Angiotensin-converting enzyme, a dipeptidyl carboxypeptidase, catalyzes the conversion of angiotensin I to the vasoactive peptide angiotensin II. The finding of angiotensin-converting enzyme in dexamethasone-stimulated cultured monocytes and alveolar macrophages prompted the examination of a human monocyte-like cell line (U937) for angiotensin I-converting activity. Conversion of angiotensin I (5 x 10^(-3) mol/L) to angiotensin II by U937 cell extracts (10^4 - 4 x 10^4 cells) was detected, and the pH optimum for the reaction was 7.0 to 8.0. The U937 cell angiotensin I-converting activity was purified to homogeneity by carboxymethylcellulose chromatography and trypsin affinity chromatography. The purified protein performed similarly to purified human neutrophil cathepsin G on sodium dodecyl sulfate-gradient polyacrylamide gel electrophoresis (SDS-gradient PAGE), eliciting a reaction of complete identity with neutrophil cathepsin G when diffused against anti-cathepsin G antiserum, and had quantitatively similar angiotensin I-converting activity as neutrophil cathepsin G. Neutrophils and U937 cells had 143 and 52 times greater angiotensin I-converting capability than cultured monocytes or peripheral blood mononuclear cells, suggesting the relative importance of mobile cells containing cathepsin G in the local generation of angiotensin II. These data identify the angiotensin I-converting activity of the U937 cell as leukocyte cathepsin G and provide evidence that the U937 cell has neutrophil-like as well as monocyte-like characteristics.

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Measurement of Angiotensin I-Converting Activity

Unless otherwise noted, specimens were incubated with 5 x 10^{-7} mol/L angiotensin I for 15 minutes at 37 °C in 500 μL of DPBS, pH 7.4. Reactions were stopped by addition of 500 μL of HPLC solvent and either assayed directly or frozen until assayed. Angiotensin I-converting activity was assayed by isocratic reverse-phase ion-pair high-performance liquid chromatography (HPLC) in a buffer containing 0.017 mol/L H₃PO₄, 0.1 mol/L NaClO₄, in aqueous acetonitrile (60% H₂O; 40% CH₃CN, vol/vol), using a Beckman model 144 system.¹⁹ Dipetidyl peptidase angiotensin converting enzyme activity was measured by a fluorimetric assay²⁰ in which His-Leu, derived from Hip-His-Leu, is quantified spectrophluorimetrically by formation of a fluorescent adduct with O-phthaldialdehyde.

Preparation of Cells

U937 cells were grown in liquid phase RPMI 1640 medium that contained 10% (vol/vol) heat inactivated (30 minutes at 56 °C) fetal calf serum and 20 U/mL penicillin and streptomycin (RPMI-FCS). The cells were maintained at 37 °C in 5% CO₂ and passaged every second or third day. Before use in experiments, cells were washed twice in DPBS, pH 7.4. Cell extracts were prepared by sonication of cells for 45 seconds at 4 °C, freeze-thawing, and resonication for 45 seconds at 4 °C.

Human peripheral blood mononuclear cells and neutrophils were isolated by dextran sedimentation and Ficoll-Hypaque density centrifugation.²¹ Human monocytes were cultured by incubating peripheral blood mononuclear cell preparations as obtained above in RPMI 1640 with 20% autologous serum for two hours at 37 °C in T25 flasks (Corning, Corning, NY). The non-adherent cell population was removed by washing twice with HBSS. The adherent cells were then cultured in RPMI FCS. The media was replaced every two or three days. Cultured monocytes were harvested after at least seven days by scraping culture flasks with a rubber policeman. Adherent cells were 100% viable, as determined by trypan blue exclusion, and were 100% non-specific esterase staining positive.²² Extracts of neutrophils, peripheral blood mononuclear cells, and cultured human monocytes were obtained by sonication of suspended cells for 45 seconds, freeze-thawing, and resonication for 45 seconds at 4 °C.

Preparation of Lymphokine-Rich Supernatants

Human peripheral blood mononuclear cells obtained as previously described were incubated in RPMI FCS for 48 hours at 37 °C in the presence of 2 μg/mL phytohemagglutinin.²³ After incubation, the cell supernatants were collected and stored at -70 °C until used.

