Constitutive production of angiotensin converting enzyme from rheumatoid nodule cells under serum free conditions

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Abstract

Angiotensin converting enzyme was assayed in serum free culture supernatants from unstimulated rheumatoid nodule cells. Angiotensin converting enzyme was released spontaneously and the angiotensin converting enzyme derived from rheumatoid nodule cells was suppressed in a dose and time dependent manner by the protein synthesis inhibitor cycloheximide. These data suggest the constitutive de novo synthesis of angiotensin converting enzyme by rheumatoid nodule cells.

Rheumatoid arthritis (RA) is characterised by the formation of non-necrotising granulomas including pannus and subcutaneous nodules rich in monocyte lineage cells. There is evidence of monocyte lineage cell activation in patients with RA. For example, HLA-DR expression in rheumatoid synovial membrane macrophages is increased¹; and cloned synovial membrane cells, including macrophage-like cells, produce interleukin 1 and prostaglandin E₂ without stimulation in patients with RA.²⁻³ The spontaneous release of interleukin 1 β and angiotensin converting enzyme,⁴⁻⁵ and enhanced phospholipase activity⁶ and Fc receptor activity⁷ in peripheral blood monocytes from patients with RA, have been described.

Rheumatoid subcutaneous nodules mainly consist of macrophage-like cells.⁸ In an earlier paper⁸ we reported that rheumatoid nodule cells released interleukin 1 β and prostaglandin E₂ spontaneously, in a similar manner to rheumatoid peripheral blood monocytes⁴⁻⁵ and synovial membrane cells.²⁻³ Experiments now in progress indicate the increased release of angiotensin converting enzyme and interleukin 1 from cloned cells in rheumatoid synovial membranes.

The increased angiotensin converting enzyme activity of monocytes and synovial cells from patients with RA raises the question of whether similar cell types distributed in inflammatory lesions such as rheumatoid nodules also release angiotensin converting enzyme, as do cells in sarcoidosis nodules.⁹⁻¹⁰

Patients and methods

PATIENTS

Studies were carried out on four female patients (aged about 27-58 years) with classical RA as defined by the American Rheumatism Association criteria.¹¹ All subjects were seropositive

for rheumatoid factor (RAHA 1/320-1/2560). They were treated with non-steroidal anti-inflammatory drugs and small doses (<5 mg/day) of prednisolone.

ISOLATION OF RHEUMATOID NODULE CELLS

Subcutaneous nodules were removed from the olecranon region while the patients were under local anaesthesia. The rheumatoid nodules were immediately minced with scissors, washed extensively, and plated into tissue culture plates in HAM F-10 medium (Gibco, Grand Island, NY, USA) supplemented with 10% endotoxin free fetal bovine serum (Gibco), 100 U/ml penicillin, 100 μ g/ml streptomycin, and 10 mM HEPES buffer (Sigma, St Louis, MO, USA). When cell outgrowths were observed during the culture period, the cells were collected and examined for the release of angiotensin converting enzyme as described in the following.

RELEASE OF ANGIOTENSIN CONVERTING ENZYME FROM RHEUMATOID NODULE CELLS

Cell outgrowths from rheumatoid nodules were collected and resuspended in culture medium. The cells were plated in 96 well tissue culture plates and cultured for several days. A monolayer of macrophage-like cells (about 5000 cells) was obtained and incubated for 24 hours in serum free medium. Cloned synovial fibroblast-like cells from patients with RA (about 5000 cells) and normal skin fibroblast-like cells (about 5000 cells) served as controls. In separate experiments, various concentrations (about 100 pg/ml to 100 μ g/ml) of cycloheximide (Sigma) were added for 24 hours or the cells were cultured with 100 ng/ml cycloheximide for up to 48 hours. The medium was collected and centrifuged to remove any suspended cells.

