Production of prostaglandin E₂ induced by histamine by cloned rheumatoid synovial cells

M Sasano, M Goto, K Nishioka

Abstract

Production of prostaglandin E₂, with or without histamine stimulation, by three different types of cloned rheumatoid synovial cells (macrophage like, dendritic, and fibroblast like) was evaluated. The ability of these cloned cells to respond to histamine on a cell to cell basis was as follows: macrophage like cells responded most strongly, followed by dendritic cells, followed by fibroblast like cells. Production of prostaglandin E₂, stimulated by histamine, may have a role in bony destruction in rheumatoid joints.

Articular synovium is considered to be the most important site of chronic inflammation in rheumatoid arthritis (RA). Dayer *et al* have shown that cultured adherent synovial cells (ASC) dispersed from the lining of rheumatoid synovium produce large amounts of prostaglandin E₂.¹ Prostaglandin E₂ probably contributes to the destruction of cartilage and to changes in immune functions,² and its synthesis increases in response to various inflammatory stimuli, including interleukin-1.³ Recent histochemical studies have shown that both mast cells and macrophages accumulate at sites of cartilage erosion⁴ and synovium^{5,6} in rheumatoid joints, as well as a large amount of histamine in some rheumatoid synovial fluids.⁷ Histamine can also stimulate ASC to produce prostaglandin E₂. These studies did not provide any information, however, on which types of cells are responsible for the production of prostaglandin E₂ as synovial cells consisted of heterogeneous cell populations. To overcome this we cloned these synovial cells. In our previous paper⁹ cloned adherent synovial cells comprised three major populations, classified morphologically as fibroblast like, dendritic, and macrophage like cells. In this study we tried to investigate prostaglandin E₂ production induced by histamine by rheumatoid synovial cells, using three different types of cloned cells.

Methods

PREPARATION OF SYNOVIAL CELLS

Synovial tissue specimens were obtained from the knee joints of nine patients (eight women, one man; age 52.7 (10.1), range 40-73 years) with classic or definite RA (duration of disease 11.3 (9.2), range two to 26 years, erythrocyte sedimentation rate: 58.5 (29.3), range 26-108 mm/first hour) at the time of synovectomy. The specimens were dissociated enzymatically, as previously described.⁹ All of the patients were receiving non-steroidal anti-inflammatory agents; seven patients were also receiving small doses of corticosteroids (≤ 5 mg/day). After removal of the adipose tissues the specimens were minced into small pieces, then washed three times in phosphate buffered saline

(PBS). These synovial tissues were treated with PBS+0.25% trypsin (Nakarai Chemicals Ltd, Kyoto, Japan) for 40 minutes at 37°C, after which the cells in the supernatants were collected. The cells were washed three times in PBS, suspended in Nutrient Mixture F12 (HAM-F12; Gibco Laboratories, Grand Island, New York, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 5×10^{-5} M 2-mercaptoethanol (Nakarai), 100 U/ml penicillin, and 100 µg/ml streptomycin (Gibco), and cultured at 37°C in a humidified atmosphere of 95% air and 5% carbon dioxide. Fibroblast like and macrophage like cells each comprised 40-50% and dendritic cells $\approx 5\%$ of the total cell population from the original synovial specimens. Subcultured ASC were established by treating the primary adherent synovial cell culture with 0.05% trypsin for five minutes at 37°C, then replating at a reduced cell density.

CLONING OF SYNOVIAL CELLS

Synovial cell cloning was performed on nine patients by limiting dilution. Dispersed cells were suspended in the culture medium and distributed in each well of Microtest plates (Falcon 3072) at a density of 0.6 cells/well. They were incubated at 37°C in a humidified atmosphere of 95% air and 5% carbon dioxide. The day after the cloning of the synovial cells each well was carefully examined with a microscope to ascertain that only a single cell existed. If two or more cells were observed the well was excluded from the study. After 30 days of primary culture each adherent cloned cell was passaged once in two weeks by trypsin treatment.

PROSTAGLANDIN E₂ PRODUCTION OF SUBCULTURED, AND CLONED ASC

The subcultured (second to fourth passage) ASC from five patients were placed in 96-well plates at about 3×10^3 cells/well in 0.2 ml of culture medium, and incubated with 2-mercaptoethanol free medium for 24 hours in the presence or absence of histamine (histamine dihydrochloride; Kanto Chemical Co, Tokyo, Japan) at varying concentrations (1×10^{-8} – 5×10^{-5} mol/l). The culture supernatants of each well were stored at -70°C until prostaglandin E₂ assay. After the supernatants had been collected the cells were removed from each well by trypsin and the number of viable cells was counted with trypan blue dye exclusion. Most of these subcultured ASC were fibroblast like cells.

