Chemistry of a Human Monocyte-Derived Cell Line (U937): Identification of the Angiotensin I-Converting Activity as Leukocyte Cathepsin G

By Robert A. Snyder, Carol E. Kaempfer, and Bruce U. Wintroub

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 10⁻⁶ mol/L) to angiotensin II by U937 cell extracts (10⁶ - 4 x 10⁶ 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 trasylol 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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ANGIOTENSIN II is a vasoactive octapeptide that is thought to play a central role in the control of blood pressure and fluid homeostasis in man.¹ The initial step in the generation of this peptide is the cleavage of angiotensin I from the plasma glycoprotein angiotensinogen by renin.² Angiotensin I is then converted to angiotensin II by a dipeptidyl carboxypeptidase, designated angiotensin-converting enzyme (kininase II, E.C. 3.4.15.1).³,⁴ Angiotensin-converting enzyme has been primarily localized to the endothelial cells of most mammalian tissues.⁵

Recently, two other human cell-dependent angiotensin II-generating pathways have been described. First, cathepsin G, a neutrophil lysosomal enzyme converts angiotensin I to angiotensin II⁶ and also generates angiotensin II directly from angiotensinogen,⁷ reactions carried out without a requirement for renin or angiotensin-converting enzyme. Second, a chymotryptic enzyme probably derived from human skin mast cells⁸ rapidly converts angiotensin I to angiotensin II.⁹

In addition to its presence in endothelial cells of many mammalian tissues,⁵ brain,¹⁰ kidney,¹¹ and intestinal epithelial cells,¹² angiotensin-converting enzyme has been found to be present in human monocytes,¹³ alveolar macrophages,¹⁴ and in the granulomas of some human¹⁵ and animal¹⁶ granulomatous diseases. In the granulomas of sarcoidosis, angiotensin-converting enzyme has been localized by immunofluorescent techniques to cells believed to be derived from peripheral blood monocytes.¹⁷ For these reasons, the nature of the human monocyte-dependent angiotensin I metabolism is of interest. To overcome the difficulty of obtaining a homogeneous population of human monocytes in amounts needed for chemical studies, we examined the U937 cell, a neoplastic cell thought to be monocyte-like,¹₈ for angiotensin I-metabolizing activity. This report describes the presence of a U937 cell activity that converts angiotensin I to angiotensin II, identifies it as leukocyte cathepsin G, rather than angiotensin-converting enzyme, and examines the relative angiotensin I-converting activities of human neutrophils and mononuclear cells.

MATERIALS AND METHODS

Angiotensin I, angiotensin II, hippuryl-histidyl-leucine (Hip-His-Leu) (Vega, Tucson), N-benzoyl-l-tyrosine ethyl ester (BTEE), N-formyl-l-methionyl-l-leucyl-l-phenylalanine (fMet-Leu-Phe), cytochalasin B, dimethyl sulfoxide, dexamethasone, phenylmethane-sulfonyl fluoride (PMSF), soybean trypsin inhibitor (SBTI), pepstatin, mersalyl acid (Sigma Chemical Co., St Louis), Ficoll-Hypaque, Pharmacia Fine Pharmaceuticals, Piscataway, NJ, captopril, bradykinin-potentiating peptide (SQ 20881) (Squibb, Princeton, NJ), O-phthalaldehyde (Eastman, Rochester, NY), phytohemagglutinin (PHA) (Difco, Detroit) and porcine pancreatic α-chymotrypsin (Millipore, Bedford, Mass) were obtained as noted. U937 cells were obtained from Dr John Stobo (University of California, San Francisco). RPMI-1640, Hank's balanced salt solution (HBBS), and Dulbecco's phosphate-buffered saline (DPBS) were obtained from the University of California, San Francisco, Cell Culture Facility.

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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 H_3PO_4, 0.1 mol/L NaClO_4, in aqueous acetonitrile (60% H_2O; 40% CH_3CN, vol/vol), using a Beckman model 144 system. 

Dipeptidyl peptidase angiotensin converting enzyme activity was measured by a fluorimetric assay in which His-Leu, derived from Hip-His-Leu, is quantified spectrofluorimetrically by formation of a fluorescent adduct with O-phthalaldehyde.

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% CO_2 and passaged every two or three days. Cultured monocytes were harvested after at least seven days by scraping culture flasks with a rubber policeman.

Human peripheral blood mononuclear cells and neutrophils were isolated by dextran sedimentation and Ficoll-Hypaque density centrifugation. 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.

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. Human peripheral blood mononuclear cells and neutrophils were isolated by dextran sedimentation and Ficoll-Hypaque density centrifugation. 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.

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. 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 methods of Pike et al. 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^-1 - 10^-3 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, lactic dehydrogenase (LDH), 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. A monospecific, polyclonal antiserum to cathepsin G was obtained as described.

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 10^5, 10^6, and 4 x 10^6 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 10^6 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://example.com/fig1.png)

(A) Dose-response of U937 cell angiotensin I-converting activity. (B) Time course of U937 cell angiotensin I-converting activity (cell number = 5 x 10^5 U937 cells).
The pH optimum of angiotensin I conversion was determined by keeping the pH constant at 7.0 to 8.0 and by raising the pH by 1.0 pH unit intervals. The pH optimum was found to be between pH 7.0 and 8.0, with 95% of the activity remaining at each pH interval.

Cell extracts were examined for susceptibility to a panel of inhibitors. Samples (500 μL) containing an extract of U937 cells were exposed to each inhibitor for 10 minutes at 37 °C prior to a 5-minute incubation with angiotensin I (5 × 10^-5 mol/L). Captopril (10^-4 mol/L) inhibited the angiotensin I-converting activity of 100 μL of human serum by 100%, as assessed by HPLC assay.