MEASUREMENT OF ANGIOTENSIN CONVERTING ENZYME ACTIVITY

Angiotensin converting enzyme activity was measured by the addition of angiotensin I to the reaction mixture and measurement of the angiotensin II generated by radioimmunoassay.¹⁰ Bradykinin potentiating peptide B, angiotensin I, angiotensin II, and bestatin were purchased from Protein Research Foundation (Osaka, Japan). Angiotensin I and angiotensin II labelled with iodine-125 were obtained from New England Nuclear (Boston, MA, USA). Other reagents were of analytical-reagent grade and were obtained from commercial sources.

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Results

Table 1 shows that when the rheumatoid nodule cells were cultured in serum free HAM F-10 medium, angiotensin converting enzyme activity appeared spontaneously. The amount of angiotensin converting enzyme released from the rheumatoid nodule cells over 24 hours was about 20–40 pg/20 minutes/well. Angiotensin converting enzyme activity could not be detected in the culture supernatants from cloned rheumatoid synovial fibroblast-like cells and normal skin fibroblasts. Table 2 shows that the angiotensin converting enzyme activity was inhibited in a dose dependent manner when the cells were cultured with 1 ng/ml to 100 µg/ml cycloheximide for 24 hours. Cycloheximide at a concentration of 100 µg/ml completely suppressed the angiotensin converting enzyme activity in the culture supernatant. When the cells were treated with 100 ng/ml cycloheximide for about 12–48 hours in culture before assay, the angiotensin converting enzyme activity of rheumatoid nodule cells was suppressed in a time dependent manner (table 3).

Table 1 Angiotensin converting enzyme (ACE) activity in rheumatoid nodule cells.

Rheumatoid nodule cells ^a	ACE activity (pg/20 minutes/well/24 hours)
Patient No 1	40
Patient No 2	36
Patient No 3	20
Patient No 4	20

^aRheumatoid nodule cells (about 5000 cells/well) were cultured for 24 hours in serum free HAM F-10 medium.

Table 2 Dose dependent suppression of rheumatoid nodule cell derived angiotensin converting enzyme (ACE) activity by cycloheximide.

Concentration of cycloheximide ^a	ACE activity (pg/20 minutes/well/24 hours)
100 µg/ml	0
10 µg/ml	4
1 µg/ml	4
100 ng/ml	4
10 ng/ml	16
1 ng/ml	32
100 pg/ml	36
0 pg/ml	36

^aRheumatoid nodule cells (about 5000 cells/well) were cultured for 24 hours in the presence of cycloheximide at the concentrations indicated.

Table 3 Time dependent suppression of rheumatoid nodule cell derived angiotensin converting enzyme (ACE) activity by cycloheximide.

Culture duration ^a (hours)	ACE activity (pg/20 minutes/well)
0	20
12	16
24	8
48	0

^aRheumatoid nodule cells (about 5000 cells/well) were cultured with 100 ng/ml of cycloheximide for the times indicated.

Discussion

The spontaneous production of angiotensin converting enzyme from rheumatoid nodule cells was observed in this experiment in a similar manner to the production of peripheral blood monocytes in RA.⁵ Rheumatoid nodule cells are heterogenous in nature; in a separate experiment, however, most of the rheumatoid nodule cells used were shown to be macrophage-like cells HLA-DR and CD14 positive, but negative for factor VIII.⁸

The experiment with cycloheximide, a protein synthesis inhibitor, indicated that angiotensin converting enzyme was synthesised in vitro and secreted rather than released from the previously processed protein stores. Therefore rheumatoid nodule derived macrophage-like cells are activated in vitro.

Macrophage-like cells in rheumatoid synovial cells produce interleukin 1β and prostaglandin E₂ in vitro without stimulation and are HLA-DR positive.^{2,3} Macrophages in rheumatoid synovial fluid are also activated.

These results suggest that rheumatoid monocyte lineage cells are activated even in in vitro culture. This is the first report of the spontaneous production and release of angiotensin converting enzyme from rheumatoid nodule cells.

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