synovial fibroblasts, although the pattern was not like that of smooth muscle cells in response to PGE_2. However, the addition of indomethacin, an inhibitor of PG synthesis, inhibited the elevation of cAMP induced by histamine. Histamine has also been reported to stimulate human rheumatoid synovial cells and articular chondrocytes to produce PGE_2 [42], which prostanoid alters vascular tone and permeability of the synovial vessels. A pharmacological study using antagonists against H_1- and H_2-receptors suggested that H_1-receptors are expressed in rheumatoid synovial cellls and human articular chondrocytes [41]. Therefore, this elevation of cAMP levels, which was inhibited by the addition of indomethacin, is considered to be due to histamine-induced PGE_2, and not to a direct effect of histamine. Therefore, it would appear that synovial fibroblasts express H_1- but not H_2-receptors.

We conclude that expression of large amounts of histamine H_1-receptor by rheumatoid synovial fibroblasts and production of histamine by mast cells and macrophages may play an important role in both remodelling and disruption of the joints of patients suffering from rheumatoid arthritis.

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