Production of prostaglandin E\textsubscript{2} induced by histamine by cloned rheumatoid synovial cells

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Abstract
Production of prostaglandin E\textsubscript{2}, 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 E\textsubscript{2}, stimulated by histamine, may have a role in bony destruction in rheumatoid joints.

Articular synovium is considered to be the most important cause of chronic inflammation in rheumatoid arthritis (RA). Dayer \textit{et al} have shown that cultured adherent synovial cells (ASC) dispersed from the lining of rheumatoid synovium produce large amounts of prostaglandin E\textsubscript{2}. Prostaglandin E\textsubscript{2} probably contributes to the destruction of cartilage and to changes in immune functions, and its synthesis increases in response to various inflammatory stimuli, including interleukin-1. Recent histochemical studies have shown that both mast cells and macrophages accumulate at sites of cartilage erosion and synovium in rheumatoid joints, as well as a large amount of histamine in some rheumatoid synovial fluids. Histamine can also stimulate ASC to produce prostaglandin E\textsubscript{2}. These studies did not provide any information, however, on which types of cells are responsible for the production of prostaglandin E\textsubscript{2} as synovial cells consisted of heterogeneous cell populations. To overcome this we cloned these synovial cells. In our previous paper\textsuperscript{6} 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 E\textsubscript{2} 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.\textsuperscript{9} All of the patients were receiving non-stereoidal anti-inflammatory agents; seven patients were also receiving small doses of corticosteroids (<5 mg/day). After removal of the adipose tissue 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\textsuperscript{-5} M 2-mercaptoethanol (Nakarai), 100 U/ml penicillin, and 100 \mu\text{g} ml\textsuperscript{-1} 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 =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 E\textsubscript{2} 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\textsuperscript{4} 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\textsuperscript{-8}-5×10\textsuperscript{-5} mol/l). The culture supernatants of each well were stored at −70°C until prostaglandin E\textsubscript{2} 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 trypsin 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.\textsuperscript{9} To assay spontaneous or histamine induced prostaglandin E\textsubscript{2} production, the cloned dendritic, macrophage like, and fibro-
Production of prostaglandin $E_2$ by cloned rheumatoid synovial cells

Prostaglandin $E_2$ production induced by histamine by adherent rheumatoid synovial cells.

Each point indicates prostaglandin $E_2$ concentration during 24 hour culture of adherent synovial cells from four different experiments, with or without addition of $1.10^{-8}-5.10^{-8}$ M histamine.

Horizontal lines with hatched bars represent the mean (SEM) (n=5). (*) A significant increase (p<0.05) compared with control (without histamine).

PGE$_2$=prostaglandin $E_2$.

Results

PROSTAGLANDIN E$_2$ 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 $E_2$. 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 $E_2$ 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 $E_2$ spontaneously (84-634 pg/ml).

Considering the number of cells in the culture wells, the most efficient spontaneous producers of prostaglandin $E_2$ 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 $E_2$ than fibroblast like cells on a cell to cell basis, and the differences between fibroblast like and macrophage like or dendritic like 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 $E_2$ by all types of adherent synovial cell clones. Prostaglandin $E_2$ 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 $E_2$ in macrophage like cells was not significant because of the large variability among samples, the mean concentration of increased prostaglandin $E_2$ stimulated by histamine in macrophage like cells was about four times as great as that for spontaneous release. The ability of

No significant changes in prostaglandin $E_2$ production by cloned adherent rheumatoid synovial cells