As the dipeptidyl carboxypeptidase, angiotensin-converting enzyme, is known to cleave Hip-His-Leu, the Hip-His-Leu cleaving activities of the extract from 5.0 × 10^6 U937 cells and of 100 μL of human serum were assessed. U937 cell-catalyzed Hip-His-Leu cleavage was not detected, while human serum hydrolyzed 62.3 nmol Hip-His-Leu/min/mL of serum.

Since the pH optimum, and the cleavage of the Phe^4-His^5 bond of angiotensin I could be explained by the presence of a chymotryptic neutral serine protease, the U937 cell angiotensin I-converting activity was studied with respect to its relationship to neutrophil cathepsin G. The U937 cell angiotensin I-converting activity was subjected to a procedure devised for the purification of neutrophil cathepsin G (Table 2). The recovery of starting activity was 28%, and the material was purified 2.7 × 10^5-fold.

The purified U937 cell angiotensin I-converting activity behaved during the purification procedure as if it were cathepsin G, the purified material was compared to neutrophil cathepsin G with respect to physicochemical, antigenic, and functional characteristics. Two micrograms of the U937 cell angiotensin I-converting activity and 2 μg of neutrophil cathepsin G were each subjected to sodium dodecyl sulfate-gradient polyacrylamide gel electrophoresis (SDS-gradient PAGE) (Fig 2). The U937 cell angiotensin I-converting activity was purified 2.7 × 10^5-fold.

### Table 1. Inhibition of U937 Cell Angiotensin I-Converting Activity

<table>
<thead>
<tr>
<th>Inhibitor (10^-4 mol/L)</th>
<th>Inhibition (%)</th>
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</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>B-Hydroxyquinolone</td>
<td>2</td>
</tr>
<tr>
<td>Captopril</td>
<td>2</td>
</tr>
<tr>
<td>SO 20881</td>
<td>2</td>
</tr>
<tr>
<td>1-10 Phenanthraline</td>
<td>1</td>
</tr>
<tr>
<td>Mersalyl acid</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 2. 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>1,766.00</td>
<td>4.55</td>
<td>100</td>
<td>2.5 × 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 × 10^-6 mol/L) in 15 minutes.

†Trasylol affinity chromatography was performed in a 35 × 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 of 2 mol/L NaCl, 1 mol/L NaAc, pH 4.5. The .5 mol/L NaCl effluent 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 of 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.

Angiotensin I-converting activity showed bands at 30,000 and 29,000 mol wt. Neutrophil cathepsin G showed major bands at 30,000 and 28,000 mol wt. The antigenic identity of neutrophil cathepsin G and the U937 cell angiotensin I-converting activity was then investigated by immunodiffusion. Anti-cathepsin G antiserum elicited a reaction of complete identity when diffused against 3 μg of purified neutrophil cathepsin G, and 3 μg of U937 cell angiotensin I-converting activity (Fig 3). Each enzyme (1 μg) was examined for angiotensin I conversion by 15-minute incubation with 5 x 10⁻⁵ mol/L angiotensin I. The U937 cell-derived activity generated 1.16 nmol of angiotensin II, and neutrophil cathepsin G generated 10.4 nmol of angiotensin II.

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 character 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, 10⁻⁶ mol/L, 10⁻⁷ mol/L, and 10⁻⁸ 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 10⁵ 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 × 10⁵ dexamethasone-treated U937 cells had no appreciable Hip-His-Leu cleaving activity. The extract from 10⁵ monocytes cultured in the absence of dexamethasone cleaved 0.57 nmol Hip-His-Leu/min, while the extract from 10⁵ 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 10⁵ neutrophils and U937 cells, and 10⁶ peripheral blood mononuclear cells and cultured monocytes were examined by incubation of cell extracts with 10⁻⁴ mol/L angiotensin I in 500 μL 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 (10⁻⁴), 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 10⁵ peripheral blood mononuclear cells could be attributed to neutrophil cathepsin G contamination, this activity was inhibited 92% by SBTI (10⁻⁴) 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 10⁶ 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 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 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.

The U937 cell has been used by others as a model for the human monocyte-macroage. This cell stains positive for nonspecific esterase activity, and spontaneously releases lysozyme and endogenous pyrogen. After exposure to lymphokines, the U937 cell demonstrates phagocytic capacity, antibody-dependent cellular cytotoxicity, Fc receptors, and fMet-Leu-Phe-dependent chemotaxis and lysosomal enzyme secretion. This cell has recently been shown to contain elastase activity that is similar to that found in human monocytes and neutrophils. Although the U937 cell has many monocytic characteristics, it differs from the monocyte by the presence of cathepsin G, an enzyme previously found only in the human neutrophil, and not found in significant quantities in peripheral blood mononuclear cells or cultured monocytes (Table 3). This observation raises questions about the

<p>| Table 3. Relative Angiotensin I-Converting Activity of U937 Cells, Cultured Monocytes, Peripheral Blood Mononuclear Cells (PBMCs), and Neutrophils |
|-------------------------------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Angiotensin II Generated (nmol) per 10⁶ Cells*</th>
<th>Relative Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>200.3</td>
</tr>
<tr>
<td>U937 cells</td>
<td>73.0</td>
</tr>
<tr>
<td>Cultured monocytes</td>
<td>1.4</td>
</tr>
<tr>
<td>PBMCs†</td>
<td>1.4</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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