U937 Cell Release Experiments

U937 cell release experiments were performed using the method of Pike et al.²³ U937 cells that had been cultured for 48 hours in the presence or absence of lymphokine-enriched supernatants (5.0% vol/vol RPMI FCS) were suspended in the presence of cytochalasin B (5 μg/mL) in RPMI 1640. After incubation for ten minutes at 37 °C, the cells were exposed to various concentrations of fMet-Leu-Phe (10^{-6} – 10^{-8} mol/L) for 15 minutes at 37 °C. The tubes were then immediately centrifuged at 400 g for ten minutes at 4 °C, and the supernatants were removed. The pellets were resuspended to the starting volume in RPMI 1640 and then were sonicated for 45 seconds at 4 °C. The supernatants and sonicated pellets were assayed for B-glucuronidase,²⁴ lactic dehydrogenase (LDH),²⁵ and angiotensin-converting activity. Enzyme activity released into the supernatant was expressed as a percentage of the total enzyme activity of cells not stimulated with fMet-Leu-Phe. The amount of enzyme activity released by cells cultured in the absence of lymphokine-enriched supernatants, which was always less than 3% for each enzyme, was subtracted as background from the release of lymphokine-treated cells. LDH release was consistently less than or equal to 6% at each dose of fMet-Leu-Phe.

Isolation of Neutrophil Cathepsin G and Preparation of Antibody to Cathepsin G

Human neutrophil cathepsin G was purified from peripheral blood neutrophils using a procedure previously described.²⁶ A monospecific, polyclonal antiserum to cathepsin G was obtained as described.²⁶

RESULTS

Angiotensin I-Converting Activity of U937 Cells

The angiotensin I-converting activity of U937 cells was examined by incubating extracts from varying numbers of U937 cells with 5 x 10^{-7} mol/L angiotensin I. The extracts from 10⁴, 10⁵, 10⁶, and 4 x 10⁵ cells generated 0.32, 5.1, 12.9, and 17.8 nmol angiotensin II, respectively (Fig 1A). The time course of angiotensin II formation was examined by incubating the extract from 5 x 10⁵ cells with 5 x 10^{-5} mol/L angiotensin I in 500 μL DPBS, pH 7.4, at 37 °C (Fig 1B). At various time intervals up to 60 minutes, 100 μL aliquots were removed, and the reaction was stopped by placing specimens on ice and adding an equal volume of HPLC buffer. Samples were then subjected to HPLC assay. The reaction proceeded rapidly and was linear for the first 15 minutes. At each time point, the sum of the angiotensin II formed, and the angioten-

![Fig 1](https://www.bloodjournal.org)  
(A) Dose-response of U937 cell angiotensin I-converting activity. (B) Time course of U937 cell angiotensin I-converting activity (cell number = 5 x 10⁵ U937 cells).
Table 1. Inhibition of U937 Cell Angiotensin I-Converting Activity

<table>
<thead>
<tr>
<th>Inhibitor (10^-4 mol/L)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBTI</td>
<td>100</td>
</tr>
<tr>
<td>PMSF</td>
<td>76</td>
</tr>
<tr>
<td>Pepstatin</td>
<td>18</td>
</tr>
<tr>
<td>EDTA</td>
<td>8</td>
</tr>
<tr>
<td>8-Hydroxyquinolone</td>
<td>2</td>
</tr>
<tr>
<td>Captopril</td>
<td>2</td>
</tr>
<tr>
<td>SQ 20881</td>
<td>2</td>
</tr>
<tr>
<td>1-10 Phenanthrolane</td>
<td>1</td>
</tr>
<tr>
<td>Merasyl acid</td>
<td>0</td>
</tr>
</tbody>
</table>

sin I remaining was greater than 95% of the amount of angiotensin I in the starting reaction mixture. To determine the pH optimum of angiotensin I conversion extracts from 5 x 10^5 U937 cells were incubated with angiotensin I for 15 minutes at 37 °C in separate 500 μL samples in 0.01 mol/L TRIS, 0.20 mol/L acetate, 0.02 mol/L phosphate, and 0.15 mol/L NaCl, which differed in pH by 1.0 pH unit intervals. The pH optimum of angiotensin I conversion was 7.0 to 8.0. No angiotensin II was generated below pH 5.0, and only 15% of the angiotensin II generated at pH 8.0 was generated at pH 10.0.