Three different types of rheumatoid synovial cells (dendritic, macrophage like, and fibroblast like) were cloned and distinguished morphologically in every experiment.⁹ To assay spontaneous or histamine induced prostaglandin E₂ production, the cloned dendritic, macrophage like, and fibro-

Institute of
Rheumatology,
Tokyo Women's Medical
College, Tokyo
M Sasano
M Goto
K Nishioka

Division of Rheumatic
Diseases, Metropolitan
Otsuka Hospital
M Goto

Correspondence to:
Dr M Goto,
Institute of Rheumatology,
Tokyo Women's Medical
College, Box 116, NS BLD,
7F, 2-4-1, Nishi-Shinjuku,
Shinjuku-ku, Tokyo,
163, Japan.

Accepted for publication
6 September 1989

Prostaglandin E₂ production induced by histamine by adherent rheumatoid synovial cells. Each point indicates prostaglandin E₂ concentration during 24 hour culture of adherent synovial cells from four different experiments, with or without addition of 1×10^{-8} – 5×10^{-5} M histamine. Horizontal lines with hatched bars represent the mean (SEM) (n=5). (*) A significant increase (p<0.05) compared with control (without histamine). PGE₂=prostaglandin E₂.

blast like cells were passaged with trypsin treatment and replated at a density of about 100, 500, or 1000 cells/well, respectively, in 96-well culture plates. The cultures became confluent after three to six days, and the approximate number of the cells was ≈200 cells/well for dendritic cells, 1000 cells/well for macrophage like cells, and 3000 cells/well for fibroblast like cells, respectively. After the medium was removed aliquots of 2-mercaptoethanol free medium to be tested were added in the presence or absence of 5×10^{-6} M histamine. The supernatants were collected after 24 hours of additional culture and kept at -70°C until prostaglandin E₂ assay.

Prostaglandin E₂ in the culture media was measured by radioimmunoassay kit (New England Nuclear, Boston, Massachusetts, USA) after extraction and silicic acid column chromatography, as described previously.¹⁰ Results were expressed as pg of prostaglandin E₂/ml (SEM).

Results

PROSTAGLANDIN E₂ IN THE CULTURE SUPERNATANT FROM THE SUBCULTURED ASC

The ASC spontaneously released considerable amounts of prostaglandin E₂ into the culture medium even after the second to fourth passages. No significant changes in prostaglandin E₂ pro-

duction by these subcultured ASC were observed throughout the second to fourth passages. Production of prostaglandin E₂ by ASC in response to histamine is shown in the figure. Prostaglandin E₂ production was significantly (p<0.05) enhanced in response to 5×10^{-6} – 5×10^{-5} M histamine, according to a paired *t* test. Although the prostaglandin E₂ concentrations varied considerably among individual culture samples, the mean prostaglandin E₂ concentration released from the ASC was highest at a histamine concentration of 5×10^{-6} mol/l. We thus used histamine at this concentration in the subsequent experiments. The addition of histamine increased prostaglandin E₂ release by all five adherent synovial cell cultures tested, and there was no significant change in the number of viable cells throughout the experiments.

PROSTAGLANDIN E₂ IN THE CULTURE SUPERNATANT FROM CLONED ASC

The culture supernatants from the 14 clones of rheumatoid synovial cells in the presence or absence of histamine were assayed for prostaglandin E₂. The table shows a relatively small number of dendritic cells (about 200 cells/well) and macrophage like cells (about 1000 cells/well) from patients with RA which produced considerable amounts of prostaglandin E₂ dendritic cells: 41–1211 pg/ml, macrophage like cells: 51–4393 pg/ml). Similarly, but to a lesser extent, fibroblast like cells (about 3000 cells/well) secreted prostaglandin E₂ spontaneously (84–634 pg/ml). Considering the number of cells in the culture wells, the most efficient spontaneous producers of prostaglandin E₂ were dendritic cells, followed by macrophage like cells, and fibroblast like cells. Dendritic and macrophage like cells produced about eight to 10 times more prostaglandin E₂ than fibroblast like cells on a cell to cell basis, and the differences between fibroblast like and macrophage like or dendritic cells were significant (p<0.05), according to multiple comparison test. The table also shows that 5×10^{-6} M histamine increased the production of prostaglandin E₂ by all types of adherent synovial cell clones. Prostaglandin E₂ production in dendritic and fibroblast like cells was significantly enhanced by about twice as much compared with spontaneous release when they were stimulated with histamine (fibroblast like cells=p<0.01, and dendritic cells=p<0.001 by paired *t* test). Although the increase of prostaglandin E₂ in macrophage like cells was not significant because of the large variability among samples, the mean concentration of increased prostaglandin E₂ stimulated by histamine in macrophage like cells was about four times as great as that for spontaneous release. The ability of

