Spontaneous release of angiotensin converting enzyme and interleukin 1β from peripheral blood monocytes from patients with rheumatoid arthritis under a serum free condition

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Abstract
Angiotensin converting enzyme (ACE) and interleukin 1 activities were assayed simultaneously in the serum free medium from the unstimulated peripheral blood monocytes from 32 patients with rheumatoid arthritis (RA), 11 patients with osteoarthritis, and 25 normal controls matched for age and sex. Angiotensin converting enzyme activity was raised in most (29/32) patients with RA and interleukin 1 activity (most of which was interleukin 1β) was enhanced in 11/32 patients with RA, while monocytes from only two patients with osteoarthritis, but from none of the controls, secreted a small amount of ACE activity in the culture condition. Monocytes from patients with early RA (disease duration <3 years) released significantly more ACE and interleukin 1 than those from late stage RA (disease duration ≥3 years).

Patients and methods

PATIENT SELECTION
Studies were carried out on 32 patients (27 female, 5 male) with definite or classical seropositive RA, whose diagnosis was made according to the American Rheumatism Association criteria. Their ages ranged from 33 to 69 years (mean SD 54.9 ± 12.4). The duration of disease in 19 patients with RA was less than three years (group A: 0-1-2-9 years) and for three or more years in 13 (group B: 3-25 years). Eleven patients (eight female, three male) aged 40-70 years (56 ± 3 (10-8)) were radiographically diagnosed as having osteoarthritis. Twenty five healthy volunteers matched for age and sex served as controls. Most patients were treated with non-steroidal anti-inflammatory drugs; in addition, 20 patients with RA were taking small doses of prednisolone (<10 mg/day). No patients had been given second line antirheumatic drugs.

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Rheumatoid arthritis (RA), a disease of unknown aetiology and worldwide distribution, is characterised by formation of non-necrotising granulomas, such as pannus and RA nodules rich in monocyte lineage cells, and a state of anergy to purified protein derivatives. These characteristics may be partly related to monocyte activation in RA. In patients with RA HLA-DR expression in synovial membranes was increased, interleukin 1 production by monocytes is enhanced, activities of Fc receptor and phospholipase C in monocytes are increased, and there is increased biosynthesis of complement components by monocytes.

Angiotensin converting enzyme (ACE) is a product of monocytes. Few, but controversial results on serum ACE activity in patients with RA have been published. Machin et al found no significant change in serum ACE levels of patients with Sjögren's syndrome associated with RA, systemic lupus erythematosus, or systemic sclerosis. Aoyagi et al reported decreased levels in RA, whereas Sheikh and Kaplan described increased ACE activity in serum samples from patients with RA and systemic lupus erythematosus. Therefore, we undertook this study to determine whether the serum level of ACE is increased, whether peripheral blood monocytes in RA release ACE and interleukin 1 spontaneously, and whether there is a correlation between them. The results showed that monocytes from patients with RA of less than three years' duration secrete ACE and interleukin 1 spontaneously, which is consistent with the hypothesis that there is monocyte activation in the early stage of RA.

ISOLATION OF PERIPHERAL BLOOD MONOCYTES
Heparinised blood (10 ml) was obtained from each individual, and mononuclear cells were isolated by Ficoll density gradient centrifugation. After two washes in Hams F-10 nutrient medium (Flow Laboratories, Rockville, MD) at room temperature, the cells suspended in this medium were incubated in plastic Petri dishes (60 × 15 mm; Falcon Labware, Oxnard, CA) at a concentration of 2 × 10⁶ cells/ml for two hours at 37°C in 5% CO². After this incubation period the non-adherent cells were removed and the dishes rinsed with warm F-10 medium. The adherent cells were then incubated in F-10 medium. All cell counts were made in duplicate on cell cultures grown in parallel with those used for ACE measurements. For counting, cells were harvested in F-10 medium containing 0-8% lidocaine (Fujisawa Pharmaceutical Co, Osaka, Japan). The monocyte monolayers were washed twice with 4 ml F-10 medium, then 2 ml of 0-8% lidocaine was added to each dish, and the cells were incubated at 37°C for 15 minutes. F-10 medium (2 ml) containing endotoxin-free 10% fetal bovine serum (Flow Laboratories) was then added, and the detached cells were collected and counted with a haemocytometer (~10⁵ cells). Microscopic examination showed no residual cells in the dishes after treatment. The monocyte content of this cell preparation measured by non-specific esterase activity was more than 90%. The protein content of the monocyte monolayers was assayed by the method of Lowry et al. All the media and fetal bovine serum used in this experiment were shown to be free...
from pyrogen by a conventional chromogenic Limulus test (Toxicolor test; Seikagaku-Kogyo, Tokyo). 16

ACE ACTIVITY ASSOCIATED WITH CELLS
To measure ACE activity associated with cells the cells in monolayer cultures were removed with a rubber policeman, washed with F-10 medium, sonicated at 0°C for 10-second intervals, and partially solubilised in 0-5 ml of 0-01 M TRIS.HCl, pH 7-4, in the presence of 0-5% Nonidet P-40 (Nakarai Chemicals, Kyoto, Japan), a non-ionic detergent used in the initial purification procedure for converting enzyme. 17 Samples of 100 μl were assayed for ACE activity.