<table>
<thead>
<tr>
<th>Type of cloned cell</th>
<th>Approximate number of cells/well</th>
<th>Prostaglandin $E_2$ (pg/ml)</th>
<th>Percentage increase$^*$</th>
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<tr>
<td></td>
<td></td>
<td>Spontaneous</td>
<td>Histamine induced</td>
</tr>
<tr>
<td>Macrophage like</td>
<td>1000</td>
<td>1241 (387)</td>
<td>4199 (2095)</td>
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<td></td>
<td>(0.248)</td>
<td>(0.840)</td>
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<td></td>
<td></td>
<td>303 (82)</td>
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<td></td>
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<td>(0.303)</td>
<td>(0.449)</td>
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<tr>
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<td>464 (196)</td>
<td>749 (278)</td>
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<td>(0.031)</td>
<td>(0.050)</td>
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<tr>
<td>Dendritic</td>
<td>200</td>
<td>1241 (387)</td>
<td>4199 (2095)</td>
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<td>(0.248)</td>
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<td></td>
<td>Fibroblast like</td>
<td>1241 (387)</td>
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<td>(0.248)</td>
<td>(0.840)</td>
</tr>
</tbody>
</table>

Values are the mean (SEM) of 14 separate experiments, each clone in a set of dendritic, macrophage like, and fibroblast like cells.

*Percentage increase obtained in 14 different clones with and without the addition of histamine in the medium.

$^*$Prostaglandin $E_2$ concentration on cell to cell basis (pg/cell) is indicated below in parentheses.

$^*$Percentage increase=(histamine induced prostaglandin $E_2$ concentration)/(spontaneous prostaglandin $E_2$ concentration)$\times$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 $E_2$ 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.\(^\text{3-7}\) Histamine has been shown to stimulate prostaglandin $E_2$ production in both primary and passaged cultures of rheumatoid ASC and articular chondrocytes.\(^\text{9}\) Both types of cells were activated to increase the amounts of prostaglandin $E_2$ by exposure to synovial factor (IL-1), and histamine subsequently increased the production of prostaglandin $E_2$.\(^\text{8}\) It was not known which type of cells were responsible for the induced production of prostaglandin $E_2$, 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)\(^\text{9}\) and tried to identify those responsible for the spontaneous or induced production of prostaglandin $E_2$.

The results presented here suggest that cloned ASC, as well as unseparated ASC, can be stimulated by histamine to produce prostaglandin $E_2$ even after long term culture. Each of the three types of synovial cell clones from five different patients with RA produced prostaglandin $E_2$ spontaneously and was stimulated to produce additional prostaglandin $E_2$ by histamine. None of the following indices affected the histamine mediated production of prostaglandin $E_2$ 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 > dendritic cell > fibroblast like cells. Macrophage like cells produced the most prostaglandin $E_2$ 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 $E_2$ from cultured ASC.\(^\text{11}\) 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.\(^\text{13}\) The mechanism by which histamine increases the production of prostaglandin $E_2$ by ASC is not clear, but it has been reported that production of prostaglandin $E_2$ induced by histamine is mediated via the histamine H$_2$ receptor.\(^\text{14}\)

Moreover, early in the culture ASC released large amounts of prostaglandin $E_2$—for example, 54-1 ng/ml per 24 hour culture in 23 mm diameter 12-well plate, but the prostaglandin $E_2$ concentration in culture declined rapidly with time.\(^\text{1,8}\) Spontaneous prostaglandin $E_2$ production by subcultured synovial cells with predominantly fibroblastic morphology was generally very low (<0.25 ng/ml/24 hour culture).\(^\text{3}\) Primary cultures of ASC represented a mixed cell population, classified morphologically as fibroblast like, dendritic, and macrophage like cells,\(^\text{9}\) 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 $E_2$, it has been proposed that dendritic or macrophage like cells produce most of the prostaglandin $E_2$ in primary culture. This notion was confirmed by our present observation that dendritic and macrophage like cells are the predominant spontaneous producers of prostaglandin $E_2$.

The interaction of histamine and ASC may modulate inflammatory processes in rheumatoid joints. Consequently, identification of the predominant producers of prostaglandin $E_2$ in the rheumatoid synovial tissues might provide more precise information and help in understanding the pathogenesis of inflammatory joint diseases such as RA.

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