Identification and characterization of the U937 angiotensin I-converting activity. In order to determine the relationship of the U937 cell angiotensin I-converting activity to human angiotensin converting enzyme, the angiotensin I-converting activity of U937 cell extracts was examined for susceptibility to a panel of inhibitors. Samples (500 μL) containing an extract of inhibitors. The volume was centrifuged twice at 10,000 g, and >90% of the activity was detected in the supernatant. Table 1. Purification of U937 Cell Angiotensin I-Converting Activity

<table>
<thead>
<tr>
<th>Purification Step</th>
<th>Volume (mL)</th>
<th>Total Protein (mg)</th>
<th>Total Activity (U)*</th>
<th>Recovery at Each Step (%)</th>
<th>Sp Act Units/Mg Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elution with 0.01 mol/L TRIS, pH 7.4, 1.0 mol/L NaCl for 1 h at room temperature</td>
<td>225</td>
<td>1,768.00</td>
<td>4.55</td>
<td>100</td>
<td>2.5 x 10^{-3}</td>
</tr>
<tr>
<td>Trasylol affinity chromatography</td>
<td>30</td>
<td>4.60</td>
<td>2.17</td>
<td>48</td>
<td>.47</td>
</tr>
<tr>
<td>CM-cellulose chromatography</td>
<td>1</td>
<td>.19</td>
<td>1.29</td>
<td>59</td>
<td>670.00</td>
</tr>
<tr>
<td>Overall recovery 28%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 1 unit, activity that cleaves 0.1 μmol of angiotensin I (5 x 10^{-8} mol/L) in 15 minutes.
† Trasylol affinity chromatography was performed in a 35 x 1.0-cm column (Biorad, Richmond, Calif), equilibrated with .5 mol/L NaCl. 0.15 mol/L NaHCO3, pH 7.5, and batch eluted with 200 mL 2 mol/L NaCl, 1 mol/L NaAc, pH 4.5. The .5 mol/L NaCl elution contained no angiotensin I-converting activity.
‡ CM-cellulose chromatography was carried out in a 60-mL B-D Plastipak Syringe (Becton Dickinson, Rutherford, N.J.). The column was equilibrated with .02 mol/L NaAc, .15 mol/L NaCl, pH 5.5, and was batch eluted with 200 mL each of 0.02 NaAc, .5 mol/L NaCl, pH 5.5, and .02 mol/L NaAc, 1 mol/L NaCl, pH 5.5. The effluent of the CM-cellulose column contained no angiotensin I-converting activity, the 0.5 NaCl eluate contained 5% of the angiotensin I-converting activity present in the 1.0 mol/L NaCl eluate.
U937: ANGIOTENSIN I-CONVERTING ACTIVITY

Fig 2. SDS-gradient PAGE of reduced U937 cell angiotensin I-converting activity and neutrophil cathepsin G. (A and D) Protein standards. (B) 3 μg neutrophil cathepsin G. (C) 3 μg of U937 cell angiotensin I-converting activity. (B) The low molecular weight bands are the result of neutrophil cathepsin G autodegradation.

The effect of lymphokine-enriched supernatants and dexamethasone on the angiotensin I-converting activity of U937 cells. Lymphokine-enriched supernatants from PHA-stimulated peripheral blood mononuclear cells induce U937 cell activation as evidenced by the development of fMet-Leu-Phe receptors. Therefore, experiments were designed to characterize the effect of supernatants from PHA-stimulated peripheral blood mononuclear cells (lymphokine-enriched supernatants) on the modulation and characterization of the U937 cell angiotensin I-converting activity. U937 cells were cultured in the presence or absence of lymphokine-enriched supernatants (5% vol/vol) for 48 hours and were examined for the ability to release B-glucuronidase and angiotensin I-converting activity in response to fMet-Leu-Phe. Lymphokine-treated U937 cells exposed to 10⁻⁵ mol/L, 10⁻⁴ mol/L, 10⁻³ mol/L, and 10⁻² mol/L fMet-Leu-Phe released 20.9%, 20.7%, 15.0%, and 6.9% B-glucuronidase activity and 12.1%, 6.6%, 4.9%, and 1.7% angiotensin I-converting activity. In this and other similar experiments, release of angiotensin I-converting activity by lymphokine-treated cells could not be conclusively demonstrated because LDH release by treated cells was ~6%, suggesting that low levels of release could in part be attributable to cytotoxicity. In addition, exposure of cells to lymphokine-enriched supernatants resulted in a marked suppression of total cell angiotensin I-converting activity (see following), making release of low levels of enzyme difficult to detect. U937 cells cultured in the absence of lymphokine-enriched supernatants showed ≈3% release of each measured enzyme at each dose of fMet-Leu-Phe. Other U937 cells were similarly cultured in the presence or absence of lymphokine-enriched supernatants (5% vol/vol) for 72 hours, and the extract from 5 x 10⁶ cells was assayed for total angiotensin I-converting and B-glucuronidase activities. In five separate experiments, the lymphokine-enriched supernatant-treated cells had an average of 69.8% (48.7% to 89.2%) less angiotensin I-converting activity and 23.7% (4.8% to 32.3%) greater B-glucuronidase activity than untreated U937 cells. The angiotensin I-converting activity of cells cultured in the presence of lymphokine-enriched supernatants was inhibited 100% by SBTI (10⁻⁴ mol/L) and showed no Hip-His-Leu cleaving activity, suggesting that all the angiotensin I-converting activity was attributable to cathepsin G. That the suppression of U937 cell angiotensin I-converting activity by lymphokine-enriched supernatants was not due to an inhibitor in the lymphokine-enriched supernatants was suggested by the finding that lymphokine-enriched supernatants (5% vol/vol) did not inhibit the angiotensin I-converting activity of the extract from 5 x 10⁵ U937 cells.