RELEASE OF ACE AND INTERLEUKIN 1 FROM MONOCYTES
The monolayer of monocytes obtained from peripheral blood was incubated for 24 hours in 4 ml F-10 medium without fetal bovine serum to avoid possible monocyte stimulants. 18 The collected medium was centrifuged for 20 minutes at 3000 rpm to remove any suspended cells, and ACE and interleukin 1 activities were both assayed in the same supernatants.

MEASUREMENT OF SERUM ACE ACTIVITY
Serum ACE activity was determined spectrophotometrically by a modification of the method described by Cushman and Chung. 19 One unit of ACE is defined as 1 nmol/min hippuric acid released from hippuryl-L-histidyl-L-leucine (5 mmol/l initial concentration) at 37°C.

MEASUREMENT OF ACE ACTIVITY
Angiotensin converting enzyme activity was measured by addition of angiotensin I to the reaction mixture and measurement of the angiotensin II generated by radioimmunoassay. 20 Bradykinin-potentiating peptide B, angiotensin I, angiotensin II, and bestatin were purchased from Protein Research Foundation, Osaka, Japan. Pepstatin A was kindly provided by Dr T Aoyagi, Institute of Microbial Chemistry, Tokyo, Japan. 125I-Angiotensin I and 125I-angiotensin II were obtained from New England Nuclear Co, Boston, MA. Other reagents were of analytical grade and were obtained from commercial sources.

MEASUREMENT OF INTERLEUKIN 1 ACTIVITY
Interleukin 1 activity was assayed by conventional thymocyte costimulation. 1 An ultrapure human interleukin 1 standard was purchased from Genzyme Corporation (Boston, MA; sp act: 100 U/ml).

The interleukin 1 activity in units per milliliter of supernatant samples was defined as the reciprocal of the dilution at which 50% of the maximum response was obtained. 1 In one typical experiment one unit standard interleukin 1 gave 15 000 cpm, and medium containing suboptimal phytohaemagglutin alone gave 150 cpm.

The neutralisation of interleukin 1 like activity was examined by preincubating the culture supernatant with varying concentrations (dilution 1:400-1:3200) of rabbit antihuman interleukin 1α or β serum for two hours at 4°C. 21

Results
SERUM ACE ACTIVITY
Serum ACE levels (U/ml, mean (SE)) were 26.8 (0-3) in RA, 24.9 (1-6) in osteoarthritis, and 22.6 (0-5) in controls (table 1). There was no significant difference between these levels, though the mean serum level of ACE in the patients with RA was slightly higher than that of patients with osteoarthritis or controls. No significant difference in serum ACE level was observed between groups A (disease duration <3 years) and B (disease duration ≥3 years) among patients with RA (table 2).

RELEASE OF ACE INTO MEDIUM FROM MONOCYTES
When the peripheral blood monocytes were maintained in serum free F-10 medium ACE activity appeared spontaneously in the media from patients with RA and osteoarthritis. The mean amount of ACE released from patients with RA was ~500 times more than that from patients with osteoarthritis per protein; no detectable amount of ACE was released from normal monocytes (table 1). The amount of ACE released from group A monocytes (disease duration <3 years) was four to five times more than that from group B monocytes (disease duration ≥3 years) (table 2).

Drugs such as prednisolone may affect the release of ACE from monocytes. When patients in groups A and B were divided into two groups (those taking or not taking prednisolone), and the ACE release from monocytes compared, a slight, but insignificant decrease was noted in the patients treated with prednisolone in both

Table 1: Activities of angiotensin converting enzyme and interleukin 1 in arthritic patients. Values are means (SE) and (range)

<table>
<thead>
<tr>
<th></th>
<th>RA* (n=32)</th>
<th>OA* (n=11)</th>
<th>Controls (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE serum level (U/ml)</td>
<td>26.8 (0-3) (11.6-43.6)</td>
<td>24.9 (1-6) (8.9-69.4)</td>
<td>22.6 (0-5) (15.5-55.8)</td>
</tr>
<tr>
<td>ACE culture supernatant (pg/20 min/mg protein/24 h)</td>
<td>510 (12.2) (0-1469)</td>
<td>1:2 (0-2) (0-5-8)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Intragellular ACE (pg/20 min/mg protein)</td>
<td>23.2 (1-2) (0-131)</td>
<td>10:3 (2-4) (0-94-2)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>IL-1* culture supernatant (U/ml)</td>
<td>0.5 (0-3) (0-2-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
</tr>
</tbody>
</table>

*RA=rheumatoid arthritis; OA=osteoarthritis; ACE=angiotensin converting enzyme; IL-1=interleukin 1. p<0.0001, p<0.01 compared with patients with OA or normal controls.
Figure 2. Relation between IL-1 activity and ACE activity.

Figure 3. Duration of disease (years) vs. ACE activity (U/ml).

Table 2: Effect of disease duration on the monocyte functions. Values are given as means (S.E. and range).