Induced prostaglandin E₂ production by cloned adherent rheumatoid synovial cells

Type of cloned cell	Approximate number of cells/well	Prostaglandin E ₂ (pg/ml) (SE)		Percentage increase§
		Spontaneous	Histamine induced	
Macrophage like	1000	1241 (387) (0.248)†	4199 (2095) (0.840)	378 (108)‡
Dendritic	200	303 (82) (0.303)†	449 (103) (0.449)	184 (25)‡
Fibroblast like	3000	464 (196) (0.031)†	749 (278) (0.050)	171 (13)‡

Values are the mean (SEM) of 14 separate experiments, each clone in a set of dendritic, macrophage like, and fibroblast like cells.
*p<0.05.
†Percentage increase obtained in 14 different clones with and without the addition of histamine in the medium.
‡Prostaglandin E₂ concentration on cell to cell basis (pg/cell) is indicated below in parentheses.
§Percentage increase=(histamine induced prostaglandin E₂ concentration)/(spontaneous prostaglandin E₂ concentration)×100.

dendritic, macrophage like, and fibroblast like cells to respond to histamine was greatest in macrophage like cells followed by dendritic cells, followed by fibroblast like cells. Macrophage like cells produced the most prostaglandin E₂ in response to histamine.

Discussion

Aggregation of mast cells at sites of cartilage erosion and an increased concentration of histamine in rheumatoid synovial fluid suggest that mast cells may have a role in the pathophysiology of rheumatoid synovitis.^{4-7, 11} Histamine has been shown to stimulate prostaglandin E₂ production in both primary and passaged cultures of rheumatoid ASC and articular chondrocytes.⁸ Both types of cells were activated to increase the amounts of prostaglandin E₂ by exposure to synovial factor (IL-1), and histamine subsequently increased the production of prostaglandin E₂.⁸ It was not known which type of cells were responsible for the induced production of prostaglandin E₂, however, as the cultures of unseparated ASC consisted of heterogeneous cell populations. We thus cloned three different types of synovial cells (fibroblast like, macrophage like, and dendritic)⁹ and tried to identify those responsible for the spontaneous or induced production of prostaglandin E₂.

The results presented here suggest that cloned ASC, as well as unseparated ASC, can be stimulated by histamine to produce prostaglandin E₂ even after long term culture. Each of the three types of synovial cell clones from five different patients with RA produced prostaglandin E₂ spontaneously and was stimulated to produce additional prostaglandin E₂ by histamine. None of the following indices affected the histamine mediated production of prostaglandin E₂ by ASC: disease activity; drugs; duration of disease; and patient's age. The number of experiments, however, was quite limited. The ability of cells to respond to histamine on a cell to cell basis was in the following order: macrophage like cells > dendritic cells > fibroblast like cells. Macrophage like cells produced the most prostaglandin E₂ in response to histamine.

The histamine concentrations used and the method of stimulation in the present experiments were similar to those reported to enhance the production of prostaglandin E₂ from cultured ASC.¹² As mast cells may degranulate in response to the anaphylatoxins C3a, C4a, and C5a derived from the interactions with the complement and immune complexes, mast cells present in the synovial tissues or synovial fluids might contribute to the release of histamine.¹³ The mechanism by which histamine increases the production of prostaglandin E₂ by ASC is not clear, but it has been reported that production of prostaglandin E₂ induced by histamine is mediated via the histamine H₁ receptor.^{8, 14}

Moreover, early in the culture ASC released large amounts of prostaglandin E₂—for example, 54.1 ng/ml per 24 hour culture in 23 mm diameter 12-well plate, but the prostaglandin E₂ concentration in culture declined rapidly with time.¹⁸

Spontaneous prostaglandin E₂ production by subcultured synovial cells with predominantly fibroblastic morphology was generally very low (<0.25 ng/ml/24 hour culture).⁸ Primary cultures of ASC represented a mixed cell population, classified morphologically as fibroblast like, dendritic, and macrophage like cells,¹⁵ and those heterogeneous cells were used in the conventional experiments. Because primary cultures containing large numbers of macrophage like and dendritic cells produce large amounts of prostaglandin E₂, it has been proposed that dendritic or macrophage like cells produce most of the prostaglandin E₂ in primary culture. This notion was confirmed by our present observation that dendritic and macrophage like cells are the predominant spontaneous producers of prostaglandin E₂.