Dexamethasone (4.5 x 10⁻⁷ mol/L) induces dipeptidyl carboxypeptidase angiotensin converting enzyme in cultured monocytes and alveolar macrophages as measured by Hip-His-Leu cleavage. Therefore, U937
cells and human monocytes were cultured in the presence or absence of dexamethasone (10^{-8} mol/L) for 72 hours. The extract from 5 \times 10^5 dexamethasone-treated U937 cells had no appreciable Hip-His-Leu cleaving activity. The extract from 10^6 monocytes cultured in the absence of dexamethasone cleaved 0.57 nmol Hip-His-Leu/min, while the extract from 10^6 monocytes cultured in the presence of dexamethasone cleaved 3.39 nmol Hip-His-Leu/min.

**Relative Angiotensin I-Converting Activities of Neutrophils, U937 Cells, Peripheral Blood Mononuclear Cells, and Cultured Monocytes**

The relative amounts of the angiotensin I-converting activities of 10^6 neutrophils and U937 cells, and 10^6 peripheral blood mononuclear cells and cultured monocytes were examined by incubation of cell extracts with 10^{-4} mol/L angiotensin I in 500 \mu L DPBS, pH 7.4, for 30 minutes at 37°C (Table 3). The angiotensin I-converting activity of neutrophils and U937 cells was inhibited 100% by SBTI (10^{-6}), suggesting that all of the angiotensin I-converting activity in these cells was attributable to cathepsin G. While the angiotensin I-converting activity of the extract of 10^6 peripheral blood mononuclear cells could be attributed to neutrophil cathepsin G contamination, this activity was inhibited 92% by SBTI (10^{-4} mol/L) and 11% by captopril (10^{-4} mol/L), suggesting the possibility that small amounts of dipeptidyl carboxypeptidase were present. The angiotensin I-converting activity of 10^6 cultured monocytes was at the lower limits of sensitivity of the HPLC assay, and therefore, inhibitor studies could not be reliably performed.

**DISCUSSION**

Human monocytes and alveolar macrophages have the capacity to convert angiotensin I to angiotensin II. This reaction is carried out by the dipeptidyl carboxypeptidase angiotensin converting enzyme and has been measured by cleavage of the synthetic peptide Hip-His-Leu. Utilizing an HPLC assay system that directly quantifies angiotensin I and angiotensin II, the ability of a monocyte-like cell line (U937) to convert angiotensin I to angiotensin II was detected (Fig 1). The U937 cell converts angiotensin I to angiotensin II with a pH optimum of 7.0 to 8.0, however, it is inhibited by the serine protease inhibitors SBTI and PMSF, but not by captopril or SQ20881, has no Hip-His-Leu cleaving activity, and is therefore differentiated from the angiotensin-converting enzyme of monocytes and macrophages (Table 1). The U937 cell in angiotensin I-converting activity was shown to be cathepsin G on the basis of four lines of evidence. The activity was purified to homogeneity and behaved identically to neutrophil cathepsin G when assayed in a procedure designed for the purification of neutrophil cathepsin G (Table 2). When analyzed by SDS-gradient PAGE, the neutrophil and U937 cell activities behaved similarly (Fig 2). Equivalent amounts of both enzymes were similar in their ability to convert angiotensin I to angiotensin II. Finally, the identity of the U937 cell enzyme as cathepsin G was confirmed by elicitation of a line of complete identity to neutrophil cathepsin G when diffused against an antiserum to neutrophil cathepsin G in an immunodiffusion assay (Fig. 3). In contrast to monocytes and alveolar macrophages, which have been shown to have dexamethasone-inducible angiotensin converting enzyme, U937 cells exposed to dexamethasone did not have detectable dipeptidyl carboxypeptidase angiotensin converting enzyme as measured by Hip-His-Leu cleavage. The angiotensin I-converting activity of the neutrophil and the U937 cell were found to be of similar magnitude (Table 3). The angiotensin I-converting capability of neutrophils was 143 times greater than that of peripheral blood mononuclear cell preparations and cultured monocytes.