<table>
<thead>
<tr>
<th>Group</th>
<th>Disease duration (years)</th>
<th>Number of patients</th>
<th>ESR released from medium (U/ml)</th>
<th>Monocytes released from patients (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.2 (1.1-5.6)</td>
<td>10</td>
<td>27.5 (20.8-34.2)</td>
<td>35.7 (13.6-49.8)</td>
</tr>
<tr>
<td>B</td>
<td>4.0 (2.5-5.7)</td>
<td>15</td>
<td>30.0 (22.5-37.5)</td>
<td>40.0 (18.0-57.0)</td>
</tr>
</tbody>
</table>

These groups were divided into those ACA positive and negative for the following reasons:

- **ACE activity**
  - Group A: Taking patients
  - Group B: Not taking patients

- **Monocyte function**
  - Group A: Taking patients
  - Group B: Not taking patients

**Note:** The data presented above is from a study on monocyte functions and their relation to ACE activity and disease duration. The study involved patients with osteoarthritis and rheumatoid arthritis. ACE activity was measured in U/ml, and monocyte function was assessed through the release of IL-1 from monocytes. The duration of disease was also recorded in years for each patient. The results indicate a significant correlation between ACE activity and disease duration, with higher ACE activity observed in patients with longer disease duration.
Angiotensin converting enzyme release from RA monocytes

ACE activity was slightly higher in patients treated with prednisolone than in those without such treatment in both groups A (39-9 (4-5) v 20-8 (3-9) pg/20 min/mg protein; mean (SE)) and B (14-1 (2-3) v 10-9 (3-1). The higher intracellular ACE levels in the patients taking prednisolone may be partly explained by the prednisolone-mediated suppression of ACE release. Two patients in group A were serially monitored for a few months. The activity of RA changed dramatically in one patient (No 1) after the administration of prednisolone (ESR fell from 57-9 to 27-6 mm/h), whereas the ESR in another patient (No 2) not taking prednisolone was almost stable (ESR 68 l—57-9). In patient No 1 both ACE activity (500—+444 pg/20 min/mg protein/24 h) and interleukin 1 activity (0-5—4 0 U/ml) released from monocytes decreased, but serum ACE level and intracellular ACE activity were unaffected. All the variables examined in this study were basically unchanged for patient No 2.

Discussion

Much interest has been focused on the immune status in the circulation of patients with RA, primarily in relation to the immunopathogenesis of RA. There seems to be a decrease in the percentage and function of suppressor T cells. 24-26 In addition, monocytes in peripheral blood are activated and show an increased number of IgG Fe receptors, increased prostaglandin and phospholipase C synthesis, and greater phagocytic activity. 27 Interleukin 1 and ACE are also products of activated monocytes. 4, 20 Our study showed that monocytes of the peripheral blood produce and release increased amounts of ACE and enhance the release of interleukin 1 in patients with RA, especially those in the early stages of RA, like those with sarcoidosis. 20, 28

Most interleukin 1 like activity in the serum free culture supernatant, though its level was low, may be interleukin 1β, because this interleukin 1 like activity was completely neutralised by anti-interleukin 1β, but not by anti-interleukin 1α antisera and ~10 ng/ml interleukin 1β, but a negligible amount of interleukin 1α was detected by enzyme linked immunosorbent assay (ELISA) using recombinant human interleukin 1α or β and anti-human interleukin 1α or β (Goto et al, unpublished data). 23 Monocytes from the patients with RA secreted ACE (~500 pg/20 min/10⁶ cells/24 h) spontaneously in this study at a level comparable with that in sarcoidosis (~600 pg/20 min/10⁶ cells/24 h). 20 The increased ACE activity of monocytes from patients with RA raises the question of whether other cell types distributed in the inflammatory lesions release ACE and interleukin 1 as do cells in sarcoidosis nodules and endothelium. 20, 28 Experiments now in progress in our laboratory indicate increased spontaneous release of ACE and interleukin 1 from cells in both rheumatoid synovial membranes and nodules rich in macrophage-like cells (Goto et al, unpublished data). 23 The spontaneous release of both ACE and interleukin 1β from monocytes from patients with less than three years’ duration of disease suggests a critical role of activated monocytes in the early stage of RA, though suppression of macrophage activation by prednisolone may also participate in the reduction of released ACE level in group B. The good correlation between ACE and interleukin 1β secretion and the higher levels of secretion of these two peptides in early RA rather than in late disease suggest (a) monocytes from early stage RA may be activated by unknown stimuli (or antigen), resulting in the release of interleukin 1β and ACE, whereas those from late stage may be refractory to tissue destruction and production of these two substances may be similar in part. Regulation of activated monocytes through suppression of interleukin 1 release from monocytes and synovial cells has recently been thought to be beneficial for the control of RA. 28-30

This is the first report of the spontaneous production and release of ACE and release of interleukin 1 from peripheral blood monocytes from patients in the early stage of RA. These results indicate a theoretical basis for the use of monocyte inhibitors in the treatment of early stage RA.

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16. Obayashi T, Tamura H, Tanaka S. Endotoxin-inactivating


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