The interaction of histamine and ASC may modulate inflammatory processes in rheumatoid joints. Consequently, identification of the predominant producers of prostaglandin E₂ in the rheumatoid synovial tissues might provide more precise information and help in understanding the pathogenesis of inflammatory joint diseases such as RA.

- 1 Dayer J M, Krane S M, Russell R G G, Robinson D R. Production of collagenase and prostaglandins by isolated adherent rheumatoid synovial cells. *Proc Natl Acad Sci USA* 1976; 73: 945-9.
- 2 Wolinski S, Goodwin J S, Messner R P, Williams R C J. Role of prostaglandins in the depressed cell-mediated immune response in rheumatoid arthritis. *Clin Immunol Immunopathol* 1980; 17: 31-6.
- 3 Dayer J M, Breard J, Chess L, Krane S M. Participation of monocyte-macrophages and lymphocytes in the production of a factor that stimulates collagenase and prostaglandin release by rheumatoid synovial cells. *J Clin Invest* 1979; 64: 1386-92.
- 4 Bromley M, Fisher W D, Woolley D E. Mast cells at sites of cartilage erosion in the rheumatoid joint. *Ann Rheum Dis* 1984; 43: 76-9.
- 5 Crisp A J, Chapman C M, Kirkham S E, Schiller A L, Krane S M. Articular mastocytosis in rheumatoid arthritis. *Arthritis Rheum* 1984; 27: 845-51.
- 6 Godfrey H P, Ilardi C, Engber W, Graziano F M. Quantitation of human synovial mast cells in rheumatoid arthritis and other rheumatic diseases. *Arthritis Rheum* 1984; 27: 852-6.
- 7 Partsch G, Schwagerl W, Eberl R. Histamine in rheumatoid diseases. *Z Rheumatol* 1982; 41: 19-22.
- 8 Taylor D J, Yoffe J R, Brown D M, Woolley D E. Histamine stimulates prostaglandin E production by rheumatoid synovial cells and human articular chondrocytes in culture. *Arthritis Rheum* 1986; 29: 160-5.
- 9 Goto M, Sasano M, Yamanaka H, et al. Spontaneous production of an interleukin-1 like factor by cloned rheumatoid synovial cells in long-term culture. *J Clin Invest* 1987; 80: 786-96.
- 10 Ciabattini G, Pugliese F, Spaldi M, Cinotti G A, Patrono C. Radio-immunoassay of prostaglandin E₂ and F₂ in human urine. *J Endocrinol Invest* 1979; 2: 173-7.
- 11 Yoffe J R, Taylor D J, Woolley D E. Mast cell products stimulate collagenase and prostaglandin E production by cultures of adherent rheumatoid synovial cells. *Biochem Biophys Res Commun* 1984; 122: 270-6.
- 12 Alhenc-Gelas F, Tsai S J, Callahan K S, Campbell W B, Johnson A R. Stimulation of prostaglandin formation by vasoactive mediators in cultured human endothelial cells. *Prostaglandins* 1982; 24: 723-42.
- 13 Dias da Silva W, Eisele J W, Lepow I H. Complement as a mediator of inflammation III. *J Exp Med* 1967; 126: 1027-48.
- 14 Taylor D J, Woolley D E. Histamine H₁ receptors on adherent rheumatoid synovial cells in culture: demonstration by radioligand binding and inhibition of histamine-stimulated prostaglandin E production by histamine H₁ antagonists. *Ann Rheum Dis* 1987; 46: 425-30.
- 15 Burmester G R, Dimitriu-Bona A, Waters S J, Winchester R J. Identification of three major synovial lining cell populations by monoclonal antibodies directed to Ia antigen and antigens associated with monocytes/macrophages and fibroblasts. *Scand J Immunol* 1983; 17: 69-82.