**Table 3. Relative Angiotensin I-Converting Activity of U937 Cells, Cultured Monocytes, Peripheral Blood Mononuclear Cells (PBMCs), and Neutrophils**

<table>
<thead>
<tr>
<th></th>
<th>Angiotensin II Generated (nmol) per 10^6 Cells</th>
<th>Relative Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>200.3</td>
<td>143</td>
</tr>
<tr>
<td>U937 cells</td>
<td>73.0</td>
<td>52</td>
</tr>
<tr>
<td>Cultured monocytes</td>
<td>1.4</td>
<td>1</td>
</tr>
<tr>
<td>PBMCs†</td>
<td>1.4</td>
<td>1</td>
</tr>
</tbody>
</table>

*Starting concentration of angiotensin I was 10^{-4} mol/L, incubation time was 30 minutes.
†The PBMC preparation contained 0.6% neutrophils, 88.6% lymphocytes, and 10.8% monocytes. Neutrophil contamination was adequate to account for angiotensin II generated.
origin of the U937 cell and suggests that this cell may be derived from a precursor of both the monocyte and the neutrophil. This view is supported by the finding that lymphokine-enriched supernatants decreased U937 cell cathepsin G activity by 58% to 89%, suggesting that the activated cell has become more monocyte-like.

The renin–angiotensin system has been considered the major angiotensin II generating pathway in man. Renin cleavage of angiotensinogen is believed to occur in the peripheral circulation, and angiotensin I conversion to angiotensin II is thought to occur on the endothelial cell surface. Human monocytes and alveolar macrophages also have dipeptidyl carboxypeptidase angiotensin converting activity that has similar characteristics to the endothelial cell-derived enzyme. While the functional role of the monocyte-derived enzyme is unclear, the observations that captopril treatment diminishes granulomatous inflammation in schistosomal and BCG-induced murine granuloma models suggest that angiotensin II generating pathways may have modulating effects on some inflammatory processes. Recently, an angiotensin II generating pathway has been characterized in the neutrophil. The neutrophil angiotensin II-generating activity has been identified as cathepsin G, a releasable lysosomal protease. It is of interest that the endothelial cell and monocyte angiotensin-converting enzymes are localized to the plasma membrane, while the neutrophil angiotensin II-generating enzyme (cathepsin G) is localized to the lysosome. While the biologic importance of this difference is unclear, the plasma membrane localization of angiotensin-converting enzyme allows this enzyme ready access to its circulating substrates angiotensin I and bradykinin. The localization of cathepsin G to the neutrophil lysosome, on the other hand, provides a mechanism by which this enzyme may become recruited from a releasable compartment at sites of neutrophil accumulation.

Angiotensin II is viewed as a major mediator of blood pressure control and fluid homeostasis in man. In addition, angiotensin II has several direct biologic effects on vascular tissues. These include smooth muscle contraction, vasoconstriction, enhancement of vascular permeability, and stimulation of local prostaglandin synthesis by endothelial cells. These biologic effects have suggested a possible role for angiotensin II as a modulator of inflammation. The presence of angiotensin II-generating pathways in the monocyte (angiotensin-converting enzyme) and the neutrophil and U937 cell (cathepsin G) provide mechanisms by which angiotensin II can be generated locally by circulating cells. Such pathways of angiotensin II formation may enable these cells to alter their local microvascular environment by generation of a locally active vasoactive peptide.

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Chemistry of a human monocyte-derived cell line (U937): identification of the angiotensin I-converting activity as leukocyte cathepsin G

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